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## LIFE HISTORY AND HABITAT OF THE RARE PATCH-NOSED SALAMANDER (*URSPELERPES BRUCEI*)

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**Abstract.**—We examined the life history and habitat characteristics for the Patch-nosed Salamander, *Urspelerpes brucei*. Body-size measurements of individuals captured using litter bags and by hand from 2008 to 2010 indicated that the larval period lasts at least 2 y, salamanders attain reproductive maturity at or shortly after metamorphosis, and adults have very little variation in body size. Occupied streams are characterized by small size, little water, and narrow, steep-walled ravines. Within occupied streams, larval capture rate was significantly and negatively related to mean water depth, underscoring the importance of protecting headwaters. We hypothesize that the only known population of *U. brucei* east of the Tugaloo River was isolated from the west-bank populations by the tremendous increase in water flow caused by the capture of the Tallulah and Chattooga rivers by the Tugaloo as recently as the Pleistocene.

**Key Words.**—amphibian; headwater; larvae; salamander; plethodontid; development; stream capture; metamorphosis

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### INTRODUCTION

Understanding the life history and habitat of rare species is fundamental to making informed decisions regarding conservation and management. This is particularly true for enigmatic or poorly studied species where there is limited data on population dynamics or for species that may occur among patchy habitats with small geographic ranges. For example, many species within the salamander family Plethodontidae, which comprise two-thirds of all known salamander species, have been described in the last few decades as the result of taxonomic splitting (e.g., Jacobs 1987; Highton 1989; Tilley and Mahoney 1996; Highton and Peabody 2000; Campbell et al. 2010). A few previously unknown, morphologically distinct species have also been described (e.g., Wynn et al. 1988). Many of these species are poorly studied ecologically, and their natural history is characterized as unknown or, in the case of members of larger species complexes, presumed to be similar to other, better-known relatives (Drukker et al. 2018). There is a need to describe basic life and natural histories for all of these species to determine whether they have unique life histories and habitat requirements that distinguish them from their better-known relatives.

The Patch-nosed Salamander, *Urspelerpes brucei* (Fig. 1), is a recently described, enigmatic species endemic to northeastern Georgia and adjacent South

Carolina, USA (Camp et al. 2009 2012). This is the only member of the genus *Urspelerpes*, which is a sister taxon to *Eurycea* within the tribe Spelerpini (Wake 2012). *Urspelerpes brucei* is restricted to a very small geographic area where the Tugaloo River bisects the contact zone between the Blue Ridge and Piedmont physiographic provinces. Known as the Tugaloo Mosaic, this region is unique in its soil and floral composition (Garst and Sullivan 1993; Menzel et al. 2016). It is akin to the Piedmont in elevation, but due to its topographic heterogeneity and montane flora, it is sometimes physiographically classified as Blue Ridge (Jensen et al. 2008). *Urspelerpes brucei* occurs in 1<sup>st</sup>- and 2<sup>nd</sup>-order streams that flow through steep-walled ravines (Camp et al. 2012; Pierson et al. 2016). It is currently known from only 17 such streams, all but one of them occurring in Georgia. Given its small geographic range and limited number of known sites, *U. brucei* is a species of conservation concern.

The life history of this species is poorly known but given its evolutionary and morphological distinctiveness (Camp et al. 2009), its habitat and life history may be relatively unique among related and syntopic species. Like other members of the tribe Spelerpini (Ryan and Bruce 2000), it has a biphasic life cycle (Camp et al. 2009); however, the length of the larval stage has not been described. Adult males and females, while dimorphic in color and pattern, do not differ in body



FIGURE 1. Female (left) and male Patched-nosed Salamander (*Urspeleperpes brucei*). (Photographed by Todd Pierson).

size and presumably metamorphose at the same time. No post-metamorphic, immature specimens have been reported. Our purpose was to describe specific variables associated with the habitat of *U. brucei* and report data on larval development and metamorphosis.

#### MATERIALS AND METHODS

From 2008–2010, we collected larval *U. brucei* partly as the result of distributional surveys and partly to collect tissue for later analysis of population genetics. We collected specimens from mid-March through early October, primarily using litter bags (Dodd et al. 2012). We placed litter bags systematically in shallow, flowing water. We also opportunistically collected specimens by hand. We measured snout-vent length (SVL) in the field using a small, metric ruler. Taking measurements in this manner undoubtedly introduced error; however, we declined to take the salamanders from the field site or to anesthetize them to get more accurate measurements because of the rarity of the species and the possible lethal effects that anesthesia has on small, plethodontid larvae (Camp et al. 2014). Following measurement, we released all larvae on site.

We attempted to determine larval period by plotting SVL in two ways. First, we pooled SVLs of larvae captured from different streams across years to maximize sample size. We plotted these measurements against day of the year. Because of inherent year-to-year and stream-to-stream variance in growth, we also plotted SVLs of larvae from the largest sample ( $n = 11$ ) taken at one time from the same site (8 June 2010). We took any adults encountered to the lab for more accurate measurements and later returned them to their resident stream. During early fall of 2009 we discovered a single metamorphosing individual; at the same time in 2010 we found three additional metamorphosing salamanders. We sacrificed the one collected in 2009, and we measured and dissected it to determine reproductive state. We measured all of the other metamorphosing

individuals in the field and released them on site. We examined all of them for potential external secondary sex characteristics.

As the result of the above survey efforts combined with a survey using environmental DNA (eDNA; Pierson et al. 2016), we discovered 17 independent streams in which *U. brucei* occurs. During 2018, we took five measurements of each of five variables that appeared to be important to the habitat for 14 of the known streams for *U. brucei*. We measured water depth (cm) in the center of the stream. We estimated flow velocity by measuring the time in sec it took a Styrofoam fishing cork to travel 1 m; we then converted this to  $\text{m sec}^{-1}$ . We took these measurements during a relatively rainy period for the region (scattered thunderstorms daily); therefore, water depth and flow velocity were higher than is typical; however, we were primarily concerned with relative, not absolute values. Because all measurements were completed within a few days of each other, water depth and velocity should be reflective of differences among streams. We determined aspect of stream flow using a compass, and we noted stream order and any unique characteristics of the vegetation. We measured width of the entire stream bed, both wet and dry portions, as a relatively permanent indicator of high-water flow. We took the transverse ground-to-ground distance at 1 m above the stream as an indicator of the immediately adjacent topography. We estimated slope by measuring the two legs of a right triangle, setting the substrate distance as the hypotenuse. We established the short leg vertically as 0.5 m by placing a meter stick vertically on the substrate in the center of the stream. We then measured the long leg as the upstream distance from the half-meter mark on the meter stick to the point where the measurement intersected the substrate. We then determined slope in degrees based on the trigonometry of a right triangle. We took five replicate measurements for each variable at widely distributed, selected points that reflected the full range of variation of the stream. We used standard correlation statistics to describe relationships among variables. To compare overall variation among variables, we calculated the coefficient of variation for each variable by dividing its standard deviation by its mean.

We did not design the collection protocol to account for variation in detectability, an important consideration in drawing conclusions regarding density or abundance (Mazerolle et al. 2007); however, we felt that an analysis of our crude data on capture rate would be suggestive of the important habitat of this species. Because this species has only been found in both 1<sup>st</sup>- or 2<sup>nd</sup>-order streams, we analyzed whether larval capture rate was related to water depth, which we took as an indicator of relative stream size. Because a primary goal for trapping larvae was to collect tissue for analysis of population genetics,

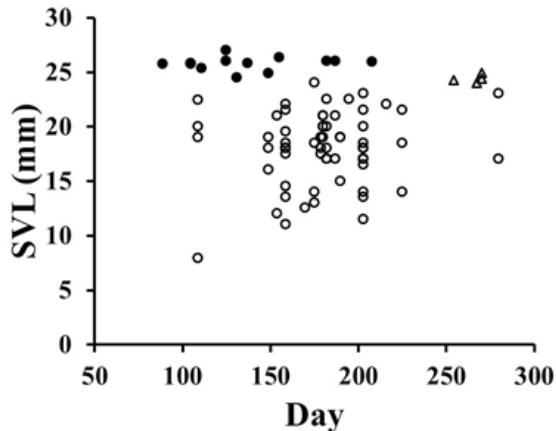


FIGURE 2. Distribution of measurements of snout-vent length (SVL) of the Patch-nosed Salamander (*Urspelerpes brucei*) taken from six streams during 2009–2011. Day represents day of the year from 1 January. Open circles represent larvae; closed circles represent adults; open triangles represent metamorphosing individuals.

we focused our trapping effort on representative streams across the known geographic range of the species. Therefore, we based our determination of capture rate ( $n \times 10^{-3}$  per trap night) from the eight streams that had been heavily trapped using litter bags. We did not use specimens from other streams or any that we incidentally collected. Although between-bag variance of larval counts can be high (Chalmers and Droege 2002; Dodd et al. 2012), we used large sample sizes of trap-nights (900–3,500 per stream) to generate reliable estimates of capture rates. For our analysis, we regressed capture rate ( $n \text{ trap-night}^{-1}$ ) against mean water depth for the eight streams. Because we used the data for water depth in two analyses (regression and correlation), we tested for statistical significance involving this variable with an adjusted alpha level of 0.025.

### RESULTS

Mean SVL ( $\pm 1$  SE) of eight adult males was  $25.83 \pm 0.22$  mm and of five adult females averaged  $26.11 \pm 0.26$  mm. There was no significant difference in SVL between the sexes ( $t = 0.790$ ,  $df = 11$ ,  $P = 0.446$ ). All adults averaged  $25.76 \pm 0.17$  mm SVL. The four metamorphosing individuals averaged  $24.42 \pm 0.20$  mm SVL. At first glance all four had the muted coloration characteristic of adult females; however, two had very obscure dorsolateral stripes, which are found only in adult males, and one had very short nasal cirri. The individual dissected lacked the cirri but contained fully developed, pigmented testes and pigmented vasa, although they were uncoiled. These observations led to our conclusion that reproductive maturity likely occurs simultaneous to metamorphosis without an extended post-metamorphic, immature period. The other two

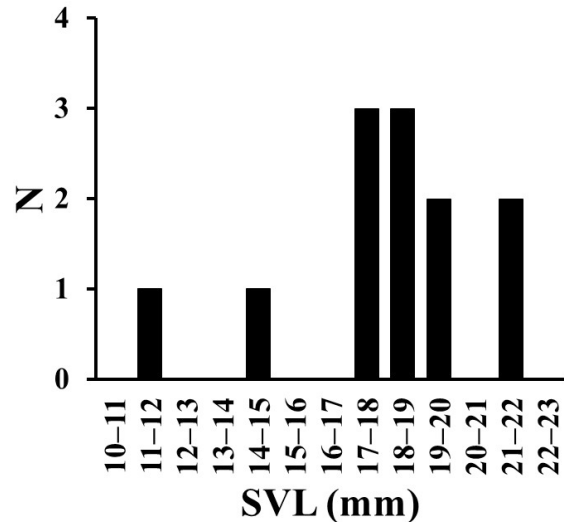


FIGURE 3. Distribution of snout-vent length (SVL) measurements of 11 larval Patch-nosed Salamanders (*Urspelerpes brucei*) collected from the same stream 8 June 2010.

individuals had no signs of external characteristics found in males and were presumed female.

The 65 larvae we measured had a mean SVL of  $18.01 \pm 0.41$  mm. Variance, however, was high, and at least two, possibly more, size classes were present in both the pooled data across streams and years (Fig. 2) and the data from the single collection of 11 larvae (Fig. 3). Although stream variables were measured during a rainy period, values for mean water depth were below 10 cm in all but one stream. Mean water depth was significantly correlated with mean width of the stream bed ( $r = 0.779$ ;  $t = 4.307$ ;  $df = 1, 12$ ;  $P < 0.001$ ), which exceeded 3 m in only one stream (Table 1). Slope and flow rate were also significantly correlated ( $r = 0.592$ ;  $t = 2.546$ ;  $df = 1, 12$ ;  $P = 0.024$ ). Mean slope ranged

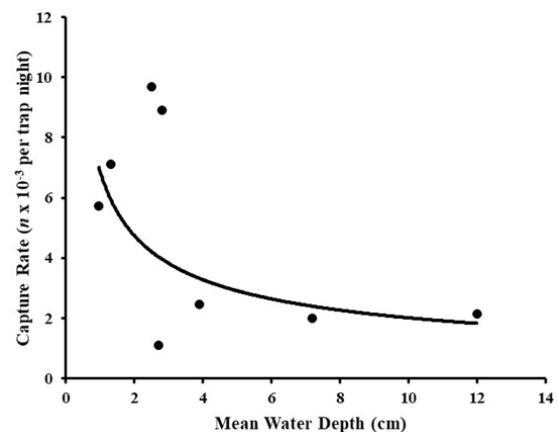


FIGURE 4. Regression (power curve:  $\text{Capture Rate} = 6.87 \times \text{Mean Water Depth}^{-0.533}$ ) analysis of larval capture rate of the Patch-nosed Salamander (*Urspelerpes brucei*) and mean water depth of occupied streams.

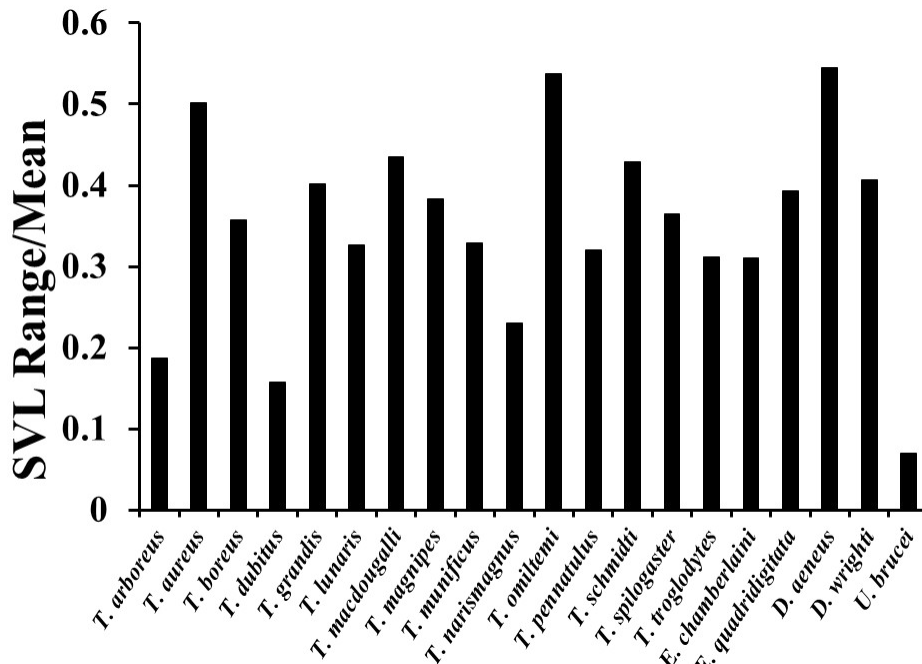


FIGURE 5. Comparison of the ratio of range to mean snout-vent length (SVL) in *Urspelerpes brucei* to other tiny (< 30 mm SVL) plethodontid salamanders. Comparative data are from the genera *Thorius* (Bruce 2000), *Eurycea* (Harrison and Guttman 2003), and *Desmognathus* (Hining and Bruce 2005).

from less than 4° to over 23°, and flow rate ranged from 0.2 to 0.6 m sec<sup>-2</sup> (Table 1). No other variables were significantly correlated to one another.

Water depth had the highest coefficient of variation (0.91), and ravine width had the lowest (0.28). Aspect was highly variable, with stream-flow direction ranging from due north to due south (Table 1). The analysis of capture rate among streams with known *Urspelerpes* occupancy indicated a higher capture rate in streams with shallow water. The relationship between capture rate and water depth fit a negative power curve ( $t = 4.197$ ,  $df = 7$ ,  $P = 0.006$ ; Fig. 4).

All streams in which we found *Urspelerpes* flowed through mature deciduous forest with a closed canopy. Trees were typical of a mesic slope forest (Wharton 1978), and common species were various oaks (*Quercus*), Tulip Poplar (*Liriodendron tulipifera*), and American Beech (*Fagus grandifolia*). All streams were edged with at least one species of heath (*Rhododendron*). In all but one stream, Great Laurel (*Rhododendron maximum*) was abundant. In the other stream the heath was Piedmont Rhododendron (*Rhododendron minus*). Both species of heath were present at most streams.

## DISCUSSION

*Urspelerpes brucei* is among the smallest plethodontid salamanders, having SVLs comparable to the smallest species of *Eurycea* (*E. chamberlaini*

and *E. quadridigitata*) and *Desmognathus* (*D. aeneus*, *D. organi*, and *D. wrightii*) as well as the minute salamanders of the genus *Thorius* (Bruce 2000). As was previously reported (Camp et al. 2009), no sexual size dimorphism is apparent in *U. brucei*, a characteristic that it shares with other miniaturized species of *Eurycea* (Semlitsch and McMillan 1980), *Desmognathus* (Organ 1961; Hining and Bruce 2005), and many *Thorius* (Bruce 2000). What is unique about body size of adult *Urspelerpes*, however, is the very small variance in adult body size. It has the smallest amount of variance relative to its body size of any plethodontid studied to date (Fig. 5). Therefore, we assume that very little growth occurs after reproductive maturity is attained.

Reproductive maturity is likely achieved simultaneous to metamorphosis or very shortly thereafter (Camp et al. 2012), suggested in this current study by metamorphosing individual *Urspelerpes* that were almost as large as adults, possessing maturing gonads, and showing signs of sexually dimorphic characters. Although the virtual absence of a post-metamorphic, immature period is unusual in plethodontids, *Urspelerpes* shares this characteristic with some populations of another spelerpine, *Gyrinophilus porphyriticus* (Bruce 1972). The presence of enlarged gonads at metamorphosis in *U. brucei* suggests that sexual maturation actually begins during the larval stage. This is perhaps unsurprising, as the tribe Spelerpini is characterized by numerous examples of smaller clades and species that have

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**TABLE 1.** Characteristics of 14 streams occupied by the Patch-nosed Salamander (*Urspeleperpes brucei*). Water Depth, Bed Width, Ravine Width, Slope, and Flow Rate are given as mean  $\pm$  1 standard deviation. Data summary for each of those variables is based on five replicates; the last row represents grand means for each. Numbers in parentheses for variables other than aspect represent range of values. More than one value for aspect indicates change in direction of stream flow.

Dominant Heath	Order	Aspect (° from N)	Water Depth (cm)	Bed Width (m)	Ravine Width (m)	Slope (°)	Flow Rate (m sec <sup>-2</sup> )
<i>Rhododendron</i>	1 <sup>st</sup>	32	3.6 $\pm$ 2.4 (1.5–7.5)	1.7 $\pm$ 0.5 (0.9–2.3)	7.6 $\pm$ 2.5 (5.2–11.0)	5.3 $\pm$ 3.4 (2.0–10.9)	0.31 $\pm$ 0.08 (0.27–0.46)
<i>R. maximum</i>	1 <sup>st</sup>	182	4.7 $\pm$ 2.3 (1.5–7.5)	1.7 $\pm$ 0.2 (1.5–2.0)	6.8 $\pm$ 0.9 (5.5–7.9)	5.6 $\pm$ 4.3 (2.9–13.2)	0.31 $\pm$ 0.12 (0.20–0.46)
<i>R. maximum</i>	1 <sup>st</sup>	116, 135	2.0 $\pm$ 1.5 (2.0–5.0)	1.2 $\pm$ 0.3 (0.8–7.51.5)	6.4 $\pm$ 2.5 (3.7–9.1)	3.9 $\pm$ 1.5 (2.4–6.2)	0.30 $\pm$ 0.13 (0.13–0.44)
<i>R. maximum</i>	2 <sup>nd</sup>	30, 270, 312	2.8 $\pm$ 1.4 (1.0–4.5)	1.4 $\pm$ 0.3 (1.0–1.8)	3.6 $\pm$ 1.2 (2.5–4.6)	3.3 $\pm$ 1.9 (2.1–6.7)	0.27 $\pm$ 0.06 (0.18–0.33)
<i>R. minus</i>	1 <sup>st</sup>	0	3.9 $\pm$ 1.9 (2.0–7.0)	1.7 $\pm$ 0.2 (1.4–2.0)	5.5 $\pm$ 2.2 (3.7–8.8)	7.9 $\pm$ 1.9 (5.9–10.3)	0.35 $\pm$ 0.14 (0.18–0.55)
<i>R. maximum</i>	1 <sup>st</sup>	32	2.7 $\pm$ 1.3 (1.0–4.0)	2.6 $\pm$ 0.8 (2.0–4.0)	6.9 $\pm$ 1.8 (4.9–9.1)	5.4 $\pm$ 5.5 (2.5–15.3)	0.41 $\pm$ 0.09 (0.33–0.53)
<i>R. maximum</i>	1 <sup>st</sup>	0	1.0 $\pm$ 0.7 (0.3–2.0)	1.5 $\pm$ 0.4 (1.0–2.1)	4.2 $\pm$ 1.2 (3.1–6.0)	23.4 $\pm$ 10.5 (11.6–39.3)	0.41 $\pm$ 0.24 (0.25–0.80)
<i>R. maximum</i>	2 <sup>nd</sup>	240	12.0 $\pm$ 3.9 (7.5–18.0)	3.7 $\pm$ 0.7 (2.7–4.6)	5.5 $\pm$ 0.6 (4.9–6.4)	5.3 $\pm$ 3.1 (2.5–10.3)	0.45 $\pm$ 0.09 (0.33–0.57)
<i>R. maximum</i>	2 <sup>nd</sup>	180	7.2 $\pm$ 4.5 (1.5–13.0)	2.7 $\pm$ 0.7 (1.7–3.4)	6.6 $\pm$ 2.0 (4.0–8.8)	4.7 $\pm$ 1.9 (1.8–6.2)	0.34 $\pm$ 0.03 (0.30–0.36)
<i>R. maximum</i>	1 <sup>st</sup>	292	1.3 $\pm$ 0.8 (0.5–2.5)	1.7 $\pm$ 0.6 (0.9–2.4)	4.6 $\pm$ 1.5 (3.4–7.0)	7.3 $\pm$ 5.1 (3.5–15.3)	0.23 $\pm$ 0.05 (0.20–0.31)
<i>R. maximum</i>	1 <sup>st</sup>	35	1.1 $\pm$ 0.5 (0.5–2.0)	2.2 $\pm$ 0.5 (1.8–2.7)	5.4 $\pm$ 0.5 (4.3–5.8)	3.9 $\pm$ 0.7 (3.1–4.9)	0.20 $\pm$ 0.03 (0.17–0.24)
<i>R. maximum</i>	1 <sup>st</sup>	17	0.4 $\pm$ 0.4 (0.1–1.0)	1.7 $\pm$ 0.8 (1.0–1.8)	6.6 $\pm$ 1.6 (4.6–8.5)	7.4 $\pm$ 2.4 (4.7–10.3)	0.31 $\pm$ 0.06 (0.24–0.41)
<i>R. maximum</i>	1 <sup>st</sup>	42	2.5 $\pm$ 1.5 (1.0–5.0)	1.4 $\pm$ 0.4 (1.1–2.0)	3.1 $\pm$ 1.1 (2.1–4.6)	6.3 $\pm$ 0.6 (5.2–6.7)	0.33 $\pm$ 0.04 (0.31–0.39)
<i>R. maximum</i>	2 <sup>nd</sup>	75	6.2 $\pm$ 3.7 (2.0–11.0)	1.7 $\pm$ 0.5 (1.0–2.3)	3.0 $\pm$ 0.7 (2.4–4.0)	17.3 $\pm$ 10.8 (6.7–35.1)	0.61 $\pm$ 0.31 (0.33–0.97)
—	—	—	3.67	1.93	5.42	7.63	0.345

independently evolved paedomorphosis (Bonett et al. 2014).

Virtually all of the variation in body size of *U. brucei* occurs during larval development. Graphic representation of larval SVLs suggests that individuals spend at least 2 y as larvae. Small sample sizes, high variance, and size-class overlap makes it impossible to determine the exact length of the larval period at this time. Though it is possible that some variance in SVL measurements in the pooled data seen in Figure 1 is the result of variation among streams and years, the high variance in body size among larvae collected in a single, simultaneous sample in Figure 2 suggests that is unlikely. It is more likely that the variation in body size

reflects the presence of multiple age cohorts of larvae. Possibly, there is asynchronous reproduction; however, across multiple years of this study, we have only detected metamorphosing individuals at a single time of year. This suggests a synchronous metamorphosis and lends no support for the hypothesis of asynchronous reproduction. Moreover, asynchronous reproduction is not common among plethodontids, including other spelerpines.

The first described localities for *U. brucei* were all small, 1<sup>st</sup>- or 2<sup>nd</sup>-order streams in steep-walled ravines. Our survey of a larger number of sites confirmed these earlier observations. Ravine width showed the smallest coefficient of variation among the variables that we

measured. Although aspect was not consistent, the narrow, steep walls of the ravines ensures that these habitats remain moist. This is further indicated by the abundance of heath, particularly *R. maximum*, which requires high levels of moisture (Duncan and Duncan 1988).

Although water depth showed a relatively high coefficient of variation among the streams in which this species exists, there was a negative association between larval capture rate and water depth in occupied streams. This agrees with our personal observations in which larvae are most easily found at the origins of streams where they first emerge as seepages. Our analysis has not accounted for detectability and how that might vary as a function of stream depth; therefore, capture rate may conflate true variation in larval density with variation in detectability. Accordingly, we interpret those results with caution; however, our data combined with the apparent absence in larger streams certainly suggest that the smallest streams are the most important habitat. Other small and miniature Appalachian salamanders (e.g., small *Desmognathus* and *Eurycea*) are also associated with headwater streams and often occur along the margins of larger streams or, given sufficient substrate moisture, out in the forest floor away from the stream itself. Unlike those species, however, *U. brucei* has never been found in either situation. This species appears to be a headwater endemic and adds to the emerging recognition of the importance of conserving these habitats (Lowe and Likens 2005; Meyer et al. 2007).

The dependence of *Urspelerpes* on headwater streams is also of interest biogeographically. *Urspelerpes* is known from a single stream in South Carolina and is separated from all other occupied streams by the Tugaloo River. The Tugaloo has a channel more than 50 m wide at the entrance of the South Carolina *Urspelerpes* stream and has a mean discharge rate of 55 m<sup>3</sup> sec<sup>-1</sup> (DuBose 2017). This river is formed by the confluence of the Tallulah and Chattooga rivers, both of which originate in the Blue Ridge Mountains of North Carolina, USA. Like other similar rivers of the region (Wharton 1978), it possesses a robust fish fauna. Intensive efforts to discover *U. brucei* in nearby, seemingly suitable streams in South Carolina by both trapping and eDNA have proven unsuccessful (Pierson et al. 2016). These negative results suggest that this small population is the only one, or one of very few, located on the east side of the river and is functionally isolated from the known populations in Georgia.

It is difficult to conceive how such a small salamander that is dependent on small, headwater streams could disperse across such a significant waterway. A possible explanation for the distribution of this species lies in the drainage history of the Tugaloo. The Tallulah and

Chattooga rivers drained into the Chattahoochee River prior to their capture by the Tugaloo as recently as the Pleistocene (Johnson 1907; Voss et al. 1995; DuBose 2017). Water was diverted from the Chattahoochee, which eventually empties into the Gulf of Mexico via the Apalachicola River. Those mountain waters now flow through the Tugaloo and drain directly into the Atlantic Ocean by way of the Savannah River. The original headwaters of the Tugaloo were located near the current range of *U. brucei* (Voss et al. 1995). We hypothesize that *U. brucei* was originally associated with the headwaters of the Tugaloo River, and the South Carolina population was isolated when the range of this species was disrupted by the stream-capture event that led to the tremendous increase in water volume flowing along the Tugaloo. Genetic analysis is underway to test this hypothesis and to determine if the South Carolina population is sufficiently divergent from those in Georgia to warrant special conservation attention.

Additional questions remain unanswered regarding this elusive species and invite investigation beyond what we have so far been able to do. For example, why is there so little variance in adult body size compared to other plethodontids? Is it possible that adults are short-lived and the species approaches semelparity? Regarding their conservation, what are critical differences between small streams in which these salamanders occur and those in which they are absent? What is the effect of invasive species, e.g., wild hogs, which are abundant in the area? A great deal is still unknown regarding the biology of *U. brucei*; however, we hope that the results presented herein will help in determining appropriate steps in ensuring its long-term health as a species.

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