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AN ESTIMATE OF HOME RANGE SIZE AND TEMPORAL ASPECTS OF HIBERNATION FOR THE EASTERN BOX TURTLE (*TERRAPENE CAROLINA CAROLINA*) IN A SUBURBAN WETLAND HABITAT OF MIDDLE TENNESSEE

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***Abstract.*—**Eastern Box Turtles (*Terrapene carolina carolina*) are known for spending most of their lives within a defined home range, but there is variation in home range size depending upon both biotic and abiotic factors. Our objective was to use radio-telemetry to estimate home range for Eastern Box Turtles ($n = 6$) in a suburban wetland habitat in Murfreesboro, Tennessee, USA. In addition, we characterized temporal aspects of hibernation, including immergence, emergence, and duration. The 95% fixed kernel (FK) home range estimates for two female turtles and one male turtle averaged 1.50 ± 1.18 ha. The 50% FK core areas for the same three turtles were small and averaged 0.19 ± 0.15 ha. The 95% minimum convex polygon (MCP) home range estimates for four female and two male box turtles averaged 1.19 ± 1.67 ha. Turtles entered their hibernacula in November and emerged in April, spending an average of 149 ± 9.44 d in hibernation. To our knowledge, this is the first study to quantify home range and describe temporal aspects of hibernation for Eastern Box Turtles in middle Tennessee, as well as one of the few studies examining these aspects in a suburban wetland habitat. This baseline information provides general aspects of box turtle ecology and can be used for identifying minimum conservation areas necessary to preserve box turtle populations considering that habitat quality and fragmentation affect home range size and regional variation affects timing of hibernation.

Key Words.—conservation, fidelity, habitat, hibernaculum, management, mark-recapture, radio-telemetry

As habitat fragmentation and loss continues, it is becoming increasingly important to understand all aspects of box turtle ecology such as home range size and timing of hibernation. Much of the remaining box turtle habitat only exists in small patches, which can lead to wandering behavior and larger home range sizes as turtles search for resources and suitable habitat (Dodd 2001). Additionally, changes in the biophysical characteristics (e.g., microclimate, landscape structure) of the habitat due to improper management or fragmentation can result in habitat that is unsuitable for box turtle populations, thus altering their behavior and ultimately affecting their overall survival (Curtin 1995, 1997; Dodd 2001). For example, Eastern Box Turtles (*Terrapene carolina carolina*) in an urbanized landscape in North Carolina exhibited lower adult survivorship and delayed maturity due to anthropogenic factors (Budischak et al. 2006). Consequently, gathering basic information on the life history and natural history of a species can be critical for proper management and conservation, especially in long-lived species such as box turtles where decline might not be apparent until years into the future (Belzer and Steisslinger 1999; Dodd 2001).

While Eastern Box Turtles are generally not territorial, they do have a home range in which they spend most of their lives (Stickel 1950, 1989). Box turtles have excellent homing abilities and have been known to orient towards their home range if they are displaced or translocated (Dodd 2001; Cook 2004). These home ranges are typically small and often overlap with the home ranges of other conspecifics in the area (Stickel 1950; Madden 1975). Within the home range, there is often a "core area" where most normal daily activities occur (Madden 1975; Dodd 2001). Box turtles generally have an average home range size between 0.25 and 5 ha (e.g., Legler 1960; Schwartz and Schwartz 1974; Stickel 1989; Fredericksen 2014; Williamson 2014), with occasional outliers having home range sizes of 10 ha or more, typically due to searching for mates, nesting sites, or hibernacula (e.g., Stickel 1950; Willey 2010; Greenspan et al. 2015). In some instances, there are seasonal shifts in home range usage but generally not yearly shifts (Madden 1975; Stickel 1989). Reported home range size tends to fall within the smaller end (0.25–2.5 ha) of the typical size range (0.25–5 ha). For example, in one of the most noted long-term studies on Eastern Box Turtle home range size, Stickel (1989) estimated home ranges of females to be 1.13 ha and males to be 1.20 ha based on multiple captures between 1944 and 1981. However, home range estimates are quite variable and are influenced by tracking technique, calculation and estimation method, displacement, and by both biotic and abiotic factors, such as habitat type, level of urbanization, or level of forest fragmentation.

There are several biotic and abiotic factors that can affect home range size in box turtles, but in most cases, home ranges tend to be smaller with higher quality habitat (i.e., resource availability, mate availability, suitable nesting and hibernation sites, etc.), less habitat structure (i.e., waterways, roadways, artificial structures, etc.), decreased levels of urbanization (i.e., requiring less movement in search of suitable resources), and younger age classes (Madden 1975; Dodd 2001; Willey 2010). Habitat availability and variability have major influences on turtle movements, and turtles in high quality habitat or more diverse habitat are expected to have smaller home ranges because they presumably do not need to travel as far to find resources, such as food, mates, or nesting sites. (Madden 1975; Stickel 1989; Willey 2010). Alternatively, box turtles may be forced to exhibit smaller home range sizes in more urbanized landscapes simply because there is less habitat available, and barriers may block movement outside of urban habitat patches. However, if they are able, Eastern Box Turtles will leave their established home range on occasion if resources such as appropriate nesting sites (Stickel 1950) or a water source (Donaldson and Echternacht 2005) are not available.

Within their home range, box turtles generally have a suitable site for overwintering. However, there is regional variation in timing of immergence into and emergence from the hibernaculum, as well as duration of time spent in the hibernaculum. For example, in Ohio, Eastern Box Turtles entered their hibernacula in mid-October through mid-November and emerged in late February through early April (generally in March), with an average duration of 142 d in the hibernaculum (Claussen et al. 1991). Conversely, in Illinois, Ornate Box Turtles (*Terrapene ornata ornata*) exhibited earlier immergence and later emergence patterns than Eastern Box Turtles in Ohio, where turtles entered their hibernacula in early September to early October, emerged in early April to early May, and spent anywhere from 187 to 225 d hibernating (Milanovich et al. 2017).

Knowledge of Eastern Box Turtle home range size and timing of hibernation in Tennessee is limited to a few studies in the eastern part of the state (Dolbeer 1969; Davis 1981; Donaldson and Echternacht 2005), and with the subspecies in decline across its distributional range (van Dijk 2011), including Tennessee (Tennessee Wildlife Resources Agency 2015), home range estimates and timing of hibernation will provide managers with important information for monitoring and conservation efforts for both turtles and their habitats. Furthermore, most movement studies have been conducted in habitats located away from urban or suburban areas, and there are no studies in Tennessee on home range or hibernation in wetlands. Therefore, the objective of this study was to estimate home range size and characterize temporal aspects of hibernation for the Eastern Box Turtle in a suburban wetland habitat of middle Tennessee. We hypothesized that turtles would have a home range size within the typical range (0.25 – 5 ha) and that timing of immergence into hibernaculum would be similar to findings of studies in east Tennessee.

METHODS AND MATERIALS

Study Species and Study Area—. The Eastern Box Turtle (*Terrapene carolina carolina*) is one of six subspecies of the common box turtle and belongs to the family Emydidae. Between 2013 and 2015, we studied Eastern Box Turtles in a 23.5 ha suburban wetland habitat known as Nickajack Trace and Black Fox Wetlands (Murfreesboro, TN, USA). Nickajack Trace is predominantly a Hardwood Forest with areas of Palustrine Emergent and Forested Wetlands (5%; U.S. Army Corps of Engineers 2000). The site is divided into two sections by a road and is surrounded by a housing community. One side of the road is 3.5 ha in size and contains a small pond fed by Black Fox Spring, whereas the area on the other side of the road is 20 ha with Lytle Creek running through the interior of the forest (U.S. Army Corps of Engineers 2000).

Field Methods—. We captured Eastern Box Turtles opportunistically by walking through the field site and visually searching for individuals. Upon initial capture, all box turtles were measured, weighed, sexed, and marked. We used digital calipers (203 mm, Neiko Tools, Homewood, Illinois, USA) to obtain shell measurements to the nearest 0.1 mm (see West and Klukowski 2016). We measured mass to the nearest 1 g using a 1,000 g spring scale (Pesola, Forestry Suppliers, Jackson, Mississippi, USA). We assigned a unique code to each individual by filing three marginal scutes (Cagle 1939; Somers et al. 2017). Additionally, we estimated age of each individual by counting annular rings on the pleural scutes (Ewing 1939). We also collected GPS coordinates (WGS 1984) and a photograph for each turtle.

Between June 2013 and October 2014, we captured and equipped six adult box turtles with VHF RI-2B radio transmitters (frequencies between 151.122 and 151.926—Holohil Systems Ltd., Carp, Ontario, Canada). We affixed the transmitters to the posterior region (pleural scutes) of the carapace using epoxy glue, and we held turtles overnight (> 8 h) to allow the glue to dry before releasing. The

TABLE 1. Tracking and demographic information for six adult Eastern Box Turtles (*Terrapene carolina carolina*) tracked with radio transmitters. M = male, F = Female.

ID	Tracking Start	Tracking Finish	Age Class (y)	Sex	Mass (g)
AMW	04 June 2013	02 May 2014	11–14	F	508
APQ	07 August 2013	03 April 2015	11–14	F	376
CHL	18 September 2013	21 July 2014	15–19	M	415
BCX	17 August 2013	13 September 2013	11–14	F	232
AHL	11 October 2014	05 June 2015	15–19	F	466
BLX	14 October 2014	03 April 2015	15–19	M	440

transmitters had an average lifespan of 12 mo, weighed 9.7 g, and did not exceed 5% of an individuals' body mass. We used a collapsible 3-element Yagi directional antenna (Wildlife Materials, Inc., Murphysboro, IL, USA) with a R1000 handheld receiver (Communication Specialists, Inc., Orange, CA, USA) to locate turtles. We tracked different individuals between June 2013 and June 2015, but no turtle was tracked during this entire time frame (Table 1).

This project was concurrent with another larger project; consequently, turtles were located when time permitted, generally at least once or twice a week in 2013, with most data points separated by only a few days, although a small percentage (< 5%) of points (mostly from 2014 and 2015) were separated by weeks due to time constraints towards the end of the study. Once we located an individual, we used a GPS device (Garmin Etrex 30, Olathe, KS, USA) to record coordinates for home range analyses. We tracked five of the six box turtles into hibernation, but one individual lost the transmitter before the overwintering period. The dates of immergence and emergence, as well as the duration spent in the hibernaculum, are approximate, as we did not monitor turtles every single day during late fall and early spring.

Data Analyses—. We used fixed kernel (FK—Worton 1987; Seaman and Powell 1996) density estimates with least squares cross validation and minimum convex polygon (MCP—Mohr 1947) methods to estimate home ranges of box turtles. Elimination of autocorrelation among locations is required to satisfy statistical assumptions of kernel methods, so all locations were recorded at least 24 h apart (De Solla 1999; Kie et al. 2010). Repeat coordinates recorded at hibernation sites were used only once in FK analyses. Only individuals with ≥ 20 independent locations were used in FK analyses, whereas all turtles and all coordinates were included in MCP analyses (Table 1). Of the six radio-tracked individuals, sufficient location data (≥ 20 locations) for FK estimates were collected for two female box turtles and one male box turtle. All FK and MCP home range estimates were made with the default bandwidth settings and calculated in program R 3.4.0 (R Core Team 2014) using the package 'adehabitatHR' (Calenge 2006). Home ranges were plotted using 95% FK estimates and 95% MCP estimates, and core areas were delineated using 50% FK estimates. Maps were created in ArcMap 10.6 (ESRI, Redlands, CA, USA). All values are reported as mean ± standard deviation.

Table 2. Fixed kernel (FK) and minimum convex polygon (MCP) home range estimates (hectares) for six adult Eastern Box Turtles (*Terrapene carolina carolina*). The average home range is reported with standard deviation, and *n* indicates the number of GPS location points for each turtle. Repeat hibernation coordinates are included in MCP analyses but are not included in FK analyses.

ID	95% FK	50% FK	Core Areas	*n*	95% MCP	*n*
AMW	1.66	0.27	3	32	1.10	46
APQ	2.59	0.28	7	50	4.42	62
CHL	0.25	0.01	4	24	1.12	34
BCX	-	-	-	-	0.40	14
AHL	-	-	-	-	0.04	11
BLX	-	-	-	-	0.04	9
Average	1.50 ± 1.18	0.19 ± 0.15	4.7	35	1.19 ± 1.67	24

TABLE 3. Approximate dates of immergence into and emergence from hibernacula for five radiotracked adult Eastern Box Turtles (*Terrapene carolina carolina*) along with days spent in the hibernaculum (duration). The mean duration is reported with standard deviation.

ID	Immergence	Emergence	Duration
AMW	01 November 2013	02 April 2014	152
APQ	24 November 2013	05 April 2014	132
CHL	15 November 2013	16 April 2014	152
AHL	07 November 2014	03 April 2015	154
BLX	07 November 2014	03 April 2015	154
Average			149 ± 9.44

RESULTS

We successfully tracked six adult box turtles using radio transmitters (Table 1). The 95% fixed kernel (FK) home ranges for two female turtles and one male turtle averaged 1.50 ± 1.18 ha (min.: 0.25 ha, max.: 2.59 ha; Table 2; Fig. 1). The 50% FK core areas for the same three turtles were small and averaged 0.19 ± 0.15 ha (min.: 0.01, max.: 0.28), with an average of 4.7 core areas per turtle (Table 2; Fig. 1). Similar to the FK estimates, the 95% minimum convex polygon (MCP) home range estimates for four female and two male box turtles averaged 1.19 ± 1.67 ha (min.: 0.04 ha, max.: 4.42 ha; Table 2; Fig. 2).

For the five turtles monitored during winter dormancy, approximate immergence was in November and approximate emergence was in April (Table 3). Turtles spent an average of 149 ± 9.44 d (132–154 d) in their hibernaculum, and we did not witness any turtles emerging from their hibernaculum and moving during this period. We also observed hibernation site fidelity at our field site, with two turtles each using their exact same hibernaculum for two consecutive years. All turtles used hibernacula that were well within (at least 50 m from edge) the forest (i.e., not in any open fields, along edges, etc.). One turtle (BLX) hibernated in a tree root complex that was flooded with water throughout the entire winter dormancy period.

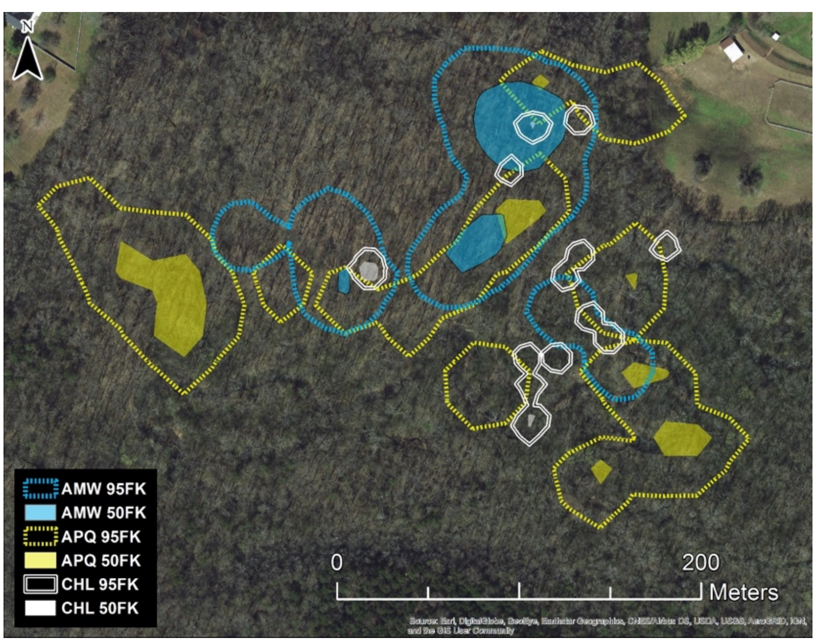

FIG. 1. 95% fixed kernel (FK) home range estimates and 50% FK core areas (shaded areas) for three Eastern Box Turtles (*Terrapene carolina carolina*).

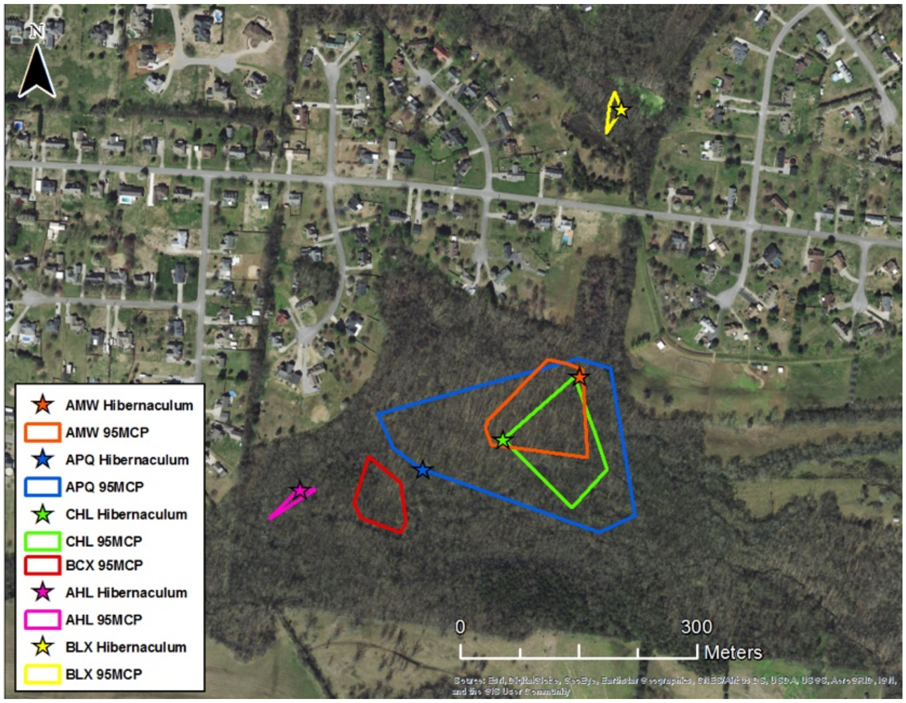

FIG. 2. 95% minimum convex polygon (MCP) home range estimates for all six radiotracked Eastern Box Turtles (*Terrapene carolina carolina*). Stars indicate hibernaculum location for each of the five turtles tracked into hibernation (i.e., all except individual BCX whose transmitter fell off before hibernation).

DISCUSSION

The average home range estimates of 1.50 ha (FK) and 1.19 ha (MCP) fell within the range of what is typically reported for adult Eastern Box Turtles (Table 4). These small home ranges had multiple core areas that likely served different purposes (e.g., resource availability) for box turtles at this site. Several of the turtles had overlapping home ranges, so conservation areas can ideally support multiple individuals. Conservation areas should seek to provide a large enough area of suitable habitat to support core areas with a surrounding buffer up to at least 5 ha to accommodate the typical home ranges of most box turtles throughout their range, keeping in mind that there may be different requirements in different habitat types and even for different individuals. However, 5 ha of suitable habitat is ideally enough for most box turtles as long as it contains the appropriate resources.

Although our home range estimates were on the smaller side, two east Tennessee studies calculated home ranges much smaller than ours (i.e., 0.45 ha—Dolbeer 1969; 0.38 ha—Davis 1981), which may be the result of differences in habitat, tracking methods, and computation and estimation methods. Based on a tracking period of four months, Marchand et al. (2004) calculated an average MCP home range of 1.19 ha for three radiotracked Eastern Box Turtles in a sanctuary with both wetland and upland habitats, which mirrors our MCP estimate. In comparison, Donaldson and Echtnernacht (2005) used thread-trailers and radio-transmitters to track Eastern Box Turtles in east Tennessee and calculated an average home range of 1.88 ha (MCP) and 2.26 ha (95% FK), which is also comparable to the findings in our study. By removing two outliers, Greenspan et al. (2015) found that the average MCP home range for box turtles went from 10.33 ha to 5.87 ha in a Longleaf Pine forest, which is still rather large but emphasizes the importance of spatial variation as well differences in individuals and differences due to habitat type and quality.

Not only is habitat a factor in determining home range size, but box turtles may exhibit seasonal differences in home range usage,

TABLE 4. Comparison of tracking methodology, home range computational methods, and home range size estimates from other Eastern Box Turtle (*Terrapene carolina carolina*) home range studies. FK = fixed kernel, MCP = minimum convex polygon, M = male, F = female.

Study	Location	Tracking Method	Estimation/ Computation Method	Home Range Size (ha)
Dolbeer (1969)	east Tennessee	mark-recapture	diameter	0.45
Davis (1981)	east Tennessee	radio-telemetry	convex polygon	0.38
Stickel (1989)	Maryland	mark-recapture	ellipse	M: 1.20, F: 1,13
Cook (2004)	New York	translocation and radio-telemetry	95% bivariate normal and 95% harmonic mean	bivariate: 9.77, harmonic: 4.82
Marchand et al. (2004)	Maryland	radio-telemetry	100 % MCP	1.19
Donaldson and Echternacht (2005)	east Tennessee	thread-trailer and radio-telemetry	MCP and 95% FK	MCP: 1.88, FK: 2.26
Greenspan et al. (2015)	Georgia	radio-telemetry	MCP and 50% FK	MCP: 10.33, FK: 2.08

generally with a larger average home range in the summer than in the spring and fall (Aall 2011). We only tracked most turtles over the course of one (or part of one and part of another) active season and sometimes only during certain seasons (i.e., spring, summer, and/or fall), and our tracking schedule was not structured due to time constraints from a simultaneous project. While our small home range sizes could be indicative of high-quality habitat, our MCP estimates were inherently smaller due to a lack of data points collected for most individuals (especially BCX, AHL, and BLX), and the average FK home range estimate was likely biased by individual CHL. Individual CHL was tracked only between the months of September through November (i.e., fall season) just prior to hibernation and a few times in July. When CHL's home range was removed, the average 95% FK home range value increased from 1.50 ha to 2.13 ha and the average 50% FK estimates shifted from 0.19 ha to 0.28 ha. Comparatively, when individuals BCX, AHL, and BLX are removed from MCP home range estimates, the average increased from 1.19 ha to 2.21 ha. A home range study for a longer time-period over multiple seasons with a more rigorous tracking protocol is warranted for valid comparison to other studies. However, the presented data can serve as a baseline comparison and a starting point for future studies.

In general, turtles entered their hibernaculum in November and emerged in April, with an average of 149 d spent in the hibernaculum, which is similar to other Eastern Box Turtle studies. For example, in Ohio, Eastern Box Turtles immerged in mid-October through mid-November and generally emerged in March, with an average of 142 d spent in the hibernaculum (Claussen et al. 1991). In east Tennessee, no study has reported hibernaculum emergence dates for Eastern Box Turtles, but turtles generally entered their hibernaculum in late October through November with movements recorded up until mid-December (Dolbeer 1969). In Virginia, Eastern Box Turtles immerged in late November through late December and emerged in mid-March through early April; however, some turtles emerged early and relocated during this time frame, with an average of 82.2 d between entry and relocation (Boucher 1999). During our study, we recorded no movements of turtles to other locations between November and April, so we believe turtles were sedentary during that entire time frame.

Hibernation site fidelity has been documented in Eastern Box Turtles (e.g., Claussen et al. 1991; Seibert and Belzer 2015; Vannatta and Klukowski 2015), but it is more common to find turtles hibernating in the same general area rather than in the exact same hibernaculum. At our study site, two individuals exhibited hibernation site fidelity (Vannatta and Klukowski 2015 and this study). In both instances, each turtle was found in the exact same hibernaculum two winters in a row, and these two hibernacula were within ~15 cm of one another, indicating that it might be an optimal hibernation location. All five turtles hibernated well within (at least 50 m from the edge) the forest (i.e., not in an open area or close to the edge) where they were buried under leaf litter. However, one individual in our study chose to hibernate in an area under tree roots that was flooded throughout the winter dormancy period. This has been documented on several other occasions, indicating the resiliency of box turtles and their ability to hibernate in water (e.g., Cahn 1933; Madden 1975; Boucher 1999; Koester 2011).

Overall, as urbanization, fragmentation, and general decline of box turtle habitat continues, we can use this information on home range size and hibernation ecology to better understand the way box turtles utilize their habitat to move and overwinter and to infer best management and conservation strategies for turtles and their habitats. We first recommend a better standardization of methods because of the wide variety of tracking and home range estimation techniques (Table 4) makes comparisons difficult. Across the range, we recommend maintaining a conservation area of suitable habitat that is at least 5 ha to support box turtle home ranges, knowing that home ranges often overlap, and multiple turtles can use a given area, but there may be slight differences in home range needs in different habitat types or for different individuals. Curtin (1995) discussed how habitat fragmentation and degradation can negatively affect habitat characteristics, such as microclimate and landscape structure, which can lead to changes in turtle movements (i.e., increasing their home range size to find proper habitat), lower their overall survival, and influence their activity patterns (i.e., growth and reproduction). Therefore, understanding how box turtles utilize their habitats and which habitat variables are necessary can aid in determining the best course of long-term management for this declining species and their vanishing habitat.

As there are no known home range studies on Eastern Box Turtles in middle Tennessee, there are no data on timing of hibernation events in middle Tennessee, and very few studies have been conducted in suburban or wetland habitats, this study can act as a baseline for future studies. However, future studies should seek to employ long-term monitoring efforts in different habitat types with an increased sample size over all seasons for home range size estimation and for describing hibernation activity in Eastern Box Turtles.

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LIMITED IMPACTS OF ACID RUNOFF FROM PYRITE-BEARING ROCK FORMATIONS ON STREAM SALAMANDERS IN MIDDLE TENNESSEE HEADWATER STREAMS

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Abstract.— In addition to the myriad anthropogenic disturbances that impact biota and conservation efforts, acidification of aquatic habitats is an important threat to aquatic biodiversity. In this study, we examined the effects of acid rock drainage (ARD) on stream salamander assemblages in streams associated with rock cuts and pyrite-bearing Chattanooga and Fentress Shale formations in Middle Tennessee. We selected two streams monitored by the United States Geological Survey for changes in water quality and used a paired study design to evaluate the impacts of ARD by monitoring sites above and below the ARD disturbance. We surveyed a single transect and two quadrats (each surveyed once) in each paired stream reach at both sites and captured 158 larval and adult stream salamanders of 6 species. Salamander counts were similar for adult and larval salamanders between ARD-impacted and unimpacted stream reaches. Biodiversity measures (species richness, diversity, and evenness) did not differ between ARD-impacted and unimpacted stream reaches. Similarly, adult and larval counts for the Spotted Dusky Salamander (*Desmognathus conanti*) and Southern Two-lined Salamander (*Eurycea cirrigera*) did not differ between reaches. In terms of species composition, adult and larval *E. cirrigera* captures represented 37.9% and 94.2% of captures in ARD-impacted reaches versus 6.7% and 84.2% of captures in unimpacted reaches. We did not detect significant differences in water quality measurements (pH, temperature, and dissolved oxygen) between ARD-impacted and unimpacted stream reaches. We attribute the lack of stream disturbance effects to mitigation measures (limestone rock abatements) that were implemented above ARD-impacted stream reaches after road cut disturbance. Collectively, our pilot study provides an initial examination of the impacts of ARD on stream salamander assemblages in Tennessee, and suggests that impacts at these two sites are limited. However, a broader scale and replicated field study is necessary before larger conclusions can be established.

Key Words.—acid rock drainage; *Desmognathus*; disturbance; *Eurycea*; lotic; Plethodontidae; riparian

The disappearance of amphibians is occurring globally at a rate of nearly 200 times the background extinction rate primarily through anthropogenic means, including commercial use, disease, climate change, pollution, introduction of invasive species, and habitat destruction (Blaustein et al. 1994; Stuart et al. 2004; Beebee et al. 2005; Cushman 2006; McCallum 2007). Given the large-scale decline in amphibian biodiversity, it is important to evaluate the impacts of human disturbances on potentially vulnerable species and create management plans to mitigate the effects of human disturbance on population, biodiversity, and ecosystem health.

Amphibians contribute directly to ecosystem function through a variety of ecosystem services. In particular, salamanders contribute directly to carbon sequestration (Wyman 1998; Best et al. 2014), predation of detritivores (Brodman and Dorton 2006), as prey in trophic food webs (Davic and Welsh 2004), through movement of nutrients between aquatic and terrestrial environments (Regester et al. 2008), and as biological indicators of community fitness (Welsh and Droege 2001).

The southeastern United States exhibits high salamander diversity and provides an excellent opportunity to utilize these amphibians as biological indicators of ecosystem condition (Micacchion 2002; Bailey et al. 2004; Welsh and Droege 2007). Many of these salamander species occur in low-order, headwater streams that originate from ground-water seeps and represent the top predators in these ecosystems (Davic and Welsh 2004). Understanding abundance and richness of stream salamander assemblages can provide valuable data relating to water quality, habitat suitability, and impacts of ecological disturbance. These species are also sensitive to stream side disturbances that impair riparian buffers or water quality (Barrett and Price 2014), especially when these disturbances impact the underlying geology of riparian zones.

Pyrite and similar minerals that contain sulfur and trace metals occur in rock formations associated with low-order streams throughout the eastern and southeastern United States (Cook et al. 1994; Daniels and Orndorff 2003; Anderson 2008). When pyrite is exposed to oxygen and water through disturbance of bedrock layers, the minerals decompose and the sulfur can react to form sulfuric acid, resulting in environmental contamination (Bacon and Maas 1979; Daniels and Orndorff 2003; Hammarstrom et al. 2004). When these pyrite-bearing formations are exposed in a road cut (e.g. an area where a section of rock or soils is cut or blasted away to make space for transportation infrastructure), there is potential for acid rock drainage (ARD hereafter) that can also contain elevated levels of heavy metals, which may have a negative impact on stream ecosystems (Bacon and Maas 1979). These compounds can be released and transported during rain events (Hammarstrom et al. 2004), and if the runoff is untreated, there may be unintended negative consequences on aquatic biodiversity and environmental conditions (Kucken et al. 1994; Schorr et al. 2013).

Anthropogenic disturbances, such as mining and road construction, can greatly influence headwater stream diversity through direct habitat impacts and changes in water chemistry (Bernhardt and Palmer 2011). Previous studies have shown that aquatic acidification can negatively impact aquatic invertebrates (Niyogi et al. 2002) and vertebrates, including both fishes and amphibians (Huckabee et al. 1975; Kucken et al. 1994; Schorr et al. 2013). Stream salamanders display species- and life-stage specific sensitivities to reduced pH, with mortality occurring below pH levels of 4.2, and larvae having greater sensitivity compared to adults (Green and Peloquin 2008). Further, the geographic distribution of stream salamander larvae (*Desmognathus spp.* in particular) is negatively impacted by landscape disturbance, most notably watersheds with reduced pH and increased turbidity and sedimentation (Gore 1983). Landscape disturbances such as mountaintop removal mining result in large reductions of available habitat, but also cause rapid reductions in stream pH and increases in specific conductivity and dissolved solids, which also negatively impact stream salamanders (Muncy et al. 2014; Price et al. 2016). Studies that have evaluated the impacts of ARD on stream salamanders has been limited; however, streams impacted by ARD have displayed decreases in pH and increases in metals and near elimination of macroinvertebrates, fish, and salamanders (Mathews et al 1981; Kucken et al. 1994) which illustrates that ARD disturbance can potentially impact stream biodiversity, including salamanders.

To this end, we aimed to evaluate the impacts of ARD disturbance on stream salamander assemblages in Tennessee headwater stream ecosystems. Our small-scale survey, which was limited to two sites, examined the potential impacts of ARD on stream salamanders at streams in Middle Tennessee in two different geologic shale formations. We predicted that impaired stream reaches would have lower diversity, richness, abundance and greater evenness of adult and larval salamanders compared to unimpacted stream reaches. We also predicted that larval counts for all salamander species would be impacted to a greater extent compared to adult salamanders due to the fully aquatic nature of this life stage and greater sensitivity of this life stage to reduced water quality parameters.

METHODS AND MATERIALS

Study Sites—. We selected study sites in Chattanooga and Fentress shale formations in Middle Tennessee that coincided with an on-going ARD project implemented by the United States Geological Survey (USGS). We targeted two sites in Middle Tennessee with direct drainage from ARD disturbance into neighboring low-order streams. The first site included a road cut through a geologic formation that contained Chattanooga Shale located in south Williamson County (840 site), which drains to the headwaters of Carter's Creek, which, in turn flows into the Mill Creek watershed (Figure 1 A – D; 35.814735, -86.972729). Road construction at the site began in 2011 as a continuation of Interstate 840 construction. The impacted portion of the stream was located ~ 100 m from the road cut. The second ARD site located in Fentress County (Fentress site) cuts through the Fentress Shale formation, and was a result of a road-cut made for Highway 127 constructed between 2008 and 2009. Runoff from this ARD disturbance runs off 430 m directly into a headwater stream of the Wolf Creek watershed (Figure 1 E – H; 36.493378, -84.963707). Specific conductance and pH measurements in water runoff samples at both ARD road cuts indicated that specific conductance was elevated (840 site: 926 – 2266 µS/cm; Fentress site: 1726 – 1870 µS/cm) and pH measurements were below neutral (840 site: 2.45 – 4.00; Fentress site: 5.25 – 6.82; Byl, unpublished data). Both sites were low-order (e.g., Order 1 and 2) headwater stream environments that were mitigated with limestone rock between the riparian zone of the study streams and ARD road disturbance.

Salamander Sampling—. We conducted field surveys (each site and stream reach surveyed once) during September and October of 2015 to evaluate biological impacts of ARD on stream salamander assemblages. We used a paired experimental design that included one stream reach located above the ARD disturbance (i.e., unimpacted) and a reach that was located downstream of the ARD disturbance (i.e., ARD-impacted) at each of the two sites. This design permitted a direct and relative measure of ARD disturbance on stream salamanders. We identified stream segments within each stream reach that contained habitat features (e.g., rock cover, small riffles, permanent water) conducive for the presence of stream salamanders as recommended in Jung (2002).

We used stream quadrat and transect surveys as described in Price et al. (2011) to evaluate the impacts of ARD on adult and larval stream salamanders. We delineated one 15 m x 3 m linear transect and two 1 m x 1 m quadrats that spanned both terrestrial and aquatic habitat in ARD-impacted and unimpacted stream reaches at both sites. We

FIG. 1. Summary photographs for two headwater streams impacted by acid rock drainage (ARD) disturbance in TN. Photographs A – D represent the 840 site (Williamson County, TN) as follows: A) highway 840 road cut with iron staining, B) close-up of pyrite-bearing rock layers, C) upstream (unimpacted) section of the 840 site, D) downstream (ARD-impacted) section of the 840 site. Photographs E – H represent the Fentress site (Fentress County, TN) as follows: E) highway 127 road cut, F) ARD runoff from the base of the highway 127 road cut, G) iron staining on tree roots and soil substrate where runoff from the highway 127 road cut enters the Fentress County stream, and H) upstream (unimpacted) section of the Fentress site.

surveyed for and captured adult and larval stream salamanders opportunistically by turning over cover objects, including rocks and logs within the transect and quadrat boundaries. We surveyed transects using non-destructive methods (i.e., cover objects were briefly lifted and returned), whereas we surveyed quadrats using destructive methods (i.e., all cover objects were completely removed from the quadrat grid and returned at the conclusion of the survey). We used these two survey methods to obtain estimates of both larval and adult salamander abundance. We identified each captured salamander to species and measured (snout-vent length [mm]) and weighed mass (g) using a digital scale.

Environmental Data—. We measured stream pH, water temperature, and dissolved oxygen at the downstream end of each ARD-impacted and unimpacted stream reach prior to implementation of stream surveys. We used an Oakton® EcoTestr™ pH2^{+} meter to monitor stream pH and a YSI® EcoSense® DO200 Series meter to monitor both water temperature (°C) and dissolved oxygen (ppm). We calibrated all instruments as specified by the manufacturer.

Data Analysis—. We determined total salamander counts and percent composition for species with > 10 captures between stream reaches above and below the ARD disturbance. We also determined richness, evenness, and Simpson's Diversity Index (heterogeneity) separately for the ARD-impacted and unimpacted stream reaches as defined in Krebs (1999). We used paired t-tests in RStudio v.1.1.414 to evaluate differences among species diversity measures (richness, evenness, and heterogeneity) and relative abundance for larval and adult stream salamanders. We considered relationships statistically-significant when p-values were ≤ 0.05.

RESULTS

Salamander Sampling—. We captured 158 salamanders of 6 species (Table 1). The Spotted Dusky Salamander (*Desmognathus conanti*) was the most commonly-captured adult salamander species (47 captures) and the Southern Two-lined Salamander (*Eurycea cirrigera*) was the most commonly-captured larval salamander species (85 captures; Table

TABLE 1. Total counts and percent composition of adult and larval stream salamanders in streams monitored for biological impacts of acid rock drainage.

Species	Species Count	% of Captures
Adult		
Desmognathus conanti (Spotted Dusky Salamander)	47	74.60%
Desmognathus welteri (Black Mountain Salamander)	1	1.60%
Eurycea cirrigera (Southern Two-lined Salamander)	12	19.00%
Eurycea lucifuga (Cave Salamander)	1	1.60%
Plethodon dorsalis (Northern Zig-zag Salamander)	2	3.20%
Total Adults	**63**	**100%**
Larvae		
Desmognathus conanti (Spotted Dusky Salamander)	5	5.30%
Eurycea cirrigera (Southern Two-lined Salamander)	85	89.40%
Gyrinophilus porphyriticus (Spring Salamander)	5	5.30%
Total Larvae	**95**	**100%**

1). The Black Mountain Salamander (*Desmognathus welteri*) and the Cave Salamander (*Eurycea lucifuga*) were both captured once at the Fentress County site and the 840 site, respectively (Table 1). We captured five larval Spring Salamanders (*Gyrinophilus porphyriticus*) at the Fentress County site (Table 1).

We did not detect impacts of ARD disturbance on adult salamander diversity measures: species richness ($t = 0.00$, $df = 1$, $p = 1.00$), diversity ($t = –0.47$, $df = 1$, $p = 0.72$), and evenness ($t = 1.00$, $df = 1$, $p = 0.50$); larval diversity measures: species richness ($t = 1.00$, $df = 1$, $p = 0.50$), diversity ($t = –1.93$, $df = 1$, $p = 0.30$), and evenness ($t = –0.19$, $df = 1$, $p = 0.88$); and total salamander diversity measures: species richness ($t = 0.33$, $df = 1$, $p = 0.80$), diversity ($t = –2.32$, $df = 1$, $p = 0.26$), and evenness ($t = –0.30$, $df = 1$, $p = 0.82$) between ARD-impacted and unimpacted stream reaches (Figure 1).

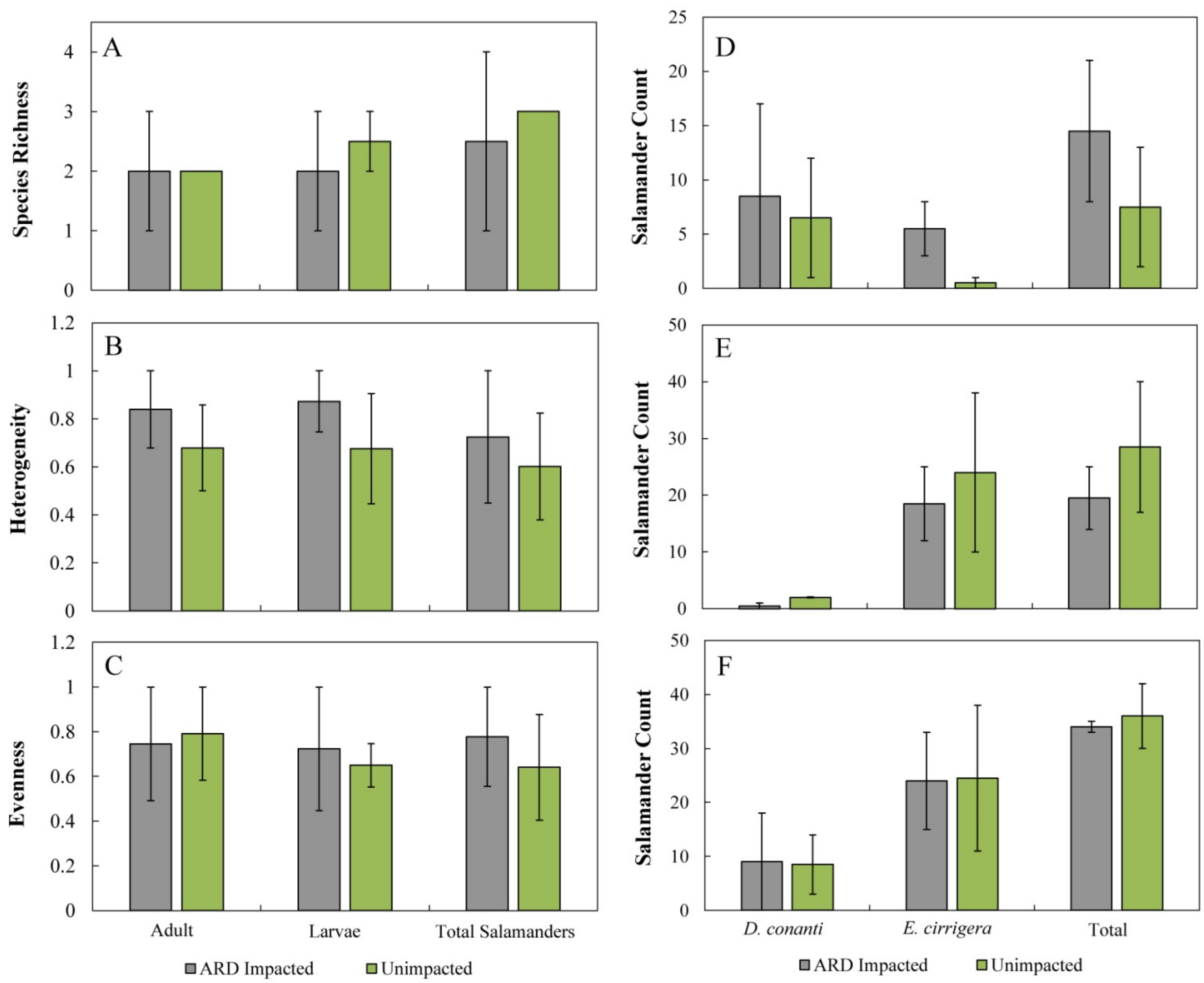

FIG. 2. Impacts of ARD disturbance on richness (A), heterogeneity (B), and evenness (C) for adult, larval, and total salamander counts. In addition, impacts of ARD disturbance on total counts for Spotted Dusky (*Desmognathus conanti*) salamanders, Southern Two-lined (*Eurycea cirrigera*) salamanders, and both species combined (Total) for adults (D), larvae (E), and total counts (F) of both larvae and adults. In all figures, gray bars represent ARD-impacted stream reaches and green bars represent unimpacted reaches.

Although total adult salamander counts were greater in ARD-impacted stream reaches compared to unimpacted reaches (Figure 2), this difference was not significant (t = –7.00, df = 1, p = 0.09; Figure 2). This trend was due to relative abundance of adult *E. cirrigera*, which although greater in ARD-impacted stream reaches (5.5 ± 2.5 captures) compared to unimpacted (0.5 ± 0.5 captures) stream reaches (Figure 2), was not statistically significant (t = –1.67, df = 1, p = 0.34). Larval counts averaged 28.5 ± 2.5 in unimpacted stream reaches and 19.5 ± 2.0 in ARD- impacted stream reaches, but were not statistically significant (t = 1.50, df = 1, p = 0.50). In addition, differences for larval *D. conanti* (t = 3.00, df = 1, p = 0.20) and *E. cirrigera* (t = 0.73, df = 1, p = 0.60) between ARD-impacted and unimpacted stream reaches were not statistically significant (Figure 2). We did not detect an effect of ARD disturbance on total salamander counts (i.e., larval and adult salamanders combined; t = 2.85, df = 1, p = 0.82) and found that percent composition was largely similar between ARD-impacted and unimpacted reaches (Figure 2). Anecdotally, we observed a trend of greater percent species composition of *E. cirrigera* in ARD-impacted stream reaches (adults: 37.9% of captures; larvae: 94.8% of captures) compared to unimpacted stream reaches (6.7% of captures; 84.2% of captures) across both sites (Figure 3).

Environmental Data—. We did not detect statistically-significant differences between ARD-impacted and unimpacted stream reaches for pH (t = 0.67, df = 1, p = 0.63; ARD-impacted: 7.45 ± 0.45, unimpacted: 7.85 ± 0.15), water temperature (t = 3.0, df = 1, p = 0.20; ARD-impacted: 12.0 ± 2.0 °C, unimpacted: 12.5 ± 2.0 °C), or dissolved oxygen (t = -2.084; df = 1, p = 0.28; ARD-impacted: 9.74 ± 0.28 ppm, unimpacted: 9.31 ± 0.31 ppm).

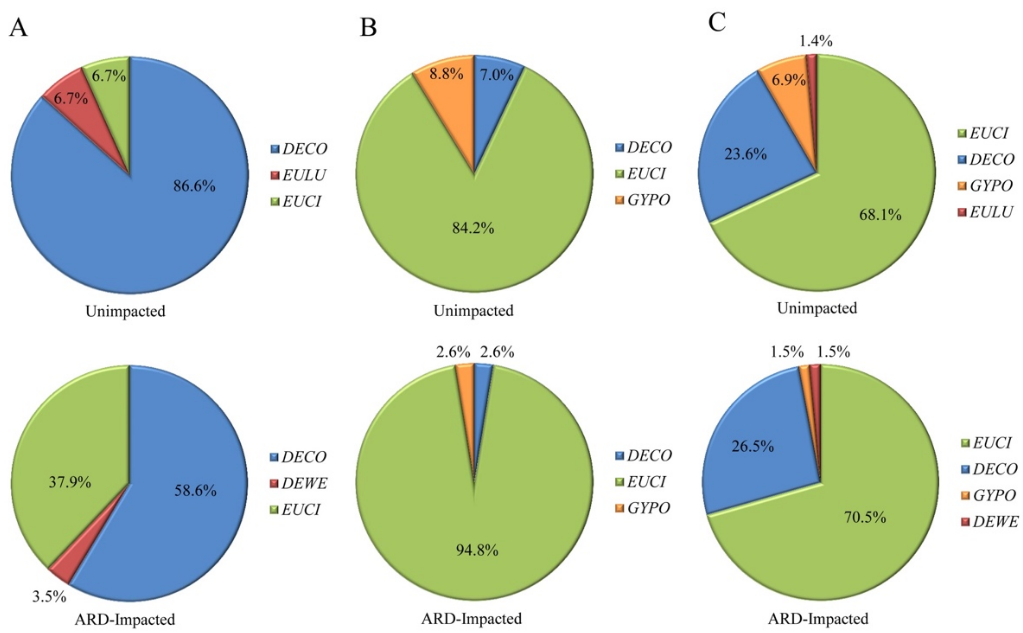

FIG. 3. Percent composition adult (A), larval (B), and total (C) stream salamanders in stream reaches unimpacted and impacted by acid rock drainage in middle Tennessee. Species abbreviations are as follows: DECO – *Desmognathus conanti* (Spotted Dusky Salamander), DEWE – *Desmognathus welteri* (Black Mountain Salamander), EUCI – *Eurycea cirrigera* (Southern Two-lined Salamander), EULU – *Eurycea lucifuga* (Cave Salamander), and GYPO – *Gyrinophilus porphyriticus* (Spring Salamander).

DISCUSSION

We originally hypothesized that salamander diversity measures and relative abundance would be lower in ARD-impacted stream reaches compared to unimpacted reaches and that larvae would be more negatively impacted by disturbance compared to adults. Our findings do not support these hypotheses and suggest that impacts of ARD disturbance on stream salamander assemblages were minimal at the two sites evaluated. Specifically, we found no difference in biodiversity measures (i.e., species richness, diversity, and evenness) between ARD-impacted and unimpacted stream reaches for adult and larval salamanders. Although few other studies have evaluated the impacts of ARD on salamander biodiversity measures, landscape-scale disturbances (such as mountaintop removal mining) result in reduced species richness compared to unimpacted streams (Muncy et al. 2014).

Although we did not find statistically significant differences in relative abundance for larval and adult *D. conanti* and *E. cirrigera* between ARD-impacted and unimpacted stream reaches, *E. cirrigera* adults and larvae tended to compose a greater proportion of adult and larval salamander captures in ARD-impacted reaches compared to unimpacted reaches. Previous research suggests that *E. cirrigera* is somewhat tolerant of stream disturbance and can maintain relatively high abundance in moderately disturbed stream sites (Southerland et al., 2004). Further, stream salamander species that inhabit disturbed stream sites (such as *E. cirrigera*) are defined as “disturbance tolerants” due to their ability to persist in stream ecosystems impacted by moderate anthropogenic disturbance (Surasinghe and Baldwin, 2015).

As we only monitored two sites during this study, continued research at a greater number of ARD-disturbed sites is necessary to better understand the impacts of ARD on stream salamander assemblages. Kucken et al. (1994) observed marked declines in relative abundance of Blue Ridge Two-lined Salamanders (*Eurycea wilderae*) and Blackbelly Salamanders (*Desmognathus quadramaculatus*) in an ARD-impacted stream in the Anakeesta shale formation of the Great Smoky Mountains National Park. Similarly, a fish kill was observed in a stream draining a roadbed fill, which continued for 10 years; downstream of the fill, the pH was acidic (4.5-5.9), whereas pH upstream of the fill was near neutral (6.5-7.0; Huckabee et al. 1975). Stream salamanders are sensitive to changes in stream conditions as evidenced by studies that have evaluated the impacts of agriculture (Willson et al. 2003), urbanization (Barrett and Guyer 2008), and mining (Price et al. 2016). Overwhelmingly, these studies suggest that land-use change in terrestrial areas surrounding stream environments causes increased levels of dissolved sediments and subsequent decline of water quality through increased conductivity and usually large decreases in stream pH (Price et al. 2016, Willson et al. 2003; Huckabee et al. 1975; Barrett and Guyer 2008).

Stream salamanders (i.e., family Plethodontidae) in particular appear to be negatively associated with changes in water quality parameters, perhaps because they rely on cutaneous respiration to acquire oxygen (Wells 2007). This adaptation requires moist environmental conditions for the diffusion of oxygen to occur, which increases the sensitivity of this species group to environmental stressors, especially water quality (Welsh and Droege 2001). For example, Grant et al. (2005) noted that stream salamander abundance was negatively associated with stream acidification across multiple sites in the Shenandoah National Park. We did not document significant differences in pH, dissolved oxygen, or temperature between stream reaches upstream and downstream of ARD disturbance in our study, which suggests that water quality was impacted minimally by ARD disturbance at these sites. Both study sites were mitigated with limestone rock, which has likely attenuated stream pH fluctuations at the sites. Limestone rock additions and drain systems are effective at mitigating pH changes due to acid mine runoff and acid rock drainage (Cravotta and Trahan 1999). Future study efforts at these sites and/or other ARD sites should measure specific conductivity (in addition to other water quality measures) to better understand changes in ion concentrations due to disturbance and to evaluate effectiveness of mitigation measures. In addition, future studies should monitor streams directly after disturbance and for multiple years following disturbance to document the initial impacts and continued long-term changes.

It is essential to monitor streams for anthropogenic impacts to preserve the unique biodiversity present in the southeastern United States, particularly for changes in water quality, which may have negative effects on stream salamander assemblages. Our study provides a preliminary examination of the impacts of rock cuts and ARD on stream salamander assemblages in Middle Tennessee. As we only evaluated the impacts of ARD on salamanders at two sites in Tennessee, and we had limited sampling events at each site, our inference of ARD impacts is limited and should be evaluated on a larger scale (e.g., statewide) to better generalize impacts. Our pilot study data suggests that ARD impacts to stream salamanders were minimal at the two sites we monitored, however, our small sample size makes it difficult to draw larger conclusions. Future studies should include a larger allocation of sample sites at the regional level, multiple ecoregions, and include streams that have a variety of impacts from ARD discharge, including time since disturbance and whether mitigation procedures were implemented. Given the global scale declines of amphibian populations, a better understanding of the primary threats to this vertebrate group is necessary for long-term conservation and management. Alteration of aquatic habitats via anthropogenic land use change represents an acute threat for lotic-dependent amphibian species and continued monitoring is necessary to assess the impacts of these disturbances on stream-dependent organisms.

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A REVIEW OF THREAD-TRAILING DEVICES FOR EASTERN BOX TURTLES (*TERRAPENE CAROLINA CAROLINA*)

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Abstract. —Thread-trailing is a tracking technique used to monitor precise movements of turtles, but there are positive and negative aspects to using this method. Thread-trailing is inexpensive but can be labor-intensive, and there are often issues with the apparatus itself. We employed thread-trailing devices to track Eastern Box Turtles (*Terrapene carolina carolina*) in a suburban wetland habitat in middle Tennessee, USA. Unfortunately, we had limited success that was likely due to insufficient attachment and wet weather conditions at our field site. However, many researchers have used thread-trailing devices successfully, and this method is often used in conjunction with other tracking methods, such as radio-telemetry or mark-recapture. We discuss the pros and cons of thread-trailing based on our experiences with this method, compare thread-trailing to other common tracking methods, and make recommendations about the thread-trailing apparatus. This information will aid other researchers in determining if the thread-trailing technique is appropriate for monitoring turtles in their study, and if so, how to best construct the thread-trailing device.

Key Words.—mark-recapture, movement, radio-telemetry, thread-trailer, tracking

Tracking is a useful way to monitor turtles and gather important information on their ecology, such as movement patterns, home range size, and habitat usage. Several tracking techniques have been used to observe movements and estimate home range size of turtles, including thread-trailers, mark-recapture, and radio-telemetry, with each having distinct advantages and disadvantages. Thread-trailing is a common tracking method for box turtles (e.g., Stickel 1950; Legler 1960; Hallgren-Scaffidi 1986; Donaldson and Echternacht 2005) and is often used in conjunction with radio-telemetry (e.g., Donaldson and Echternacht 2005) or mark-recapture (e.g., Hallgren-Scaffidi 1986) because it provides more accurate measures of movement than radio-telemetry or mark-recapture by recording actual movement pathways (Iglay et al. 2006).

Breder (1927) was the first to use a thread-trailing device in hopes of more closely following turtles to observe their day-to-day behavior. Breder (1927) created this device to better understand homing instinct, travel routes, movement within the home range, migratory patterns, mating activity, nesting activity, use of water, and behavioral patterns, which are not always easy to observe with radio-telemetry or mark-recapture alone. Breder's (1927) device consisted of a spool of thread held by a wire that was wound through a hole cut in the turtles' marginal scute. Breder's (1927) device has been modified over time, with more modern thread-trailing devices consisting of a spool of thread housed in some type of container. The container is affixed to the turtle's shell, and as the turtle moves the thread unwinds and can be collected and measured.

While thread-trailing is very useful for obtaining detailed movement information, it is vulnerable to mishaps, such as thread breaking or running out prematurely, and it is labor-intensive (Breder 1927; Dodd 2001). For thread-trailing studies, turtles need to be checked daily to add additional thread and to ensure the apparatus is still properly attached, as well as to verify that the turtle has not become entangled.

Another tracking technique, mark-recapture (i.e., marking the individual with a unique identifier for future identification), is often used in home range studies because turtles are easy to mark for future identification, and it is inherently inexpensive (Cagle 1939; Dolbeer 1969; Stickel 1989; Dodd 2001). Although the marks are semi-permanent, they can nonetheless be lost if the periphery of the carapace wears or is chewed by a predator. In mark-recapture studies, researchers are not guaranteed to find an individual turtle multiple times to accurately estimate home range size or observe movement patterns. However, use of turtle-tracking dogs has proven to be a useful technique for increasing the number of recaptured turtles (Schwartz and Schwartz 1974; Kapfer et al. 2012). Kapfer et al. (2012) used wildlife detector dogs for two consecutive days in an 11 ha study area and captured 25 box turtles (three of which were recaptures on day two), while only 22 box turtles were found in 316.5 h of visual encounter surveys in the same 11 ha site.

Radio-telemetry is a useful and efficient tracking method that is normally less labor-intensive because it involves tracking that is dependent upon a set sampling protocol (e.g., monthly, weekly, bi-weekly, tri-weekly) rather than finding turtles every day, which is typically required for thread-trailer studies (Dodd 2001). Radio-telemetry also generally allows for continuous monitoring of individual movements over longer time-periods. However, radio-telemetry is not used for real-time tracking and therefore does not track exact movement pathways resulting in the loss of detailed movement information that is available when using thread-trailing. Additionally, radio-telemetry equipment (i.e., transmitters, receivers, antennae) is more expensive than equipment required for other tracking methods (Waddell et al. 2016). While radio-transmitters are normally smaller than thread-trailing devices, they may also fall off the turtle depending on attachment method, and the battery in the transmitter can malfunction or become depleted sooner than expected, potentially resulting in loss of the turtle.

If placed and monitored correctly, thread-trailers can be used to observe many aspects of turtle movement often in conjunction with another tracking method. For example, Hallgren-Scaffidi (1986) used thread-trailers to track box turtles and estimated a home range of 0.955 ha, but with mark-recapture data from repeated captures, the average home range estimate was only 0.733 ha. Iglay et al. (2006) compared thread-trailers and radio-transmitters and found that thread length from thread-trailers was significantly longer than straight-line distance obtained from GPS locations of radio-tracked turtles. Iglay et al. (2006) emphasized how thread-trailing may provide more accurate measure of turtle movements than radio-telemetry, which often underestimates movement patterns as it relies solely on point captures and straight-line distances rather than actual distances that can be acquired with thread-trailing. This is especially the case for researchers interested in capturing information on detailed movements of turtles, such as meandering movement patterns (Iglay et al. 2006). For example, Claussen et al. (1997) used thread-trailers to study detailed movement characteristics of Ornate Box Turtles (*Terrapene ornata ornata*), such as net displacement, mean turning angle, and sinuosity. Claussen et al. (1997) described how thread-trailing is an inexpensive and efficient method for studying more exact movements of turtles, but at times it is subjective (i.e., assuming that the thread is deposited in the exact pattern that the turtle moved when mapping the thread-trail path), and analyses are time-consuming. For a long-term study, checking on turtles with thread-trailers daily can be especially tedious, and only a few turtles can be tracked at a time (Stickel 1950; Jim Basinger pers. comm.). However, for short-term studies (e.g., Claussen et al. 1997 who tracked each turtle only 1–5 d), this method may be appropriate, especially in conjunction with other tracking methods. For instance, Marchand et al. (2004) also noted that thread-trailing more accurately measured actual distance traveled than radio-telemetry that only measured straight-line distance, whereas radio-telemetry was especially useful for estimating home range size and habitat usage. Marchand et al. (2004) used thread-trailers to monitor hourly movement of Eastern Box Turtles in a wetland habitat and found that turtles moved an average of 10.3 m per hour. While we did not measure the accuracy of thread-trailing compared to radio-telemetry or mark-recapture methods in this study, we concur that all three have inherent benefits and weaknesses.

The goal of this project was to use thread-trailers to track fine-scale movements of Eastern Box Turtle (*Terrapene carolina carolina*) movements in a suburban wetland habitat in middle Tennessee in order to better understand their movement ecology as it pertains to habitat usage. We describe our experiences with the thread-trailing device and the advantages and disadvantages of thread-trailers realized from their use in this study. We also compare the use of thread-trailers to other tracking methods, namely mark-recapture and radio-telemetry, and make general recommendations based on our experiences.

THE THREAD-TRAILING DEVICE

We attached thread-trailers to six adult Eastern Box Turtles found at Nickajack Trace and Black Fox Wetlands, Murfreesboro, TN, USA between April–June 2013. We utilized several different thread-trailer models that were similar to those described in Claussen et al. (1997) and Donaldson and Echternacht (2005). Thread-trailers weighed between 30–50 g and consisted of either a small plastic pill bottle or a small plastic film canister with a spool of sewing thread inside (Fig. 1). The spool of thread was either a spool of store-bought polyester thread or a wooden spool (Woodworks Ltd., Fort Worth, TX, USA) on which we spun nylon thread. The spool was suspended by a wooden dowel and housed within the plastic container, which had a small hole drilled in it to allow the thread to unwind. The ends of the dowel generally extended well beyond the edges of the plastic container; therefore the dowel was held in place by placing wooden caps on each end (see Fig. 1). All wooden components were stained to protect them from the elements. Making sure the shell was free of dirt and debris, we attached thread-trailers to the posterior region of the carapace

FIG. 1. Eastern Box Turtle (*Terrapene carolina carolina*) AJX with an attached thread-trailer made with a film canister (A); turtle AJX with an attached thread-trailer with the thread tied to a limb at the point of capture (B); and turtle BCJ with an attached thread-trailer made with a pill bottle (C). All thread-trailers pictured are held in place by epoxy glue and epoxy putty and painted black to reduce their conspicuousness.

using some combination of epoxy glue, epoxy putty, and/or duct tape to stabilize and hold it in place. If epoxy glue or putty was used, it was allowed to set overnight (i.e., >8 h) while the subject was housed in a small plastic container or cardboard box. For the last three turtles with thread-trailers attached, we painted the epoxy and parts of the apparatus black with fingernail polish to make it less conspicuous. We tied the end of the thread to an object (e.g., small tree or log) at the point of capture, used flagging tape to mark the area, recorded a GPS point (Garmin Etrex 30, Olathe, KS, USA), and released the turtle to move freely.

Results and Discussion

Most of the thread-trailers employed in this study only remained attached to the box turtle for one day or less. All five turtles which had a thread-trailer made of a pill bottle either ran out of thread or lost the thread-trailer within a day (Table 1). The single turtle which had a thread-trailer made from a film canister ran out of thread followed by the device falling off within a week. The average length of thread collected from the six turtles was only 11.6 ± 6.89 m (2.7–23.2 m). For the five box turtles whose thread-trailer device only remained attached for at most one day, one turtle moved 23.2 m and another only moved 2.7 m before losing the device. The single turtle who retained the thread-trailer for several days moved only 7.5 m before running out of thread. Although we attempted to employ small, compact thread-trailers, we were unable to obtain proper attachment or have enough thread.

Habitat Considerations and Recommendations

It is important to consider habitat conditions when choosing an appropriate tracking method. Thread-trailers may not work well for tracking precise movements in open areas where there is no possibility for the thread to "catch" on vegetation (Claussen et al. 1997). Furthermore, thread-trailers may not stay attached in exceptionally wet habitats, which is what we experienced. It is possible that our wetland site was simply too wet for the thread-trailer device that repeatedly fell off in the water or after a rain event even with tape, epoxy putty, and epoxy glue. We believe that at least three of the six turtles lost their thread-trailer primarily due to rain events (individuals HJK, BCV, and ABX). Turtle ABX most likely lost their thread-trailer due to a combination of weather conditions and entanglement because we found the thread-trailer amongst tree roots in the creek after a rain event. Stickel (1950) mentioned the importance of replacing tape periodically, especially after heavy rainfall. Jennings (2003) experienced loss of thread-trailers on juvenile Florida Box Turtles (*T. c. bauri*) due to humid and wet conditions in Florida. It is also necessary to wipe away any moisture from inside the thread-trailer each time you check on a turtle because, as Basinger (pers. comm. — http://boxturtle.dreamhosters.com/Thread%20 Trailing.html; http://archive.fo/y3B0o) noted, if the thread-trailer becomes wet, the thread may jam inside the apparatus, but cotton or polyester thread will generally break so a turtle does not become trapped. However, nylon thread will not break as easily and could lead to entanglement (Legler 1960). Interestingly, in nearly all thread-trailer studies, only tape (not glue or putty) was used to hold the thread-trailer on the shell (e.g., Stickel 1950, Claussen et al. 1997, Iglay et al. 2006). We are uncertain why in all cases duct tape, glue, and epoxy putty were not sufficient for securing thread-trailers on the shell for extended time frames, but we do feel that the weather conditions contributed to the problem. Perhaps the large size and rounded shape of the apparatus also inhibited secure attachment or maybe the pill bottle surface was too smooth or made of a harder plastic that the tape, glue, and putty could not adhere to.

TABLE 1. Tracking and demographic information for six adult Eastern Box Turtles (*Terrapene carolina carolina*) tracked with thread-trailers. The approximate length of thread represents the thread released before loss of the device, which was collected and measured.

Turtle Code	Tracking Start	Tracking Finish	Type of Thread Used	Attachment Method	Approximate Length of Thread (m)	Age Class (y)	Sex	Mass (g)
HJK	19 April 2013	20 April 2013	Polyester	Duct Tape	23.2	20+	F	390
BCV	10 May 2013	11 May 2013	Nylon	Duct Tape and Epoxy Glue	2.7	10–14	M	435
ABX	10 May 2013	11 May 2013	Nylon	Duct Tape and Epoxy Glue	12.6	10–14	F	350
AJX*	2 June 2013	8 June 2013	Nylon	Epoxy Glue and Epoxy Putty	7.5	10–14	M	365
AIW	10 June 2013	11 June 2013	Polyester	Epoxy Glue and Epoxy Putty	13.6	10–14	M	530
BCJ	30 June 2013	1 July 2013	Polyester	Epoxy Glue and Epoxy Putty	10.2	15–19	M	428

Notes: *Indicates the single turtle that ran out of thread but retained the thread-trailer device for up to one week. All other turtles lost their thread-trailer within one day. Turtle AJX was the only turtle that had a film canister thread-trailer rather than a pill bottle.

History of Thread-trailing

Over the years, several researchers have employed different versions of thread-trailers to find the most suitable model for studying box turtle movements (Table 2). The first model of the thread-trailer was attached by making a hole in a posterior marginal scute and running wire through the hole (Breder 1927). A spool of thread was held by the wire and dragged on the ground behind the turtle as it moved (Breder 1927). Breder (1927) found that this prototype simply did not contain enough thread and that the thread often broke. This model also did not provide any housing or protection for the spool of thread, so the apparatus was dragged through the substrate wherever the turtle moved (Breder 1927). Subsequently, Stickel (1950) attempted to use Breder's (1927) wired-spool thread-trailer, but after the device failed due to entanglement, she created a new model. Stickel's (1950) thread-trailer consisted of a spool of number 80 white thread (~503 m) contained within part of a tin can that was affixed to the turtles' shell with waterproof tape, eliminating the need for making holes in the scutes or for the spool to drag unprotected on the ground. Stickel (1950) custom cut each tin can to fit on the shell of each individual turtle and trimmed the inner core of each spool in order to add more thread. Stickel (1950) described the difficulties of the thread-trailer method due to the need to check on turtles daily and resupply the thread as it ran out, meaning that only a few individuals could be tracked at a time. Legler (1960) used Stickel's (1950) tin can model but used nylon thread instead of cotton thread. Legler (1960) also cut down the spool so it would hold ~550–730 m of thread. Hallgren-Scaffidi (1986) successfully used a device similar to Stickel's (1950). Schwartz and Schwartz (1974), Claussen et al. (1997), Donaldson and Echternacht (2005), and Iglay et al. (2006) all used a variant of Stickel's (1950) can-method by using a small, plastic 35-mm film canister to hold the thread and duct tape (and epoxy glue in the case of Donaldson and Echternacht 2005) to affix the apparatus to the shell. We most closely followed the methods of Schwartz and Schwartz (1974), Claussen et al. (1997), Donaldson and Echternacht (2005), and Iglay et al. (2006), but we were not as successful as others.

Apparatus Considerations and Recommendations

To properly construct and utilize a thread-trailer, it is important to consider all aspects of the apparatus as well as the overall goals of the study. Careful consideration should go into determining size and placement of the device as well as thread size (i.e., length and thickness), material, and color. Few published studies provide thorough details of the thread-trailer device and thread used; therefore we provide recommendations based on our experiences with thread-trailers.

We sought to minimize the overall size of the device by using small pill bottles and film canisters because we found that larger pill bottles were too bulky and too cumbersome for turtles even though they held more thread. We also realized that it would be beneficial to rethink placement and/or size of the thread-trailing device to prevent any interference with mating, especially for females (Iglay et al. 2006). Iglay et al. (2006) placed thread-trailers on the top of the shell on females, but care should be taken to ensure the thread-trailer is not protruding from the shell so much that it impedes movements when turtles go underneath objects or rest in their forms (i.e., shallow depression in the ground).

We recommend better preparing both the turtle shell and the thread-trailer for proper attachment. The shell should be washed thoroughly of any dirt and debris using alcohol or water and a cloth for cleansing. Also, an abrasive, such as sandpaper, could be used to roughen the surface of the plastic container and

TABLE 2. Description of various thread-trailer models used to study box turtle movements. The approximate length of thread indicates how much thread was on the spool used in each model.

Study	Species	Device used	Attachment Method	Weight of Device(s) (g)	Approximate Length of Thread (m)	Color and Type of Thread
Breder (1927)	*T. c. carolina*	Wired spool	Wire	30	229–274	cotton, basting
Stickel (1950)	*T. c. carolina*	Tin can	Waterproof tape	unknown	503	white, number 80
Legler (1960)	*T. o. ornata*	Tin can	Duct tape	unknown	550–730	nylon
Schwartz and Schwartz (1976)	*T. c. triunguis*	Film canister	Duct tape	unknown	unknown	white
Hallgren-Scaffidi (1986)	*T. c. carolina*	Tin can	Duct tape	unknown	320	white
Claussen et al. (1997)	*T. o. ornata*	Film canister	Duct tape	25	300	white, cotton
Jennings (2003)	*T. c. bauri*	Bobbin	Duct tape	<2	250	unknown
Marchand et al. (2004)	*T. c. carolina*	Bobbin	Unknown	<10	50	unknown
Donaldson and Echternacht (2005)	*T. c. carolina*	Film canister	Duct tape and epoxy glue	19 and 24	180 and 250	extra-strength cotton
Iglay et al. (2006)	*T. c. carolina*	Film canister	Duct tape	unknown	unknown	unknown
Basinger (unpub.)	*T. c. carolina*	Bobbin	Epoxy glue	unknown	205–250	white, polyester or cotton-poly mix
Dodd (unpub.)	*T. c. bauri*	Bobbin	Aquarium sealer	unknown	unknown	white

allow more surface area for the glue to set. Basinger (pers. comm.) drills holes in the bottom of plastic caps to allow glue to bead and solidify inside the holes. Because both the turtle's shell and the plastic containers had a rounded surface, there was little overall surface area where the glue came into contact with both the shell and the plastic container in our study. While epoxy putty helped with stabilization, perhaps using a plastic container with a flatter surface, such as Basinger's flat cap, would allow better attachment.

The thick nylon thread used in this study was stronger and more resistant than polyester or cotton thread, and the neon orange color was easy to spot against the leaf litter, although white cotton thread is often chosen (e.g., Stickel 1950; Claussen et al. 1997). Legler (1960) emphasized that turtles were unable to break nylon thread, and while cattle tangled the thread, they were also unable to break it. Therefore, it may be difficult for turtles to break nylon thread should they become caught or entangled. Consequently, if the researcher frequently finds entangled turtles, they should consider switching to a type of thread that breaks more readily than nylon.

It is also important to consider the length of thread and frequency of checking, knowing that less thread equates to checking on the turtle more often (i.e., at least once every day) or potentially losing the turtle. We predict that the thick nylon thread likely prevented us from winding enough thread on the spool, especially considering that turtle AJX's thread-trailer only contained 7.5 m of thread (Table 1). Interestingly, individual AJX was the only turtle to have a film canister thread-trailer (all others had pill bottle thread-trailers) and the only turtle to retain the thread-trailer more than one day. AJX's device remained attached even after the thread ran out. If we had been able to add more thread, it is possible that the film canister model may have been more successful. However, Turtle AJX's thread-trailer was subsequently found lying on the trail one week later, and we cannot say for certain that the thread-trailer was attached during that entire time frame. Turtle AJX's thread-trailer appeared to have been chewed, meaning a predator may have removed it from the turtle, or it may have been chewed after falling off the turtle. Turtle AJX was found again several times throughout the study, indicating that if it was a predator that removed the thread-trailer, it did not seem to harm the turtle. We realize that applying fingernail polish of an appropriate color on the whole apparatus may have been a better choice for making the thread-trailer blend in with the turtles' shell in order to prevent increased predation risk.

The bobbin thread-trailer method (e.g., Wilson 1994; Carter et al; 2000; Waddell et al. 2016; Fig. 2; Table 2) may be a solution to housing more thread in a smaller, less conspicuous apparatus. This device consists of a bobbin-type spool of thread that is more compact and often contains more thread than traditional spools of thread. Wilson (1994) encapsulated cocoon bobbins in plastic wrap and plastic dip, glued them to the shell, and successfully tracked Striped Mud Turtles (*Kinosternon baurii*) to their nesting sites. Wilson's (1994) bobbins weighed between 2.3 and 5.3 g and were between 4 and 5 cm long and 1 and 1.5 cm wide. Similarly, John Roe (pers. comm.) used heat-shrink tubing to encapsulate a thread bobbin (white with 1 km of thread), while leaving a small hole on one end for the thread to unwind. Roe (pers. comm.) used duct tape for attachment for short-term purposes but used epoxy glue for extended tracking of Eastern Long-necked Turtles (*Chelodina longicollis*) in Australia. Basinger (pers. comm.) used small, plastic make-up containers or bottle caps to house a

FIG. 2. An example of a bobbin thread-trailer attached to an adult Florida Box Turtle (*Terrapene carolina bauri*). This device is made of flexible plastic tubing and contains a cocoon bobbin of white thread. One end of the tubing is sealed with aquarium sealer, and the apparatus is attached to the shell with the same aquarium sealer. Photographed by C. Kenneth Dodd, Jr. and used with permission.

205-m bobbin, which was attached with 5-minute epoxy glue to Eastern Box Turtles. Kenneth Dodd (pers. comm.) used a small piece of flexible, plastic tubing to house a cocoon bobbin and aquarium sealer to seal one end of the tubing and to attach the apparatus to adult Florida Box Turtles (Fig. 2). Dodd (pers. comm.) experienced issues with thread breakage and device attachment and suggested that epoxy rather than aquarium sealer would be necessary for long-term use due to brushy habitat and rainy/humid weather conditions. Waddell et al. (2016) used a 500-m nylon thread cocoon bobbin held in an elastic harness around the carapace to successfully track movements of the Twist-neck Turtle (*Platemys platycephala*) and other wildlife in a wet tropical rainforest. Carter et al. (2000) used Wilson's (1994) bobbin method and attached thread-trailers with epoxy putty to Bog Turtles (*Glyptemys muhlenbergii*) that already had radio-transmitters. Carter et al. (2000) found that thread distances were 6.5 times longer than point distances. Jennings (2003) successfully used Wilson's (1994) bobbin technique to study the microhabitat of Florida Box Turtles with an emphasis on juvenile movement patterns (see also Hamilton 2000 and Jennings 2007). We think the cocoon bobbin technique may prove to be a more successful and efficient method for tracking box turtles as it is much smaller (e.g. 2 g versus 50 g) than traditional thread-trailers yet contains just as much, if not more, thread. Additionally, it is capable of providing more accurate data on movement patterns that may be overlooked with radio telemetry or mark-recapture alone (e.g., Carter et al. 2000)

Ultimately, it is often beneficial to use a combination of tracking methods to estimate home range and describe movement patterns of turtles, given the advantages and disadvantages to each method. Thread-trailers are economical and can be used to obtain more exact movement data, but the method is very time consuming, allows tracking of only a few individuals at a time, and there are often issues with the apparatus itself. Mark-recapture can also be used in conjunction with other tracking methodologies, but without a tracking device, the same individual turtles are not guaranteed to be found again for observing patterns of movement, and marks may wear over time. While radio-telemetry allows tracking of multiple turtles for a long time-period, it is more expensive than other methods, may suffer from attachment issues or device malfunction, and does not provide as much detail on movement patterns as thread-trailing. Therefore, radio-telemetry may underestimate true distance moved. We recommend experimenting with various thread-trailing models in order to minimize size of the apparatus while maximizing length of thread, which can be accomplished with the bobbin method. Additionally, as several others noted (e.g., Stickel 1950; Jennings 2003; Waddell et al. 2016), wet weather conditions can be problematic when using thread-trailers, and this should be taken into consideration when selecting a field site and tracking method. Future box turtle movement studies can utilize this information when choosing appropriate tracking methods for turtles at their particular field site and for designing thread-trailing devices if that is the chosen method.

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AMBYSTOMA OPACUM (Marbled Salamander). **COLORATION**. At 1100 h on 7 November 2007, I found one adult male *A. opacum* (57 mm snout-vent length) that lacked the normal color pattern for this species (Fig. 1). Adults typically have white or gray saddles that run the full length of the dorsal surface. Some specimens may lack crossbands and have two longitudinal stripes (Trauth and Richards 1988. Bull. Chicago Herpetol. Soc. 23:87); however, this specimen had no saddles on the dorsal surface of the body. It did, however, have numerous small flecks of gray color across the dorsum and extremely faint, nearly imperceptible saddles on the tail. I discovered this specimen in Big Cypress Tree State Park, Weakly County, Tennessee (36.199260 °N, 88.890351 °W; WGS84) and photographed and released it at the site of capture.

Several color variants have been reported for marbled salamanders. Albinism is the most widely documented. It has been noted to occur in larvae in Rhode Island, Connecticut, Maryland, Mississippi (Deegan et al. 1998. Herpetol. Rev. 29:229), and Illinois (Walston and Regester 2004. Herpetol. Rev. 35: 365) and in adults from Tennessee (Campbell 2011. Herpetol. Rev. 42: 80-81) and Illinois (Walston and Regester *op. cit.*). There are also reports of a hypomelanistic adult from Tennessee (Simpson and Wilson 2010. Herpetol. Rev. 41: 185-186) and leucistic larvae from Virginia (Mitchell and Church 2002. Banisteria 20:67-69). Two reports document hypermelanism (i.e. "melanoid mutants" per Richards and Nace 1983. Copeia 1983: 979-990). Connior (2013. Herpetol. Rev. 44: 114) discovered a variant in Arkansas that was characterized by a significant reduction, but not absence, of the white saddles; however, Simpson & Wilson (*op. cit.*) reported the first known specimens of a completely melanoid *A. opacum*. These were collected at the Volunteer Army Ammunition Plant in Hamilton Co., Tennessee. They found one adult male and two females. The male was completely black and lacked any trace of the white saddles; however, both females expressed the normal pattern, but the back pattern was faint and nearly undetectable. The specimen I discovered appears to be a variant like those reported by Simpson and Wilson (*op. cit.*); however, they did not report the presence of small flecks of gray on the dorsal surface. No photograph was published in their report for comparison.

To my knowledge, this is only the third report of a color variant for *A. opacum* in Tennessee and the first report of such an occurrence in West, Tennessee. Thus, color variants have now been reported in East (Hamilton Co.; melanoid – Simpson and Wilson 2010, *op. cit.*), Middle (Franklin Co.; albinism – Campbell 2011, *op. cit.*), and West (Weakley Co.; melanoid - this report) Tennessee. The presence of melanoid specimens in both East and West Tennessee indicates that such variants may exist at low frequencies across the full extent of this species' range within the state.

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FIG. 1. A melanoid adult marbled salamander (*Ambystoma opacum*) discovered on 7 November 2007 at Big Cypress Tree State Park, Weakley County, Tennessee. Photograph by Joshua M Hall.

LITHOBATES SYLVATICUS (Wood Frog). **DAVIAN BEHAVIOR.** Davian behavior, mating attempts by males with dead females, is a well-documented phenomenon observed in mammals, birds, amphibians, and reptiles (Costa et al. 2010. Herpetology Notes 3:79). Among anurans it has been reported in Bombinatoridae (Sinovas 2009. Herptological Review 40:199), Bufonidae (Miller 2018. Tennessee Journal of Herpetology 1:23), Ranidae (Pearl et al. 2005. American Midland Naturalist 154:126-134), and Hylidae (Bedoya et al. 2014. Herpetology Notes 7:515-516). Wood frogs are an explosive breeder that have been shown to exhibit Davian behavior with a heterospecific spotted salamander (*Ambystoma maculatum*) (Moldowan et al. 2013. Herpetological Review 44:296-297). *Rhinella proboscidea,* another explosive breeding anuran, has been shown to practice functional necrophilia in which males are able to squeeze oocytes out of a dead female and fertilize them. It is hypothesized that amplexus with a dead conspecific may provide an adaptive advantage and could select for more aggressive males in explosive breeding anurans (Izzo et al. 2012. Journal of Natural History 46:2961-2967). A literature search yielded no previous records of Davian behavior between conspecific wood frogs. On 12 February 2019 at 2030 h, we found an amplexed pair of wood frogs exhibiting Davian behavior in an explosive breeding area among three other conspecific amplexed pairs and a large collection of fertilized egg masses (Fig. 1). The breeding group was located in a large, roadside wetland in Roan Mountain State Park, Carter County, Tennessee, USA (36.169943°N 82.098942°W, WGS 84, 852 m elev.). The female was dead and bloated but the male appeared healthy. The female was fully submerged with the male being partially submerged. We did not observe any other dead conspecifics in the breeding area. The amplexing pair was observed for approximately 30 minutes with the male making no apparent movement or sounds. It is unknown when amplexus began or ended.

Submitted by: **TYLER D. WICKS** (e-mail: wickstd@etsu.edu) and **TREVOR L. CHAPMAN,** Department of Biological Sciences, East Tennessee State University, Johnson City, Tennessee 37614.

FIG. 1. A male Wood Frog (*Lithobates sylvaticus*) amplexed with a dead conspecific female on 12 February 2019 in a wetland, Roan Mountain State Park, Carter County, Tennessee.

CHELYDRA SERPENTINA (Common Snapping Turtle). **REPRODUCTION.** *Chelydra serpentina* is a wide-ranging species that occurs throughout much of central and eastern North America (Powell et al. 2016. Peterson Field Guide to Reptiles and Amphibians of Eastern and Central North America. 4th Ed. Houghton Mifflin Harcourt Publishing Company, New York, New York. 512 pp.). While this species has been extensively researched, there is a limited amount of literature on breeding behavior and copulation of wild turtles. Breeding season occurs from early spring throughout late summer (Niemiller et al. 2013. The Reptiles of Tennessee. University of Tennessee Press, Knoxville, Tennessee. 366 pp.). Schwab (1988, Catesbeiana 8(2):33) described copulation as a violent act. In both Schwab's (1988, *op. cit.*) and Hamilton's (1940, Copeia 1940:124-6) accounts, the male was observed on top of the female gripping her with his claws. He then forces the vent of his tail underneath the tail of the female so that their vents align and sperm is transferred to the female (Hamilton 1940, *op. cit.*). Snapping turtles have also been observed to mate plastron to plastron (Schwab 1988, *op. cit.*). Depending on the time of year that mating occurs, the female will either store sperm for delayed fertilization or use it to fertilize and lay eggs between mid-May and late June (Niemiller et al. 2013, *op. cit.*). Copulation has been reported twice in shallow water and in both instances the female attempted to escape but was thwarted by the male's advances (Hamilton 1940, *op. cit.*; Schwab 1988, *op. cit.*). The male bit and scratched the female while attempting to maintain a solid grip (Schwab 1988, *op. cit.*). The act lasted upwards of twenty minutes, leaving both animals exhausted (Schwab 1988, *op. cit.*).

On 4 April 2016 at 1851 h, a mating pair of common snapping turtles was found in a shallow muddy pool between Sugar Creek and the South Fork of the Forked Deer River in Chester Co., TN (35.432999, -88.625880). The pool was ca. 18.29 x 9.14 m and was ca. 0.76 m deep. It was partially in forest and partially in a strip cleared for power lines. The male grasped the female's carapace with all four feet (Fig. 1) and bit the head of the female, holding her head under water and then periodically allowed both animals to breathe. The male wrestled the female until they were both on their sides (Figs. 1, 2), and then until both were upside down. The male remained tightly attached to the female's carapace throughout these movements. The female seemed to submit, however she did make a few attempts to walk at which time the male would bite her head and hiss. The male's tail was wrapped around the female's tail (Fig. 2). The duration of the event was approximately eight minutes, ending with the male and female quickly departing the site in different directions. It is uncertain how long the two turtles had been breeding before they were discovered.

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FIG. 1. A male common snapping turtle, *Chelydra serpentina*, grasps a female's carapace tightly with his claws during copulation.

FIG. 2. Mating pair of *Chelydra serpentina* in copula. The male's tail has been wrapped firmly underneath the female in order to line up both individual's cloacae.

NERODIA SIPEDON (Northern Water Snake). **TERRESTRIAL FORAGING**. On 7 October 2017 at 1230 h, we discovered a juvenile Northern Water Snake (*N. sipedon*, approx. total length 25 cm) attempting to consume an adult Northern Gray-cheeked Salamander (*Plethodon montanus*, snout-vent length 3.5 cm). The interaction was observed 20 m from the south side of Birchfield Camp Lake on Rich Mountain, Unicoi County, TN (36.077292°N, 82.551618°W, elevation 1175 m). Although the location was submerged 4 months prior, the lake had retracted due to a breach below the earthen dam. The snake was initially positioned with its body exposed and its head underneath a small rock. Upon moving the rock, the snake was observed grasping the salamander around the torso with the dorsum of the salamander oriented upward (Fig. 1). T Chapman grabbed the snake as it attempted to escape, and the snake released the salamander after approximately 30 s. The salamander suffered numerous lacerations on the dorsum and autotomized its tail upon being released from the snake's mouth. Although the salamander survived the encounter due to observer intervention, it was discovered dead the following morning at 0900. Previous reports indicate that the preferred diet of *N. sipedon* consists of primarily fish (Bowen 2001. Herp. Rev. 32:264) and aquatic vertebrates (King 1993. J. Herpetol. 27:90-94). Researchers have described in *N. sipedon* a specialized aquatic foraging behavior where individuals will swim open-mouthed until they contact prey (Gillingham & Rush 1974. J. Herpetol. 8: 384-385). Predator-prey interactions between *N. sipedon* and caudates have been reported in larval Small-mouthed Salamanders (*Ambystoma texanum*) (McCallum 1995. Herp. Rev. 26:39) and Spring Salamanders (*Gyrinophilus*

FIG. 1. A juvenile Northern Water Snake (*N. sipedon*) attempting to consume an adult Northern Gray-cheeked Salamander (*P. montanus*).

porphyriticus) (Blackburn et al 2003. Herp. Rev. 17:61), adult Red Salamanders (*Pseudotriton ruber*) (T Chapman pers. obs.), and semi-aquatic adult *Desmognathus* spp. (Himes 2004. Herp. Rev. 35:123). To our knowledge, this is the first observation of *N. sipedon* attempting to consume a fully terrestrial adult of the genus *Plethodon*. We hypothesize that the unusual encounter resulted from the rapid retraction of a historic lake bed. This led to reduced aquatic foraging opportunities for *N. sipedon* and increased terrestrial habitat availability for *P. montanus*. Future research should investigate the extent to which extreme landscape alterations can influence predator foraging behavior and predator-prey relationships within overlapping communities.

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SISTRURUS MILIARIUS STRECKERI (Western Pygmy Rattlesnake). **REPRODUCTION.** Snakes have a wide diversity of reproductive strategies, which generates a large range of reproductive behaviors (Shine 2003 Proc. Roy. Soc. B 270:955–1004). Due to snake's secretive nature (Parker and Plummer 1987 in Seigel et al. Snakes: Ecology and Evolutionary Biology), observing these reproductive behaviors in the wild is extremely rare. With the aide of radio-telemetry, we can not only get a better understanding of snakes' movements, but also increase our chances of observing their reproductive behaviors. Through this note we report two courtship events and one copulation event observed while radio tracking tagged Western Pygmy Rattlesnakes in west-central Tennessee. We observed the first courtship event on September 26th, 2018 where we encountered one of the telemetered males in an open low elevation grassland field in contact with an unmarked female located underneath the male snake (Fig. 1). We restrained both individuals with snake tongs and acrylic tubes to record weight (g), total length (TL, cm), snout-vent length (SVL, cm), and sex. We also marked the female with a passive integrated transponder (PIT) tag (Biomark, Boise, ID, USA) prior to release. The female (weight = 73g, TL = 37cm, SVL = 33cm) was larger than the male (weight = 70g, TL = 43cm, SVL = 36cm). On September 28th, 2018 both individuals were again observed approximately 4 m from the original location. We observed the second courtship event on November 5th, where we encountered the same marked male from the September 26th and 28th observations basking on top of a different unmarked female (Fig. 2) at a location approximately 480 m north on a hillside within a stand of mixed deciduous forest. We did not record measurements of this female due to lack of equipment, however; we were able to confirm through photographic evidence and size difference that the snake was different than the female snake observed of September 26th and 28th and was also considerably larger than the male. In addition to the courtship events, we also observed copulation on October 4th, when we encountered one of the telemetered females (weight = 66g, TL = 41cm, SVL = 34cm) on a southern facing slope within a stand of deciduous forest with an unmarked male (Fig. 3). The snakes remained in copulation for the entirety of the observation (approximately 30 minutes) and were still connected when we left the observation site. We did not collect information on the male to not disturb copulation; however, the male was considerably larger than the female.

The Western Pygmy Rattlesnake is considered Threatened in Tennessee (TDEC 2016. A guide to the rare animals of Tennessee) due to its limited distribution, minimal reconfirmed records, and lack of physiology, ecology, and life-history data. Since 1950, there has only been ~30 confirmed sightings of this species in West-Central Tennessee (Scott and Redmond 2008. Atlas of Reptiles in Tennessee, http://www.apsubiology.org/tnreptileatlas/), and to our knowledge this is the first-time courtship and copulation has been observed and reported for this species in Tennessee. This indicates that even though Western Pygmy Rattlesnake populations in Tennessee may be small and scarcely distributed within its range in the state, Tennessee may hold functional (i.e., populations with recruitment of young) populations within suitable habitat areas.

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FIG. 1. Marked male *Sistrurus miliarius streckeri* (1) with an unmarked female of the same species underdeath it (2) in an open low elevation grassland field located in west-central Tennessee.

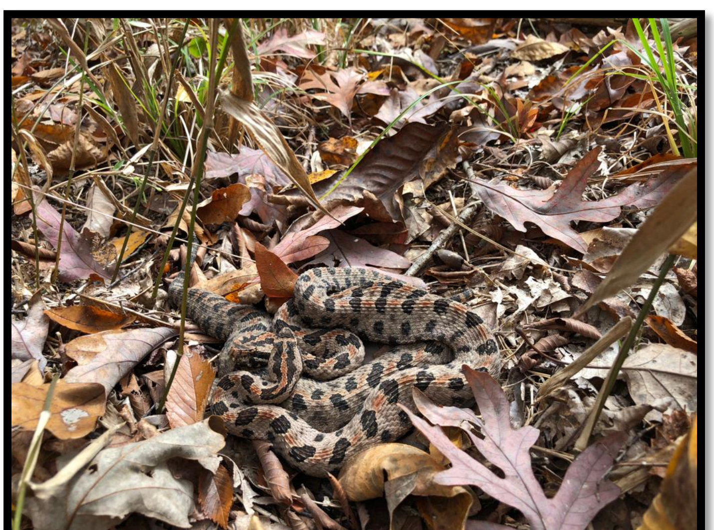
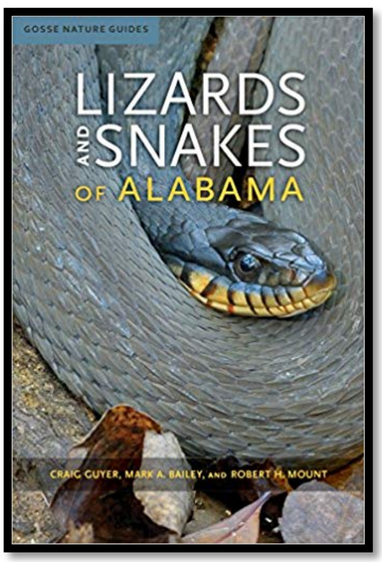


FIG. 2. Marked male *Sistrurus miliarius streckeri* with an unmarked female of the same species underdeath it on a hillside within a stand of mixed deciduous forest located in west-central Tennessee. The male in this picture is the same individual presented in Figure 1 with a different female.

FIG. 3. In situ copulation between unmarked male (2) and marked female (1) *Sistrurus miliarius streckeri*. The white circle indicates where both individuals are attached by their cloacae.



LIZARDS AND SNAKES OF ALABAMA

Craig Guyer, Mark A. Bailey, and Robert H. Mount. 2018. The University of Alabama Press, Tuscaloosa. xvi + 397 pp. Softcover, U.S. $39.73. ISBN 978-0-8173-5916-4

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Lizards and Snakes of Alabama is the second release of a four-volume set that describes Alabama's herpetofauna. These books serve as an update to the seminal work by Robert Mount: *Reptiles and Amphibians of Alabama* (1975). The first book, *Turtles of Alabama*, was released in October of 2015, the second book is described in this review, and two separate titles covering the frogs and salamanders of the state do not yet have release dates. Although this review is for a single book, *Lizards and Snakes of Alabama*, I will occasionally refer to this text along with its sister texts as "the Alabama field guides" because these books are written with similar style and structure. Thus, many of the strengths and weaknesses of one text apply to all.

The book begins with a thorough introduction that provides a list of all taxa native to the state, comments on introduced taxa, and recent taxonomic changes and problems. I use the term taxa, rather than species, because this guide recognizes subspecies. The lengthiest portion of the introduction is given to descriptions of the climate and geography of Alabama, its river basins, and the 10 physiographic units that comprise the state. These units were originally referred to as "herpetofaunal regions" by Robert Mount (1975). Unfortunately, the names of these units don't precisely correspond with the level III and level IV ecoregions defined by the Environmental Protection Agency and used nationwide. The authors do, however, describe each region, provide high-quality photographs to illustrate their characteristic habitat structure, and explain how they each influence the distribution and abundance of lizards and snakes across the state. Following the introduction are the species accounts.

The book is arranged in such a way that the taxonomic keys are interspersed through the text. These keys are easy to understand and include high-quality line drawings that highlight diagnostic morphological features when necessary. The species accounts begin with a dichotomous key to differentiate the major lineages of squamates in Alabama (Iguania, Gekkota, Scincomorpha, Anguimorpha, and Serpentes), and readers are directed to various pages pending the results of this key. For example, to key out a Pygmy Rattlesnake (*Sistrurus miliarius*), one would start on page 41 with the key to major lineages and then be directed to page 113: Serpentes (i.e. snakes). Pages 113-115 describe the group Serpentes and provide information about systematics, evolution, fossil specimens, general activity patterns, and natural history. Following this description is a dichotomous key to the families of snakes of Alabama. Via this key, the reader is directed to page 311: family Viperidae, which has a one-page, general description of vipers. Following is a key to the genera of Viperidae of Alabama, which leads to page 327: Pygmy Rattlers, genus *Sistrurus*. Here, a brief description of the genus is given along with notes on taxonomy. If there are multiple representatives of a genus in the state, a key would follow for these species; however, *Sistrurus miliarius* is the only species of this genus in Alabama. What does follow is a key to the subspecies that are recognized in the state: Dusky Pygmy Rattler (*Sistrurus miliarius barbouri*), Carolina Pygmy Rattler (*Sistrurus miliarius miliarius*), and Western Pygmy Rattler (*Sistrurus miliarius streckeri*). The authors not only recognize subspecies, they provide separate accounts for each. This contributes to the rather large size of the book (416 total pages) but also provides an opportunity for readers to learn a great deal about systematics and taxonomy. Each species/subspecies account includes at least one high-quality photo of a live specimen in the field, a physical description for the species/subspecies, and information on its distribution in Alabama, habits, conservation and management, and taxonomy. Such information is typical for field guides.

The most unique feature of the individual accounts is the distribution map, and this is my favorite feature of the Alabama field guides. Each map includes exact localities for verified species records, shaded regions indicating the assumed range for the species if it does not occur statewide, major river systems and topographic information, and boundaries between state counties. These maps are also quite large, taking up most of a page. Moreover, each map includes a small inset showing the broader distribution of the species in the United States along with the range of other subspecies, when applicable. I reviewed field guides of various states in the Southeast (Arkansas, Florida, Georgia, Kentucky, Louisiana, Missouri, North and South Carolina, Tennessee, Texas, Virginia, and West Virginia). None of these guides include all this information in their distribution maps. What is most important to consider, however, is that the Alabama field guides include an additional bit of information that is omitted from the distribution maps of every other field guide that I reviewed: ecoregional information. Each distribution map shows the locations of the 10 herpetofaunal regions that are described in the introduction, making it obvious to any reader how the distribution of each species is related to the geologic history and physiographic structure of the state. This unique feature of the distribution maps illustrates what I believe to be the greatest strength of the Alabama field guides: they do a great job of describing the herpetofauna of

Alabama within the context of the habitats in which they evolved.

Although the authors refer to the book as a field guide, its size (6 x 1.5 x 9 inches) and weight (2.6 pounds) would make it cumbersome to carry in a backpack for a long journey. This is particularly true when considering that one will eventually need four books of similar size to cover all the herpetofauna of Alabama. This, however, hardly makes the text unusual with respect to field guides for Southeastern U.S. states. I have guides for herpetofauna of Georgia, Arkansas, and Tennessee, and I wouldn't entertain carrying any of them into the field due to their size. For such adventures, nothing beats a Peterson guide. *Lizards and Snakes of Alabama*, however, is certainly robust enough to carry on field excursions if one desires. It is bound with a 'softcover'; however, this cover is thick and sturdy, and the text is printed on heavy, high quality gloss paper.

Overall, the book has all the qualities one could want in a field guide: it is easy to read, informative, accurate, relatively thorough, durable, useful, and the design is aesthetically pleasing. However, no book review would be complete without some criticism. I am not an expert, generally, on the herpetofauna of the Southeast U.S. and, thus, I cannot provide a detailed assessment of how accurate or thorough the accounts are for each species. I did, however, carefully review the accounts of species that I have studied professionally (e.g. *Norops sagrei*; *Anolis carolinensis*). The accounts are relatively thorough, for a field guide, and accurate. My primary criticism is that many of the literature references for natural history are outdated. For example, the authors twice describe the potential for competition between the native Green Anole (*A. carolinensis*) and the non-native Brown Anole (*N. sagrei*); however, they reference literature that is many decades old, even though more recent studies exist (e.g. Stuart et al. 2014). I also found one inaccuracy for the account of Brown Anoles: females are described as producing an egg "as rapidly as every twelve days or so". Reproduction for this species is much more rapid with eggs being produced as often as every four days (Fetters and McGlothlin 2017).

I don't particularly like the way the dichotomous keys are arranged (i.e. dispersed throughout the book). This requires a lot of page turning to properly key specimens. Moreover, the cover photos leave much to be desired. The front cover displays a Yellow-bellied Water Snake (*Nerodia erythrogaster*), one of the most drably adorned and common squamates in Alabama and in the Southeast, generally. Why not feature the rare and charismatic Eastern Indigo Snake (*Drymarchon couperi*) or a colorful Eastern Hog-nose (*Heterodon platirhinos*)? The back cover is even more puzzling. It displays a Brown Anole lizard (*N. sagrei*), which isn't even an Alabama native. Given the beauty and diversity of Alabama's squamates, this was a missed opportunity.

Despite the faults, few as they are, professionals and students who study Tennessee herpetology should seriously consider adding the Alabama field guides to their bookshelf. There is much overlap between Tennessee and Alabama with respect to herpetofauna. For example, of the 9 lizards and 34 snakes described in *The Reptiles of Tennessee* (Niemiller et al. 2013) all lizards and all but 2 snakes are described in *Lizards and Snakes of Alabama*. There are, however, an additional 3 lizards and 9 snake species described for Alabama. Thus, having a field guide to squamates of Alabama would provide additional information for lizards and snakes that occur in Tennessee and provide additional accounts for species native to the Southeast U.S. Furthermore, due to the recognition of subspecific status, the Alabama field guides include a wealth of information about taxonomy that may not be covered by *The Reptiles of Tennessee*. This equates to 20 additional subspecies accounts in *Lizards and Snakes of Alabama* (1 lizard and 19 snakes). Finally, Alabama shares 4 of the 8 level III ecoregions found in Tennessee: Southeastern Plains, Ridge and Valley, Southwestern Appalachians, and Interior Plateau. Within these regions, Alabama has habitats that represent most (78%) level IV ecoregions. These four major ecoregions are spread across Tennessee; thus, odds are, no matter where one studies herpetofauna in Tennessee, having an Alabama field guide will help to understand the geologic history of local habitats.

I spent the first 30 years of my life in Tennessee and sill consider it home; however, I now understand why folks refer to Alabama as "Alabama the Beautiful". The biodiversity of the state is breathtaking, and this is certainly true for its squamates. Serious students of Tennessee herpetology should not only add this field guide to their shelf; they should take some time to wander south and chase after Racerunners (*Aspidoscelis sexlineata*) and Florida Pinesnakes (*Pituophis melanoleucus mugitus*) through the upland longleaf pine habitats of Conecuh National Forest, comb the Red Hills in search of the unique and ancient Red Hills Salamander (*Phaeognathus hubrichti*), or go snorkeling around the Mobile Bay area to observe the enormous diversity of turtle species it supports. Having field guides to the herpetofauna of Alabama would certainly come in handy for such adventures.

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25th Annual Meeting of the Tennessee Herpetological Society

Gray Fossil Site, Gray, TN
September 26-27, 2019
Business Meeting Notes
Recorded by Dustin Thames

Award Recipients:
Congratulations to the 2019 recipients of the Chadwick Lewis Memorial Grant: Emilly Nolan and Amy Turpin. Emilly is a graduate student at Tennessee State University examining the cutaneous microbiome of Eastern Hellbenders before and after translocation. Amy is an undergraduate at Maryville College studying nest site selection in Bog Turtles.

The 2019 Niemiller Travel Award was given to Bradley Nissen from Tennessee State University for his work examining the effects of translocation on the spatial ecology of Eastern Hellbenders.

The Tennessee Valley Authority was recognized and presented an award for their outstanding commitment to amphibian conservation.

Conservation Committee:
Nothing new to report from the committee.

Chad Lewis Memorial Grant Committee:
Nothing new to report from the committee. They will be soliciting new applications for awards in early 2020.

Website Committee:
The committee successfully transferred the Tennessee Herpetological Society website to the Wix platform and merged it with the Tennessee Journal of Herpetology website (www.tnherpsociety.org). The committee was tasked with creating a format for species accounts on the new website and estimating a fair price per account to pay a student to transfer species accounts to the new website.

Publication/Newsletter Committee:
We continue to request new submissions. The Tennessee Journal of Herpetology may be found at:
https://www.tnherpsociety.org/tennessee-journal-of-herpetology

Treasurer's Report:
Members approved last year's report. The balance in the checking account is $14,060 and the Chadwick Lewis Memorial Grant investment balance is $13,710.

New Business:
Elections:
Vice President: Bill Sutton
Treasurer: Chris Ogle
Middle TN Representative: Matt Grisnik
East TN Representative: Laura Horton

2020 Annual Meeting of the Tennessee Herpetological Society
Tennessee Aquarium
Chattanooga, TN
We hope to see you there!