ANEIDES AENEUS (Green Salamander). BROODING AND HATCHLING BEHAVIOR. Aneides aeneus is a semi-arboreal salamander found throughout the Southern Appalachian Mountains and has been identified as a vulnerable cryptic species (Niemiller et al. 2022. Front. Conserv. Sci. 3: 890859). Characterized by their flattened, dark body covered in green to yellow lichen-like splotches and squareshaped toes, they are highly selective of their habitat, utilizing deep crevices of south-facing rocky outcrops, caves, cliffs, and large boulders for breeding and brumation (Niemiller et al. 2022. Herpetol. Conserv. Biol. 17:249-265). The IUCN Red List of Threatened Species lists A. aeneus as "Near Threatened" due to population declines resulting from disease, over-collection, climate change, largescale habitat loss, and degradation (IUCN 2025. IUCN Red List of Threatened Species; Soto et al. 2021. Conserv. Action Plan Green Salamander (A. Aeneus) Species Complex). On 3 September 2024 at 1226 h, I discovered an adult female green salamander (snout-vent length 6.0 cm) brooding her clutch of 10-15 eggs (Fig. 1A). The geographic coordinates for this observation are withheld due to the conservation status of this species. Observations of females guarding their eggs have been reported in Kentucky, North Carolina, Virginia, West Virginia, and Tennessee (Petranka 1998. Salamanders of the United States and Canada. Smithsonian Institution Press, Washington, USA). My observation adds to a very small number of recorded A. aeneus nesting observations in Tennessee, documented in two master's theses from Tennessee Tech University (Cantrell 2012. Distrib. Spec.-Habitat Relat. Green Salamander, A. aeneus, Catoosa WMA; Wyatt 2010. Status Spec.-Habitat Relat. Green Salamander A. aeneus, Myatt Creek, Catoosa WMA).

Green salamanders typically breed in May or June in the northern parts of their range, but in southern populations, breeding activity and oviposition may extend into September or October (Cupp 1971. Herpetologica 27:308-310; Petranka 1998. *op. cit.*). This female was found in a moss-enclosed crevice on a rocky outcrop in Bays Mountain Park and Planetarium, Sullivan County, Tennessee, USA. The crevice (width = 11.4 cm, height = 2.5 cm, depth = 1 cm) was unusually dry compared to nearby crevices

and covered by Pincushion Moss (Leucobryum albidum) (Fig. 1B). The egg mass (diameter = 0.4-0.5 cm) consisted of approximately 10-15 polarized and spotted eggs. While I could not get an accurate embryonic stage, the eggs were most likely nearing hatching given the time of year. Aneides typically breed in May or June and embryos take 2-2.5 months to develop (Gordon and Smith 1949. Copeia 1949:173-175). Given the number of eggs, I suspect the female was sitting on a single clutch that was most likely her own (Soto et al. 2021. op. cit.). Unlike other species, such as the Northern Two-Lined salamander (Eurycea bislineata), Green salamander females do not participate in communal oviposition (Jockusch et al. 2014. J. North Am. Herpetol. 2014: 87-92). The female was defensively positioned with her head to the left of the clutch and her tail curled on the opposite end. Females have been observed lunging and biting during the guarding period if they feel threatened (Gordon 1952. Am. Midl. Nat. 47:666-701). However, this behavior was not observed most likely due to the short duration of interaction.

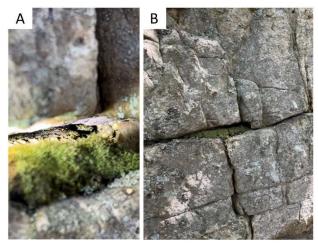


Figure 1. (A) A female Green Salamander (*Aneides aeneus*) brooding her clutch of 10-15 eggs on 3 September 2024 in a moss-covered crevice, Bays Mountain Park and Planetarium, Sullivan County, Tennessee. (B) The crevice, covered by Pincushion Moss (*Leucobryum albidum*), where the female Green Salamander (*Aneides aeneus*) was located.

On 10 October 2024 at 1236 h, I observed the same crevice, discovering six hatchlings but no sign of the female. Green salamanders' incubation period is estimated to be 67 to 91 days (Wyatt 2010. op. cit.).

Females will brood their eggs and remain with their hatchlings for 3-5 weeks post-hatching with a nest failure rate of 20-40% (Wyatt 2010. op. cit.). Successful hatching heavily depends on the female's brooding abilities to protect the eggs from predators and pathogens (Cantrell 2012. op. cit.). Green salamanders directly develop without a larval stage, so hatchlings resemble small adults ranging from 18.5-23 mm long (Cantrell 2012. op. cit.). After spending a few months in the hibernacula, the hatchlings will disperse to seek other crevices (Wyatt 2010. op. cit.). The observed green salamander hatchlings had likely spent a little over a month in this crevice, with the egg sack still intact and hanging on the right of the crevice ceiling (Fig. 2A). Assuming the eggs hatched shortly after the first observation on 3 September 2024, the hatchlings would be approximately 37 days old at the time of the second observation on 10 October 2024, which is within the maximum incubation period (Wyatt 2010. op. cit.). The hatchlings showed little to no activity, barely moving around the back of the crevice. On the same day, I found another crevice 13.27 m east with an adult green salamander and two juveniles. I was only able to photograph one juvenile due to the narrowness of the crevice (Fig. 2B). This crevice (width = 10.1 cm, height = 1.31 cm, depth = 1 cm) lacked moss at the entrance (Fig. 2C). The adult's sex could not be determined. The two juveniles sat on the right side of the crevice while the adult was on the opposite end in a defensive position with its tail in front of its face.

The current observation contributes to a growing understanding of the species' nesting behavior in

Tennessee. Only a few observations in the state have been previously recorded. Four nests were observed in 2008 and 2009 at the Myatt Creek drainage located on the Catoosa Wildlife Management Area (WMA) in Cumberland County, TN (Wyatt 2010. op. cit.). In 2008, one female was observed guarding eggs on 10 July while another female was found guarding her eggs on 23 July (Wyatt 2010. op. cit.). The eggs found on 10 July began hatching by 18 September and appeared to all hatch by 3 October; however, the second female and only one of her hatchlings were visible on 3 October as the eggs had not hatched yet (Wyatt 2010. op. cit.). On 10 October, the first female's five hatchlings were observed lacking her presence and only two hatchlings were observed with her on 20 October (Wyatt 2010. op. cit.). On the same day, the second female and three hatchlings were present in the crevice, and all eggs seemed to have hatched (Wyatt 2010. op. cit.). Two additional observations of two females brooding on different rock outcrops on 9 July 2009 were made, but follow-up observations did not occur (Wyatt 2010. op. cit.). These findings support the assertion that females guard their eggs, remain with their hatchlings for a few weeks, and hatchling success is contingent upon the female's protective care. Additionally, egg number variation and the timing of hatching across observations reinforces the idea that breeding periods and incubation times vary depending on geographic location and environmental conditions.

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Figure 2. (A) The six Green
Salamander (Aneides aeneus)
hatchlings found in the same
crevice on 10 October 2024,
Bays Mountain Park and
Planetarium, Sullivan County,
Tennessee. (B) One of the
two juvenile Green
Salamanders (Aneides
aeneus) found in a crevice
13.27 m away from the first
crevice. (C) The crevice where
one adult and two juvenile
Green Salamanders (Aneides
aeneus) were located.