

Notes on Reproduction in the Brownback Salamander (*Eurycea aquatica*)

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Photographs by the senior author.

Life history data often are lacking for species with an uncertain taxonomic status. As phylogenetic analyses improve and our understanding of species boundaries become more refined, ecological information should likewise keep pace. Here, we report information on the timing of reproduction, clutch size, and nest attendance for the Brownback Salamander (*Eurycea aquatica*), a species recently documented to be a separate lineage after a long, controversial systematic history. We confirm that this species has the largest clutch size of any lineage within the *Eurycea bislineata* complex, and suggest that male and male-female nest attendance is not uncommon in this species. We suggest that, in addition to the genetic divergence documented between *E. aquatica* and other members of the *E. bislineata* complex, ecological differences also are present and possibly are influenced by the unique springs inhabited by Brownback Salamanders.

Reporting detailed natural history information for amphibian and reptilian species is necessary for their conservation, and for analyses that describe evolutionary and ecological patterns. For some taxa, this information is currently unavailable due to their recent recognition as distinct species (e.g., cryptic species, newly described taxa) or due to controversy regarding their status as a separate evolutionary lineage. An example is the Brownback Salamander (*Eurycea aquatica*), a plethodontid salamander with a long, turbulent taxonomic history that has recently been confirmed as a monophyletic lineage distinct from nearby populations of its congener, the Southern Two-lined Salamander (*Eurycea cirrigera*; Kozak et al. 2006, Timpe et al. 2009). Previous contributions to this salamander's reproductive biology have been complicated by the uncertain taxonomic status of this species, the presence of putative "intermediates" between *E. aquatica* and *E. cirrigera*, and the subjective categorization of individuals for analysis (Jones 1980). Here, we compile information on the timing of reproduction, clutch number, nesting sites, and nest attendance in this species that was obtained coincident with collections for the morphological and phylogenetic analyses by Timpe et al. (2009). We therefore had an *a priori* method for categorizing individuals as belonging to either species. This

also allowed us to reconsider potential intermediates mentioned by other authors, and we report possible explanations for the morphological confu-



Large adult male *Eurycea aquatica*.



Spring habitat of *Eurycea aquatica*; Sander's Spring, St. Clair County, Alabama. The wellhouse is in background; note the extensive growth of Watercress (*Nasturtium officinale*) and the overturned potted plant in foreground.



Nest of *Eurycea aquatica* found under a potted plant at Sander's Spring.

sion between *E. cirrigera* and *E. aquatica*, which may have hampered earlier efforts to characterize the Brownback Salamander's life history.

Materials and Methods

Brownback Salamanders are locally abundant and inhabit springs throughout the limestone regions of northern Alabama and Georgia (Jones 1980). We sampled populations that had previously been studied by Jones (1980), and located and collected salamanders by turning cover objects in and around the springs. Tail tissue and/or whole individuals were collected for the morphological and phylogenetic analyses of Timpe et al. (2009). When clutches were found, the object covering the eggs was noted, photographs were taken of each clutch, and the number of eggs per clutch was determined from the photos. Each nest was categorized as being attended by either a male, a female, both a male and female, or no adult. Males of this species can easily be distinguished from females based on the presence of a grossly enlarged head and small but distinct cirri (Jones 1980, Timpe et al. 2009). In March 2009, we marked artificial cover objects (plastic pots for plants) at a spring site (Sander's Spring, St. Clair County, Alabama) that had previously been productive for finding clutches. At this spring, the landowner uses the spring water for potted aquatic plants, and the spring contains dozens of these pots. When found under these artificial cover objects, salamanders were photographed with or without eggs. To suggest the degree of parental care, we returned to this site three days later, re-photographed each salamander, and identified salamanders by pattern matching (e.g., Forester 1977, Bailey et al. 2004) to confirm their ongoing attendance of the egg clutch.

In addition, we examined nests for each of three divergent clades identified by Timpe et al. (2009) in their phylogenetic analysis of *E. aquatica* (e.g., those found in the Coosa Valley, Birmingham-Big Canoe Valley, and Cumberland Plateau, respectively), and compared mean clutch sizes for

nests from each of these clades using ANOVA. This analysis was conducted using JMP 8.0.1 software with $\alpha = 0.05$.

Results

Previous to the nesting period, gravid females and males in breeding condition (i.e., with enlarged heads and small but distinct cirri) were located under rocks in springs and small streams in limestone regions of northern Alabama and Georgia. Males and females were observed in springs as early as 3 January. One male in breeding condition was found crossing a road on a rainy night on 10 January 2008. Forty-one nests were located during February–March 2007–2009. The earliest recorded nests were found on 2 February, and the latest nests were observed on 16 March. In April and subsequent months, adult *E. aquatica* were less frequently observed along the edges of springs, and no nests were located. Twenty-four nests were under rocks (mostly in springs or spring runs), one under a log, and 15 under artificial cover objects (e.g., plastic pots for plants). Twenty-five nests were found with females in attendance, five with males in attendance, seven with both a male and female in attendance, and three had no adult presence/attendance. One nest was found with an adult in attendance that escaped before it could be sexed. The mean clutch size of *E. aquatica* (based upon eggs counted in nests) was 65.93 ($N = 41$; range 31–138). Mean clutch sizes for each of the three clades recovered within *E. aquatica* (Timpe et al. 2009) differed significantly ($F_{2,39} = 7.51$; $p = 0.002$), with 26 nests from the Coosa Valley exhibiting a higher clutch size (mean 72.6 ± 24.4 SD) relative to those in the Birmingham-Big Canoe Valley ($N = 5$, mean 44.0 ± 9.8 SD) or Cumberland Plateau ($N = 10$, mean 52.4 ± 9.9 SD; Tukey-Kramer post hoc test; $p = 0.01$).

The artificial cover objects at Sanders Spring contained six nests on 3 March 2009, and seven nests on 7 March 2009. On 7 March 2009,

two nests attended by females on 3 March were still attended by the same individuals. Two nests attended by females were still attended by the same females and were joined by males. One male remained with the nest he had attended, and one nest was abandoned by a male. One male replaced another male under a pot, was joined by a female, and their cover object contained a newly laid egg clutch. One male without eggs remained alone under a pot, and one male switched from one pot to another; each of these males had no eggs. Finally, a new pair arrived under a pot. No eggs were present at that time.

Discussion

The average clutch size of *Eurycea aquatica* is larger when compared to those of other members of the *E. bislineata* complex, and the mean we report in this study is larger than any population mentioned by Petranka (1998), Pauley and Watson (2005), or Sever (2005a, b) in their synopses of the complex. Although some of the larger nests we found could have been the result of communal nests (Sever 2005a), eggs in all large nests appeared to be of the exact same developmental stage, and cover objects often were found with two discrete nests with eggs in different stages of development (N = 4 observations). Similar to other plethodontid salamanders, differences in clutch size between each of the three divergent clades of *E. aquatica* recovered by Timpe et al. (2009) may be explained by variation in adult body sizes of these salamanders (Tilley 1968).

Reproductive information was used as one of many characters to distinguish *E. aquatica* from its relatives in the type description of this species (Rose and Bush 1964). Rose and Bush (1964) also reported that clutch size (based upon number of enlarged eggs counted in gravid females) was

quite large in *E. aquatica*, attributing this difference to the productive habitat of the limestone springs they inhabit. Jones (1980) also provided data on Brownback Salamander clutch size; however, in his analysis, salamanders were subjectively assigned to species based on uncertain morphological characters. Previous considerations of Brownback Salamanders have referred to “intermediate” forms between *E. aquatica* and nearby populations of *E. bislineata* (= *E. cirrigera*), and the presence of these problematic individuals contributed to the controversial assignment of this species as a spring “ecotype” of *E. cirrigera* (Folkerts 1971, Mount 1975, Jones 1980, Petranka 1998). Because these “intermediates” were included in Jones’ (1980) analysis, which species was involved is uncertain, and the reproductive information included is consequently of tenuous value.

We encountered individuals that we believe, based on coloration and patterning, would have been considered “intermediates” by previous authors, including a gravid female (AUM 37688) collected about 0.25 km from the closest spring (Sander’s Spring) harboring *E. aquatica*. This individual had yellow dorsal coloration, and otherwise was morphologically consistent with *E. aquatica*. Other salamanders collected from this site and other springs were dark brown when collected and later became considerably lighter in captivity, exhibiting a more yellow color with less distinctive brown dorsolateral stripes. The above individuals shared identical mitochondrial haplotypes to other *E. aquatica* (Timpe et al. 2009). One individual obviously assignable to *E. cirrigera* was eventually collected at this site (AUM 37836; 1 February 2009), and was confirmed to be *E. cirrigera* genetically (E.K. Timpe, unpubl. data). Thus, although these species are broadly sympatric throughout the karst regions of Alabama and Georgia, the Sander’s Spring site is currently the only known locality where these



Example of an individual *Eurycea aquatica* (AUM 37688) that could be confused for an *E. aquatica* x *E. cirrigera* “intermediate.” Note the yellow dorsal coloration and the lack of an enlarged head (this individual is female). The dark sides also are considerably lighter than in most individuals.



Comparison between an adult male *E. cirrigera* (left) and an adult male *E. aquatica* (right). These salamanders were collected on the same day ~5 km apart in Murray County, Georgia.

salamanders have been confirmed to occur syntopically. No evidence is suggestive of mitochondrial or nuclear gene exchange between these species at this site. We believe that “intermediates” noted by other authors were considered so for three reasons: (1) Female *E. aquatica* and *E. cirrigera* are very similar morphologically, (2) dorsal coloration is variable in *E. aquatica*, with some individuals occasionally exhibiting yellow coloration, and (3) male *E. aquatica* may appear more similar to *E. cirrigera* during the non-breeding season if their head size is less pronounced during this time. Otherwise, adult males of these species are unmistakably different morphologically.

Because the reproductive information we present here is based upon known populations of *E. aquatica*, these are the first data known explicitly for this species. We confirm the large clutch size (compared to other members of the *E. bislineata* complex) suggested by Rose and Bush (1964). In addition, we provide information about the location and microhabitat of several nests and information about the nesting period. Perhaps our most interesting finding is the possibility of biparental care in this species. Most reports of egg attendance in *Eurycea* have been of individual females attending nests (Wells 2007). Seven nests were attended by a male and female, five were attended by males, and 25 nests were attended by females. Interestingly, of four nests reported for the sister taxon of *E. aquatica*, the Junaluska Salamander (*E. junaluska*; Kozak et al. 2007, Timpe et al. 2009), one was attended by a male and female and two were attended by females (Bruce 1982). Although further study is needed, this suggests that these closely related salamanders might share similar reproductive strategies. An alternative explanation is that these species breed immediately prior to oviposition, and males remain with the females shortly before and/or after breeding and then disperse. This would also be an interesting characteristic for these species, since, in most plethodontids, breeding and oviposition are

temporally dissociated (Wells 2007). If this is the case, we witnessed many pairs that were in the process of breeding or about to breed. However, the number of male-only nests and our observation that females already present on nests were later joined by males suggest that male parental care and biparental care may be involved.

Interestingly, few studies have examined long-term nest attendance/fidelity in salamanders (Wells 2007). Our small experiment confirmed that many individuals found on nests were found with them after three days, suggesting that these individuals were not simply there immediately after oviposition. Follow-up studies should be performed to confirm that the individuals present with the eggs are the actual parents of the offspring, and if these individuals remain with the eggs throughout their development. This study also confirms that, in addition to the morphological and genetic differences reported by Timpe et al. (2009), disparate life history strategies are present between this species and other members of the *Eurycea bislineata* complex. As proposed by Rose and Bush (1964), these differences appear to be associated with the unique spring habitat occupied by *E. aquatica*. Future research should be directed toward comparisons between this salamander and its congeners and the extent to which their habitat and/or mating systems determine these differences.

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Perch Height Differences among Female *Anolis polylepsis* Exhibiting Dorsal Pattern Polymorphism

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Female-limited dorsal pattern variation within a species has been of interest to naturalists for years, and has been observed in animals ranging from spiders, damselflies, and dragonflies to frogs and lizards. Protection against predation by birds has been offered as a preliminary explanation for the evolution of these patterns, especially from birds that have acute color vision and which preferentially prey on females because they are less agile and more nutritious (Stamps and Gon 1983).



Fig. 1. Male *Anolis polylepsis* with dewlap extended (right) and female *A. polylepsis* (above) with the “diamond stripe” (ds) dorsal pattern (see text). Copyright © David Laurencio 2010. Used with permission.