

AMBYSTOMA MACULATUM (Spotted Salamander). **BREEDING FREQUENCY.** Previous studies have explored the breeding strategies of *Ambystoma maculatum* (Spotted Salamander) but there is a knowledge gap concerning the breeding frequency of each sex (Husting 1965, Copeia 3:352-362; Harris 1980, Copeia 4:719-722). Finkler and Cullum found that female salamanders have a much higher metabolic cost associated with breeding than males (Finkler and Cullum 2002, Copeia 3:824–829). Because males and females invest different amounts of energy into breeding, we hypothesize that there will be a difference in the breeding frequency of male and female Spotted Salamanders and that breeding frequency will be positively associated with body size. Understanding the breeding frequencies of males versus females is a key parameter in population models, and thus, describing this parameter will reduce bias in population estimates taken from breeding sites and reduce overestimates of population growth in population projection models. Reducing bias in either of these cases improves our ability to accurately identify threats to population stability.

We used data from a vernal pool surrounded by a drift fence and located on the southern Cumberland Plateau in Sewanee, TN (35.223898°N, -85.971171°W). This drift fence contains 52 evenly spaced pitfall traps. This fence is opened from January to March annually and provides a complete census of individuals moving in and out of the pond during the years sampled, starting in 2015. Each salamander that came to the pond to breed received a year specific visible elastomer implant mark, was measured (snout-vent length and mass), and their spot pattern was quantified (Chase et al. 2015, Herp. Rev. 46:192-196). Despite having 7 years of data collection, we only used individuals captured at the breeding site in 2019, 2020, and 2021 to develop individual breeding frequencies during the 7-year period because of the information necessary to accurately identify individuals. Individuals were identified via spot patterns and

capture histories as well as checked for consistency in length and mass measurements. We calculated breeding frequencies by dividing the number of years that an individual bred by the number of years between the last and first captured year. These data were separated into male (N = 357) and female (N = 204) salamanders. We assumed that all individuals traveling to the pond were investing energy in the breeding migration because they intended to engage in breeding activity, but we have no way to determine if these activities were successful.

Using a two-way ANOVA to evaluate the effect of body length and sex on breeding frequency, we discovered that body length and sex significantly affect breeding frequency ($F = 46.83$, $df = 1,652$, $p < 0.001$; $F = 7.302$, $df = 2,652$, $p < 0.001$; respectively), but there was not a significant interaction between body length and sex in their effect on breeding frequency ($F = 1.76$, $df = 2,652$, $p = 0.173$). We found that the probability of males traveling to breeding sites was 1.19 times higher than females (males, 0.8 ± 0.012 ; females, 0.67 ± 0.014). We postulate that differences in breeding frequency between the sexes is due to the energetic cost of female reproduction versus the less costly male reproduction as supported in Finkler and Cullum 2002. Alternatively, scramble competition at breeding sites may require more frequent breeding activity by males to be successful.

We also observed that larger individuals had lower breeding frequencies. There are several possible hypotheses for why smaller salamanders reproduce more frequently. (1) Spotted Salamanders reproduce more often when they are young. (2) Larger individuals have a greater reproductive output or success than smaller individuals during any single breeding season, therefore they reproduce less frequently (Rauch et al. 2014, Behavior. 151:1869-1884). (3) Spotted Salamanders could have a strong negative correlation between body size and survival appearing as reduced breeding frequencies as large individuals experience mortality through time.

Although many Ambystomatid populations are male biased at the breeding site and males remain longer at breeding sites, the relationships between breeding populations and total populations are unclear (Lannoo. 2005. Amphibian Declines: The Conservation Status of United States Species). Return probabilities here are like those observed in Kentucky and Rhode Island, but 2-3 times higher than observed in areas of Michigan, Missouri, and New York. The factors contributing to this variation are unclear. The results from this project suggest that there are sex and size-specific differences that contribute to these biases and that significant geographic variation indicates that these

STORERIA DEKAYI (Brownsnakes). REPRODUCTION AND NEONATAL DEFENSE. Although documented in other regions, no information has been published on the reproductive ecology or natural history of Brownsnakes (*Storeria dekayi*) in Tennessee. While weeding a garden in southern Cannon County, Tennessee, USA in late July 2022, I found a pregnant female Brownsnake with a total length (TL) of 228 mm. To gather information on timing of birth, litter size, and TL of neonates, I placed the female snake in a small mesocosm constructed with soil and mulch from the garden. I checked the mesocosm daily and, although they could have been born anytime during the previous 24 hours, I discovered five neonates at 0500 h on 7 August (Fig. 1A). The neonates were darker and their neck band was lighter and more distinct compared to those features of their mother (Fig. 1B, C). The mean TL of the neonates was 78 mm, but more than 15 mm separated the smallest (70.5 mm) and largest (89.5 mm) individuals.

A litter of five snakes is below average for the species ($\bar{x} = 13$, $n = 169$; Ernst and Ernst 2003. Snakes of the United States and Canada,

relationships should be established at each field site before proceeding with population modeling.

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Smithsonian Books, Washington, D.C., USA), but litters with as few as three young have been reported from other regions (e.g., Virginia, Mitchell 1994. The Reptiles of Virginia, Smithsonian Institution Press, Washington, D.C., USA). The mean TL of the neonates comprising this litter is smaller than the mean snout-vent length (SVL) of 97 mm for 109 neonates reported by Ernst and Ernst (2003, *op. cit.*), although I suspect smaller neonates occur, as they reported neonates with SVL <70 mm. The date of birth I report is within the range of typical dates reported from throughout the species' range (early June to late September; Ernst and Ernst 2003, *op. cit.*) and coincides with dates when I find neonates in southern Cannon County (late July through early September; unpublished data).

While I was photographing the neonates, one of them engaged in aggressive defensive behaviors (Fig. 2). This individual spread its neck and flattened its head and, with mouth agape, began to sway back and forth, first at my camera and then at my left hand, which I waved to the side to better photograph the gaping behavior (Fig. 2A). I slowly lowered my hand towards the neonate, which then lunged with mouth agape (Fig. 2B) several times. Although they attempted to escape by burrowing beneath mulch, none of

the other four neonates showed any signs of aggression. Furthermore, neither the neonates nor the female used other passive defense behaviors I witness routinely in adults, such as regurgitation of stomach contents, defecation, and release of a foul-smelling musk from anal glands. In addition to these behaviors, Brownsnakes throughout their range (e.g., Kansas, Michigan, Virginia) also occasionally feign death when threatened (Liner 1977. Trans.

Kansas Acad. Sci. 80:81–82; Hayes 1987 Herpetol. Rev. 18:16–17, Mitchell 1994, *op. cit.*), a behavior not yet reported for individuals in Tennessee.

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FIG. 1. (A) A litter of five Northern Brownsnakes (*Storeria dekayi*) found on 7 August 2022 in southern Cannon County, Tennessee. (B) Head and neck of a neonate, depicting the dark ground color of the body scales, dark coloration of the head blotch, distinct off-white neck band and black collar. (C) The mother and one of her offspring depicting contrasting colorations of the body, neck band, and collar.



FIG. 2. A neonatal Northern Brown Snake (*Storeria dekayi*) in a defensive pose (A) and after a strike with the anterior half of its body fully outstretched (B).

***EURYCEA WILDERAE* (Blue-Ridge Two-Lined salamander).** **MESOHABITAT PREFERENCE.** Streams are heterogeneous environments with consistent habitat characteristics at the meso-scale like riffles, runs, and pools that are driven primarily by flow and channel characteristics ranging from fast, turbulent flow to slow-moving or even stagnant reaches, respectively. Flow in these reaches also determines sedimentation rates with low frequency of sand and silt in riffles to high frequency of the same materials in pools. Two variables that are predicted to affect larval salamander survival are sedimentation and flow. Therefore, assessing mesohabitat preferences can provide important insight into the habitat necessary for successful completion of larval salamander life history. We used active dipnet surveys to capture larval Blue-Ridge Two-Lined Salamanders ($N = 209$) between 21 September – 7 November 2014 over three capture events in a 50 m stream reach on the campus of the University of the South in Sewanee, TN (35.204669°N, -85.925149°W). For each capture, we recorded their distance from a downstream culvert and the meso-scale habitat type for each capture. We performed a t-test to determine if the density of Blue-Ridge Two-Lined Salamander larvae differed between runs and pools. Riffles were not observed in this reach. Overall, we observed no preference for either habitat type with captures being

representative of the overall availability of each habitat type ($t = 0.269$, $df = 49$, $p = 0.789$). However, salamanders did not use the habitat evenly with some densities of 10-18 captures per m^2 while other areas had no captures over three sampling occasions. This stream has substrate that is primarily sand and gravel making downstream drift likely at lower velocities than streams with coarser substrate (Barrett et al. 2010. *Biol. Cons.* 143: 1998-2005). Therefore, we expected that larvae may show a preference for habitat with slower velocities like pools, but we did not observe this pattern. These data also support conclusions of others that Blue-Ridge Two-Lined Salamander larvae are not deterred from occupying areas with high sedimentation rates like pools (Keitzer et al. 2019 *Freshwater Biol.* 57: 1535-1544). More research is necessary to understand what factors contribute to habitat selection in larval salamanders and what drives high density patches.

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