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RIPARIAN HABITAT OF STREAMS USED FOR BREEDING BY THE STREAMSIDE SALAMANDER (*AMBYSTOMA BARBOURI*) IN MIDDLE TENNESSEE

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Abstract.— The Streamside Salamander (*Ambystoma barbouri*) is a stream-breeding ambystomatid that occurs in southeastern Indiana, southern Ohio, and central Kentucky, with disjunct populations forming the southern portion of the range in the Central Basin (CB) of Tennessee. Because of limited geographic distribution and association with low order, ephemeral streams that generally flow through hardwood forests, this species is under consideration by the US Fish and Wildlife Service for protection under the Endangered Species Act. The CB of Tennessee is a mosaic of habitat types with relatively small patches of forest interspersed amidst agricultural and residential lands, and many of the low-order streams have little, if any, riparian habitat that is forested. We characterized riparian habitat of 14 low-order streams in the CB that were used for breeding during the 2007–2008 and 2008–2009 seasons as forest, agriculture, or residential land. We calculated the percent coverage of these three habitat types in an area that extended 250 m and 500 m from the length of each section of stream in which we counted eggs. Riparian habitat was dominated by agricultural land (pastures and row crops), although at least a small amount of forest cover was found near most streams; thus, terrestrial stages of the Streamside Salamander likely inhabited agricultural land in the CB. Residential land was less prevalent in the vicinity of breeding sites than either agricultural land or forested land. Middle Tennessee, including the CB, is experiencing significant human population growth, a trend predicted to continue for at least the next two decades. The conversion of much of the agricultural and forested lands in the region into subdivisions potentially will negatively affect local populations of this species of conservation concern.

Key Words.— abundance, Central Basin, conservation, distribution, egg counts, population density, reproduction, terrestrial habitat.

The Streamside Salamander, *Ambystoma barbouri* Kraus and Petranka (1989), is a stream-breeding member of the Family Ambystomatidae (the Mole Salamanders) with a contiguous distribution extending from southeastern Indiana and southwestern Ohio into central Kentucky. Isolated populations occur in western and south-central Kentucky, southwestern West Virginia, and the Central Basin physiographic region (CB) of middle Tennessee (Scott et al. 1997; Petranka 1998; Niemiller et al. 2006; Niemiller et al. 2011; Anderson et al. 2014; Lockwood et al. 2016). The species is of conservation concern in most states it inhabits (NatureServe 2015. Available from http://explorer.natureserve.org [Accessed 22 February 2017]), and the IUCN lists the species as near threatened (Hammerson 2004). The Streamside Salamander is deemed in need of management by the Tennessee Wildlife

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Resources Agency (TWRA; Withers 2009), a state conservation listing used, in part, for species with either poorly understood distributions or unknown habitat needs, both types of information deemed vital for proper conservation and management (Withers 2009).

Streamside Salamanders breed in low-order streams from late fall through winter (Petranka 1984a). Many adults migrate to the streams during fall before the breeding season, and presumably live in burrows in the streambank while preparing for courtship (Petranka 1984a). Relatively little is known about terrestrial activities outside of the breeding season, but Petranka (1998) has found adults up to 400 m from streams, and he suggests that juveniles probably travel similar distances away from their natal stream. Regardless, the terrestrial stages are fossorial and inhabit burrows in the floor of hardwood forests (Petranka 1998).

Petranka (1998) indicates that terrestrial stages of the species require forested habitat adjacent to breeding streams. Although relatively little is known about population trends of the Streamside Salamander in middle Tennessee, Niemiller et al. (2009) suggest that populations are declining because of deforestation and residential development of the terrestrial landscape adjacent to streams used for breeding. Furthermore, because the Central Basin is a mosaic of habitat types, including small forest tracts interspersed among agricultural fields (pastures and cropland), cedar glades, and residential areas (urban and suburban areas) (Goodhue et al. 2000, Augustin et al. 2005), many of the low order streams flowing within the CB are not bordered by forested land. The objectives of our study are to characterize riparian habitat of streams used for breeding by the Streamside Salamander in the southern edge of its range and to compare number of females breeding, based on number of both egg masses/m and eggs/m of stream surveyed, among breeding sites with different types of riparian habitats.

METHODS AND MATERIALS

Streams surveyed—. To locate streams used for breeding by the Streamside Salamander, we searched for eggs, larvae, juveniles, and adults approximately 50 m upstream and downstream of road crossings of select low-order streams in southern Rutherford, northern Bedford, northeastern Marshall, eastern Maury, and southeastern Williamson counties from

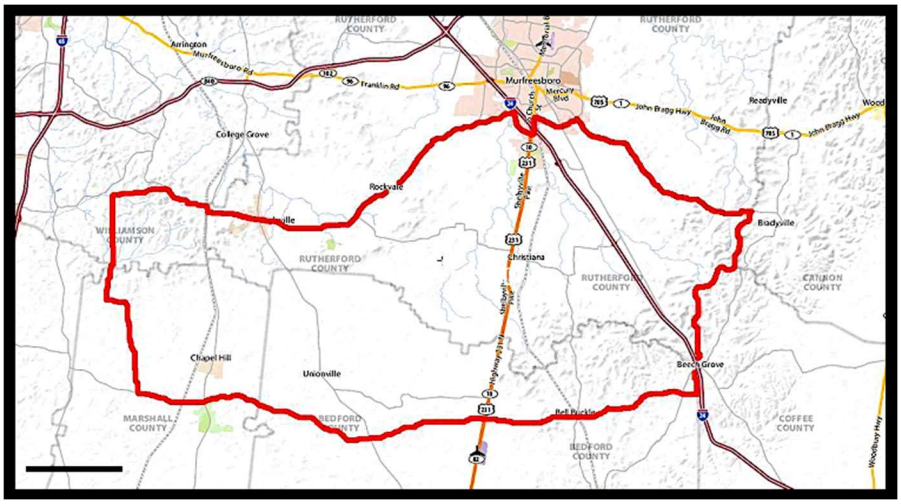


FIG. 1. The area bordered by the red polygon indicates the section of the Central Basin physiographic region in which we searched low-order streams for eggs, larvae, and adult Streamside Salamanders (*Ambystoma barbouri*) during either the 2007–2008 or the 2008–2009 breeding seasons.

December 2007 through April 2008, and from December 2008 through April 2009 (Fig. 1). If we located eggs, we would continue to search the stream until we no longer encountered eggs for a distance of approximately 50 m. We selected streams based on site access and on similarity and proximity to known breeding sites reported by Niemiller et al. (2006). All streams that we searched were clear and ephemeral, and either became reduced to isolated pools or flowed underground during summer and fall.

Relative abundance of eggs and masses—. We counted eggs at six low-order streams during either the 2007–2008 or the 2008–2009 season. We lifted rocks in both pool and riffle habitats to locate eggs, larvae, and breeding adults, and rocks and other cover objects adjacent to streams to locate juveniles and adults. We carefully returned rocks and other objects to their original positions to limit habitat destruction. In these sections of streams, we lifted rocks suitable for egg deposition and checked the undersurface of the rock for the presence of eggs. We considered all eggs and embryos on the undersurface of a rock to form a single mass unless they were at distinctly different stages of development. When we found eggs and embryos at different stages of development on the undersurface of a rock, we regarded each group of similar staged embryos to represent a distinct mass. In addition to counting the number of masses, we counted the number of eggs within each mass. We counted eggs on site if the mass was relatively small; however, we photographed large masses, and those with either eggs or embryos tightly packed, with a digital camera. To accurately count eggs on digital photographs, we used the application Windows Paint (Microsoft Corporation, Redmond, Washington, USA) and placed a dot on each egg as it was counted.

Riparian habitat—. We used aerial photographs available on Google Earth to determine the length of the section of stream surveyed, and we overlaid a grid onto aerial photographs of each site to estimate the proportion of the type of riparian habitat (forested, agricultural cropland, agricultural pasture, or residential) along the length of the section of the stream in which we found eggs or larvae. Because Streamside Salamanders have been reported to travel up to 400 m from a breeding stream (Petranka 1998), we characterized riparian habitat at distances up to 250 m and 500 m on each side of the surveyed sections. We revisited each site to verify our habitat characterization based on the aerial photographs.

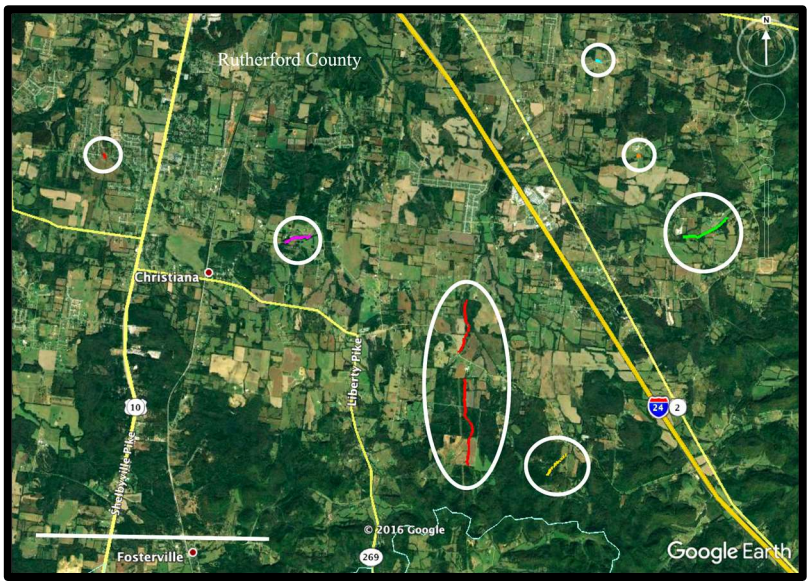
Statistical analysis—. We used Microsoft Excel 2016 (Microsoft Corporation, Redmond, Washington, USA) to perform four single linear regression analyses to elucidate the relationships between egg density (eggs/m of stream length) and the following potential predictor variables: % forest cover within 250 m of stream, % forest cover within 500 m of stream, % field cover within 250 m of stream, and % field cover within 500 m of stream. For these analyses, we used only egg count data from 2009 for the Lynch Hill stream to ensure data independence, as our 2008 counts were conducted after many of the egg masses had hatched. Egg masses from other sites were only counted during one field season each. We also omitted egg count data from the unnamed tributary of the Middle Fork Stones River north of Christiana Hoovers Gap Road, as the salamanders did not appear to be breeding throughout most of the stream area searched, thus potentially skewing the egg density measurement.

RESULTS

Streams used for breeding—. We found eggs, embryos, or larvae of the Streamside Salamander in eight streams in the Stones River watershed of southern Rutherford County (Fig. 2a) and in four streams in the Duck River watershed of northern Bedford County (Fig. 2b). Furthermore, we found one juvenile in eastern Marshall County.

Egg counts—. We counted eggs only from those streams where we discovered salamanders

(a)



(b)

FIG. 2. Aerial photograph of a portion of (a) southern Rutherford County and (b) northern Bedford County, Tennessee. Note the mosaic landscape of agricultural fields, residential developments, and forest stands of different sizes. The white ellipses encompass sections of eight low-order streams where we found Streamside Salamanders (*Ambystoma barbouri*) breeding during either the 2007–2008 or 2008–2009 breeding seasons. The length of the colored path in each circle indicates the relative length of streams searched. White scale bar in lower left of photograph is 5 km.

breeding early during the season, before eggs had started hatching. Consequently, our egg counts were limited to six streams (five streams in southern Rutherford County and one of the northwest Bedford County). We counted 42,804 eggs in 528 egg masses in these six streams (Table 1). We found more than half (55%) of the egg masses and just under half (45%) of the eggs in two low-order tributaries to the Middle Fork of the Stones River (Table 1; Fig. 2a). However, we found a greater density of egg masses (masses/m) and of eggs (eggs/m) in the nearby first-order tributary to the Middle Fork of the Stones River near Lynch Hill Road (Table 1; Fig. 2a).

Egg die offs—. During the 2007–2008 breeding season, we found 38 egg masses comprising 2,550 eggs in Dry Creek (Table 1), with 17 masses and 1,668 eggs in the 690 m upstream and 21 masses and 882 eggs in the 420 m downstream of Cobb Road. However, nearly all embryos we found upstream of the road were dead (white and motionless); whereas, those we found downstream of the road were living.

Table 1. Egg mass data for the Streamside Salamander (*Ambystoma barbouri*) at seven streams in the Central Basin, near the southern edge of the range of the species in southern Rutherford and northern Bedford counties, Tennessee, from December 2007 to May 2009. UNT = Un-named tributary.

Stream	Number of Egg Masses	Survey Length (m)	Min – Max eggs/mass	Total number of eggs	Eggs/m
UNT Middle Fork Stones River, north of Christiana Hoovers Gap Road	5	1090	25 – 205	446	0.4
UNT Middle Fork Stones River, south of Christiana Hoovers Gap Road	288	1730	1 – 439	19,371	11.0
Long Creek	64	620	2 – 345	6,064	9.8
Dry Creek	38	1110	2 – 376	2,550	2.3
Lynch Hill 2008	44	320	8 – 270	2,516	7.8
Lynch Hill 2009	65	320	6 – 910	10,249	32.0
Dolly Branch	24	230	6 – 276	1,608	7.0
Totals	528	5,420	1 – 910	42,804	7.9

During the 2008–2009 breeding season, we found only a few egg masses and larvae upstream of Cobb Road, and we found only one larva and no eggs downstream from the road.

Riparian habitat—. The riparian habitat varied among streams used as breeding sites by Streamside Salamanders during the 2007–2008 and 2008–2009 seasons (Table 2). Although the riparian habitat of most streams included some forest cover, the extent of this coverage varied from 10% or less to nearly 75% (Table 2). Furthermore, the type of field coverage varied based on watershed. For example, the percent field coverage of the seven streams of the Stones River watershed varied from 25% to 89%, but all of the field coverage was either old field or pasture; none of the riparian habitat was cropland in the Stones River watershed. In contrast, the field coverage of the four streams in the Duck River watershed varied from 31% to 90%, but all of the field was tilled cropland (Table 2).

Statistical analysis—. Within 125 m of streams, we found no significant correlation between egg density and % forest ($n = 5$, $r^2 = 0.281$, $P = 0.358$) or % field cover ($n = 5$, $r^2 = 0.298$, $P = 0.342$). Within 250 m of streams, we found a significant positive correlation between egg density and % forest cover ($n = 5$, $r^2 = 0.811$, $P = 0.037$) and a significant negative correlation between egg density and % field cover ($n = 5$, $r^2 = 0.803$, $P = 0.040$).

Table 2. Riparian habitat at 12 low-order streams used by the Streamside Salamander (*Ambystoma barbouri*) in the southern Central Basin of middle Tennessee during the 2007– 2008 and 2008–2009 breeding seasons. UNT = Un-named tributary. Values presented indicate percent coverage of the habitat type in a polygon that extended 125 m on either side of the section of the stream searched.

Watershed	Sub-Watershed	Stream	Length of stream searched (m)	Area of riparian habitat analyzed (m^2)	Forest	Field (Pasture)	Field (Cropland)	Residential
Stones River	Middle Fork	UNT to Middle Fork Stones River, north of Christiana Hoovers Gap Road	1310	327,500	41	59	0	0
		UNT to Middle Fork Stones River, south of Christiana Hoovers Gap Road	2030	507,500	28	72	0	0
		UNT to Long Creek	620	155,000	30	69	0	1
		Dry Creek	1110	277,500	44	56	0	0
		Lynch Hill						
		UNT, downstream section	320	80,000	64	36	0	0
		Upstream and downstream sections	675	168,750	74	25	0	trace
		UNT of Lytle Creek	140	35,000	9	0	0	91
		Knox Branch of Hurricane Creek	100	25,000	13	84	0	3
	West Fork	UNT of Lytle Creek	100	25,000	0	86	0	16
Duck River	North Fork Creek	Dolly Branch of Alexander Creek	230	57,500	68	0	32	0
		UNT to Weakley Creek	50	12,500	9	0	91	0
		UNT to Weakley Creek	50	12,500	69	0	31	0
	Wilson Creek	Osteen Branch of Wilson Creek	100	25,000	10	0	90	0

Table 3. Riparian habitat at 12 low-order streams used by the Streamside Salamander (*Ambystoma barbouri*) in the southern Central Basin of middle Tennessee during the 2007– 2008 and 2008–2009 breeding seasons. UNT = Un-named tributary. Values presented indicate percent coverage of the habitat type in a polygon that extended 250 m on either side of the section of the stream searched.

Watershed	Sub-Watershed	Stream	Length of stream searched (m)	Area of riparian habitat analyzed (m^2)	Forest	Field (Pasture)	Field (Cropland)	Residential (includes industrial)
Stones River	Middle Fork	UNT to Middle Fork Stones River, north of Christiana Hoovers Gap Road	1310	327,500	48	52	0	0
		UNT to Middle Fork Stones River, south of Christiana Hoovers Gap Road	2030	507,500	33	67	0	0
		UNT to Long Creek	620	155,000	15	84	0	1
		Dry Creek	1110	277,500	20	78	0	2
		Lynch Hill						
		UNT, downstream section	320	80,000	51	49	0	0
		Upstream and downstream sections	675	168,750			0	
		UNT of Lytle Creek	140	35,000	3	73	0	24
		Knox Branch of Hurricane Creek	100	25,000	9	81	0	10
	West Fork	UNT of Lytle Creek	100	25,000	16	82	0	2
Duck River	North Fork Creek	Dolly Branch of Alexander Creek	230	57,500	21	0	79	0
		UNT to Weakley Creek	50	12,500	3	0	94	3
		UNT to Weakley Creek	50	12,500				

DISCUSSION

Streamside Salamanders apparently use relatively few of the of the low-order streams available in the southern section of the CB of middle Tennessee as breeding sites (Niemiller et al. 2006, Anderson et al. 2014). Niemiller et al. (2006) found Streamside Salamanders breeding in only 5 of 40 low-order streams that they searched in this region. We searched 40 indicated by Niemiller et al. (2006) and extended our search area to include streams in southeastern Williamson County, northeastern Marshall County, and eastern Maury County, but found *A. barbouri* breeding in only seven additional streams. Thus, our results are in agreement with those of Anderson et al. (2014) who report that Streamside Salamanders breed in a small percentage of apparently suitable low-order streams in the CB of middle Tennessee.

Throughout most of their geographic range, terrestrial stages of the Streamside Salamander typically inhabit upland deciduous forests, with populations rarely found breeding in streams with riparian habitat lacking forests (Petranka 1998). Nonetheless, the CB is a mosaic of habitat types, with agricultural fields and residential land interspersed among remnant forest habitats (Goodhue et al. 2000, Augustin et al. 2005). Our data indicates that Streamside Salamanders breed in streams flowing through agricultural land (forest and cropland), but that more eggs are laid in streams with forest tracts within 500 m of the stream compared to those streams lacking forest tracts within this distance.

We cannot explain why reproduction failed at Dry Creek during the course of this study. Although used as a breeding site annually from 2001 through 2008 (Niemiller et al. 2006, this study, B. Miller pers. obs.), many eggs and embryos failed to develop through hatching upstream of Cobb Road, and we found very little evidence of reproduction during the 2008–2009 breeding season. Although we are uncertain of the cause of the die-off or if eggs have succumbed since our study ended, breeding inexplicably failed at Dry Creek for at least two consecutive years.

Terrestrial habitat—. The terrestrial habitat requirements of the Streamside Salamander are almost completely unknown, and what little is known is largely inferred from the habitat adjacent to streams used for breeding. Populations in Kentucky breed in streams that flow through large tracts of forest (James W. Petranka, pers. comm.). Perhaps in contrast to the landscape of central Kentucky inhabited by Streamside Salamanders, the CB is a mosaic of agricultural land (row crops, pasture, and old fields), residential development, commercial development, and relatively small forest remnants. The proportion of these land uses varies substantially among the smaller sub-watersheds within the Central Basin (Goodhue et al. 2000, Augustin et al. 2005). For example, the streams used as breeding sites in southern Rutherford County are tributaries of the Middle Fork and West Fork of the Stones River, and the sub-watersheds associated with these streams consist primarily of pasture land and forest (deciduous and mixed), with relatively little land devoted to row crops or development (Goodhue et al. 2000). Consequently, riparian habitat of low-order streams used for breeding by Streamside Salamanders is either forest or pasture in southern Rutherford County, but not cropland, which is what our data also indicates. However, encroachment of suburban development is occurring in this area, and residential coverage dominates at least one breeding site. Furthermore, the section of the stream where Regester and Miller (2000) found Streamside Salamanders breeding is now bordered by houses (without any forest buffer).

In contrast to the situation in southern Rutherford County, in northern Bedford County, Streamside Salamanders are restricted to the North Fork Creek sub-watershed of the Upper Duck River, where more land is devoted to pasture than deciduous forest, and more than

23% of the land is devoted to row crops (Augustin et al. 2005). Thus, the known breeding streams in Bedford County are more likely to bordered by agricultural fields than forests, and the agricultural fields are often cropland, rather than pasture land, which is substantiated by our data.

Eggs and egg masses—. The long duration of the breeding season of the Streamside Salamander in middle Tennessee, long length of streams used for breeding at some sites, and number of eggs found, prevented us from counting all eggs at each stream. For example, we did not conduct searches at previously searched stream sections after each rain event. Undoubtedly, additional eggs were laid in these sections throughout the breeding season. Consequently, our counts underestimate the number of eggs present at each stream. Nonetheless, our counts do reflect relative abundance of masses and eggs among sites. Furthermore, we found some sites late in the breeding season, after many eggs had hatched, and we did not attempt to count larvae. Consequently, in several streams we are unable to determine the number of eggs or number of masses deposited. Because of the relatively recent discovery of the Streamside Salamander in Tennessee (Scott et al. 1997), we lack data on demography and cannot comment on whether the populations in the CB are stable, declining, or increasing. Because of the relative ease in counting eggs, compared to either mark-recapture studies of adults migrating during the breeding season or counts of juveniles exiting streams after undergoing metamorphosis, egg count data (eggs/m of stream searched) can be used as a metric to evaluate trends in population dynamics (i.e., whether populations are stable, increasing, or decreasing). The use of egg-counts has been used for decades to assess trends in population dynamics of several species of amphibians that breed in ponds or pools (Cooke 1985, Crouch and Paton 2000, Grant et al. 2005, Paton and Harris 2009), and for a few species that breed in streams, including the Streamside Salamander (Kats and Sih 1992). Based on egg density, a few unnamed tributaries to the Middle Fork of the Stones River that cross Christiana Hoovers Gap Road and Lynch Hill Road are the most important breeding streams for the Streamside Salamander in middle Tennessee.

We discovered relatively late during the 2007−2008 breeding season that salamanders breed in the unnamed tributary draining the forests of Lynch Hill, and we found many larvae of various sizes in the stream channel at that time. Eggs were also present, but our count is a gross underestimate of their abundance. Nonetheless, the density estimate for this site during the 2007–2008 breeding season exceeds those that we obtained at most other sites that year. Furthermore, our count of 10,249 eggs from 65 masses during peak breeding of the 2008–2009 season yielded a density of 32 eggs/m of stream searched, which is nearly three times the density found at other sites. Because many masses of eggs were hatching, we stopped counting eggs after 320 m of stream length, but we did search an additional 675 m upstream of our study site where the riparian habitat was primarily forest. Based on the number of eggs we observed, egg density upstream of our survey section was at least equal to and potentially greater than in the section of stream in which we counted eggs. Furthermore, larvae were abundant; we found larvae near the stream source (near the summit of the hills serving as the Duck River/Stones River divide and the Rutherford County/Bedford County border). Similarly, the tributaries of the other unnamed tributaries, which also originate in the hills at the Bedford County/Rutherford County line, are important breeding streams as evidenced by the relatively high density of eggs, and include the longest known and essentially continuous breeding site for the species in Tennessee. The headwaters of these streams are adjacent to the headwaters of the streams that form the creek at Lynch Hill Road, and these tributaries share a large forested area associated with the hills at the Rutherford County/Bedford County boundary.

The forest that connects these two watersheds likely serves as a refugium for terrestrial stages.

Conservation—. Although populations of the Streamside Salamander persist along streams where forests have been converted to agricultural fields, the relatively greater density of eggs and egg masses in streams with associated with forest tracts within 500 m compared to those associated with indicates the importance of forested terrestrial habitat in the CB. Elimination of remaining forests for residential development poses a serious threat to the survival of the species in Tennessee. Terrestrial stages of the Streamside Salamander apparently are able to use agricultural land in the CB (pasture land in Rutherford County, crop land in Bedford County), but apparently, the species is not as tolerant of conversion of terrestrial habitat into residential use (Niemiller et al. 2006). Middleton and Murray (2009) project that the human population in Rutherford County will increase about 67% during the next 10 to 15 years (from 251,596 in 2010 to 420,465 in 2030). Much of this growth is projected to occur in the unincorporated areas of the county (Middleton and Murray 2009), which will result in additional destruction of terrestrial habitat. Unfortunately, destruction of some of the larger forested areas seems imminent. Water lines have been added near the unnamed tributaries on the south side of the Middle Fork of the Stones River, foreshadowing residential development. Furthermore, the land within the watersheds associated with those same unnamed tributaries and the small stream that drains Lynch Hill flow across property owned by several individuals, and a few landowners have indicated a desire to sell or develop their property.

The known breeding sites of the Streamside Salamander in southern Rutherford and northern Bedford counties, Tennessee occur entirely on private land, and, thus, there are few restrictions on how the land can be used. The Tennessee Division of Forestry (2003) requires a Streamside Management Zone (SMZ) adjacent to any permanent or ephemeral stream. This SMZ is essentially a forested buffer, for which there is no width requirement. There is, however, a recommended width, which varies from 7.6 to 44 m, depending on the slope from the cleared area to the streambed (Division of Forestry 2003). Rutherford County regulations require a 15-meter-wide conservation easement on each side of any natural waterway running through a subdivision (Rutherford County Planning & Engineering Department 2009), which is presumably similar in character to the state-defined SMZ. However, these regulations seem to be inconsistently enforced, as there is no apparent easement along at least one stream that flows through a subdivision. There is also no guarantee that a narrow strip of woodland adjacent to a stream is sufficient to continually support a population of the Streamside Salamander. Additional work is required to obtain more definite information on the terrestrial stages of the Streamside Salamander in Tennessee, including direct observations of individuals during the non-breeding phase of their life cycle and measurements of migration distances. These data would provide valuable information, which could be used to better understand and protect Tennessee populations of this species.

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