Eastern Box Turtle, Terrapene carolina, Neonate Overwintering Ecology on Long Island, New York

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**ABSTRACT.** – We studied 11 nests, and the resulting neonates of Eastern Box turtles (*Terrapene carolina*) in New York in 2001 and 2002, and documented neonate emergence and movements. None overwintered in the nest; instead, they emerged in the fall and overwintered buried shallowly close to the nest, where they were exposed to temperatures as low as -7°C. Further research is needed regarding neonate *T. carolina* behavior and the relative advantages of overwintering in the nest and overwintering terrestrially outside the nest.

**KEY WORDS.** – turtle; hatchling; *Terrapene*; overwinter

The Eastern Box Turtle (*Terrapene carolina*) is a widespread terrestrial species found in the eastern and central United States (reviewed by Dodd 2001; Ernst and Lovich 2009). The nesting ecology of *T. carolina* has been studied in several parts of the United States, and considerable information about its reproductive behavior is available (reviewed by Dodd 2001; Ernst and Lovich 2009). For example, unlike many other turtle species, copulations have been observed many times (e.g., Evans 1953), and clutch size across the range has been reported by numerous authors (reviewed by Dodd 2001). However, much less is known about the fate of *T. carolina* neonates (Forsythe et al. 2004).

Some high-latitude turtles overwinter in their nests, where they may be exposed to very cold temperatures and must survive, either through supercooling or freeze tolerance (Packard et al. 1999; Constanzo et al. 1995). *T. carolina* and the Ornate Box Turtle (*Terrapene ornata*) are the highest latitude terrestrial turtles in North America. Neonate *T. ornata* have moderate supercooling and freeze tolerance abilities (Costanzo et al. 2001; Dinkelacker et al. 2005), and adult *T. carolina* are freeze tolerant (Costanzo and Claussen 1990; Costanzo et al. 1993). However, freeze tolerance has not been tested in younger *T. carolina*. *Terrapene ornata* hatch in the fall of oviposition and, instead of emerging from the nest, burrow deeper into the soil, probably below the frost line (Constanzo et al. 1995; reviewed by Ultsch 2006). Much less is known about *T. carolina*, but, although some may overwinter in the nest (Ernst and Lovich 2009; Dodd 2001), others apparently emerge from the nest in the early fall after oviposition (Ernst and Lovich 2009) and presumably overwinter on land. However, no details of overwintering behavior have been reported in detail.

Because of this knowledge gap concerning emergent behavior, we investigated neonate behavior both immediately after nest emergence and over neonates’ first winter. Our results have both conservation and management implications, because determining where neonates live is vital to managing their habitat and predators.

**Methods.** — We studied *T. carolina* at the Wertheim National Wildlife Refuge, a 1032-ha preserve in Shirley, New York (lat 40°47’N, long 72°53’W). Wertheim National Wildlife Refuge is about 90 km east of New York City and is the southwestern portion of the Long Island Pine Barrens. There is a long history of *T. carolina* studies on Long Island (e.g., Latham 1916; Nichols 1917; Ditmars 1934; Nichols 1939a, 1939b; Madden 1975; Lee 2004; Burke and Capitano 2011), but only Madden (1975) reported data useful for comparison with this study. We actively searched for nesting females in 2001 and 2002.
We protected nests to prevent nest predation and checked them daily until emergence. Additional details are reported in Burke and Capitano (2011).

In 2001, we tracked the first 9 neonates (all those from the first 4 nests to emerge) by using fluorescent powder. We placed these neonates on the ground next to their nests and poured pink fluorescent powder in a circle around each neonate to track its movements (Butler and Graham 1993). As the neonates moved through the piles, the powder stuck to their plastrons and legs, which left trails as it fell off. We tracked the next 9 neonates (all those from the remaining nests) that emerged by gluing a 10-cm-long (0.35 g) red monofilament fishing line to the posterior edge of their carapaces. The line did not appear to interfere with hatchlings’ movements. We followed the powder trails with a hand-held ultraviolet light and replaced the powder every 24 hours by sprinkling the powder directly onto the posterior of the neonates and with a ultraviolet light. The daily locations and behavior of each neonate were observed until either the neonate could no longer be located or until it buried into the substrate and stayed in one place for more than 5 days. We did not track neonates in 2002.

We monitored overwintering temperatures indirectly by measuring ground temperatures immediately adjacent (within 5 cm) to each neonate by using Tidbit Stowaway Temperature Loggers buried at the same depth as the hatchlings. We examined the logger records to determine whether the neonates were exposed to potential freezing episodes (Costanzo et al. 1995), during which the temperature to which these neonates were exposed dropped below $-0.55\, ^\circ\text{C}$, the approximate equilibrium freezing point of turtle tissue. We also examined the temperature records for evidence of repeated freezing and thawing, and for evidence that temperatures dropped below $-5\, ^\circ\text{C}$, which is the minimum temperature at which neonate T. ornata are able to supercool and avoid freezing (Costanzo et al. 2001). We assumed that the body temperatures of the neonates were similar to those of the temperatures of loggers of similar mass (approximately 8 g), because Claussen et al. (1991) found that soil temperatures closely matched body temperatures of nearby overwintering adult T. carolina.

Statistical comparisons were made with 2-tailed $t$-tests, with tests considered significant at $p \leq 0.05$. We used the day of emergence of the first neonate from each nest as the measure of emergence date, so each nest was counted only once in analyses to avoid pseudoreplication.

**Results.** — In 2001, we protected 6 nests; 18 hatchlings emerged 11–22 September, one egg partially hatched but died before fully emerging from the eggshell. In 2002, we protected 5 nests; 2 eggs were infertile, but at least one hatchling emerged from each nest, and 14 hatchlings emerged from 22 August to 3 September. Egg viability is reported in Burke and Capitano (2011). We monitored emergence instead of hatching because hatching had occurred underground. All emergent neonates had completely absorbed their yolk sacs, which indicated that they had spent some time underground after hatching and before emerging. All neonates that emerged from each nest emerged on the same day. In 2001, the mean time from oviposition to emergence was 86.2 days ($n = 6$; range, 84–90), in 2002, this was 66.8 days ($n = 5$; range, 61–76). The difference in time to emergence between 2001 and 2002 was significant (2-tailed $t = 6.87\, ;\, \text{df} = 5\, ;\, p = 0.001$), and these times to emergence also were significantly less than reported by Madden (1975) for his combined 1969–1971 data ($n = 8$ nests, fall emergents only) from his study 42 km west of our study site (2001: 2-tailed $t = 2.20\, ;\, \text{df} = 11\, ;\, p < 0.001$; and 2002: 2-tailed $t = 2.36\, ;\, \text{df} = 7\, ;\, p < 0.001$).

We were more successful in tracking neonates by using monofilament line than fluorescent powder. Unlike the fluorescent powder, the line was not affected by rain or disturbed by other animals. We lost all 9 neonates tracked with fluorescent powder within 1 week of emergence. Three of the 9 neonates tracked with monofilament line also were lost. We found 2 more of the monofilament lines unattached to neonates; presumably the glue became unattached. The remaining 4 neonates moved only short distances from their nests in the fall after emergence (mean distance from nest, 3.4 m; range, 1.5–10.0 m). Three of these neonates buried themselves completely into the soil substrate (on 22 October, 29 October, and 15 November, 2001). These refugia were very similar to those used by adults (Carpenter 1957; Stickel 1989; Claussen et al. 1991) except that the neonates were completely covered. The fourth neonate remained on the soil surface and did not bury itself until 26 December and then its carapace was only 50% covered. Although snowfall was relatively light in winter 2001–2002, all 4 neonates were covered with snow several times over the winter; we did not disturb or uncover them. None of them moved from their refugia until 19 March, 25 March, and 1 April 2002, when the 3 more fully covered neonates emerged from their refugia. They appeared healthy and were no longer tracked. The last neonate to bury itself emerged from its refuge 1 April 2002; however, we found it dead of unknown causes the next day.

The temperature loggers on the soil surface and adjacent to the 4 neonates recorded minimum temperatures of $-13.0\, ^\circ\text{C}$ on 1 January, 11 February, and 13 February, 2001. The temperature loggers buried adjacent to and at the same depth as the neonates reached minimum temperatures of $-7\, ^\circ\text{C}$ for 3 of the neonates (including the neonate that died in April) and $-2\, ^\circ\text{C}$ for one neonate. We detected numerous potential freezing episodes that lasted 2–3 hours, and one episode that lasted 26 hours, during which the temperature to which neonates were exposed dropped below $-0.55\, ^\circ\text{C}$, the approximate equilibrium freezing point of turtle tissues. Local climate data for Shirley, New York (Weather Underground), showed that the average daily temperatures for
July and August 2002 (22.92 ± 3.4°C SD) were significantly higher than for the same period in 2001 (21.27 ± 3.3°C SD) (t = 1.996; df = 93; p = 0.012).

Discussion. — All the neonates from 11 nests (2001 and 2002) emerged by mid-September, thus, overwintering in the nest was not observed. This is in contrast to Madden (1975), who studied a T. carolina population 42 km west of our study site in similar habitat and found that neonates emerged in the fall in 5 nests and in the spring in 2 nests, and, in 1 nest, 5 neonates emerged in the fall and 2 emerged the following spring. Kipp (2003) did not observe overwintering in the nest in the T. carolina nests she monitored in Maryland. Clearly, overwintering behavior is flexible in this species.

Incubation duration is negatively correlated with incubation temperature in T. carolina (Dodge et al. 1978). We could not determine incubation duration, because we did not dig up nests until after emergence, and Madden (1975) showed that neonates may remain in the nest for days after hatching. However, the time from oviposition to emergence varied between the years we studied, being significantly shorter in the warmer summer. Both were significantly less than the average of 100.5 days (Madden 1975) for combined 1969–1971 data (t = 2.31; df = 8; p < 0.001). Thus emergence times were shorter in 2002 due to both later oviposition dates and earlier emergence dates.

We found that monofilament line made an inexpensive and effective method of tracking neonates and was more successful than florescent powder. It never tangled in vegetation and was visible with the ultraviolet light from several meters away. This technique may be useful in other studies of turtle neonates if relevant predators are nocturnal and/or lack color vision and may even work well in water, which florescent powder does not.

Madden (1975) radio-tracked (by using 1.5-g transmitters, 20% hatchling body mass) 5 fall-emerging neonate T. carolina in 1969 from a nest near our study site; 2 were quickly predated, 1 was lost, and 2 became inactive after 17–20 days. One neonate stayed in the same field as the nest and survived to emerge in May. It appears the neonates he tracked moved much farther than those we tracked. Madden (1975) predicted that initial neonate movements were limited to those necessary to finding overwintering refugia; this could explain the difference in our results.

Costanzo et al. (2001) found that turtle neonates of species that overwinter on land have lower rates of evaporative water loss than those that overwinter in water and that desiccation resistance may be an important component of winter survival. The shallow burrows we observed being used by T. carolina neonates may serve 2 functions, both to reduce temperature stress and minimize water loss. In fact, the one neonate that failed to cover itself completely over the winter did not experience colder temperatures than those that did survive, despite being much more exposed. Its cause of death may have been associated with desiccation.

Ernst and Lovich (2009) and Madden (1975) reported that juvenile T. carolina both become inactive earlier in the fall and emerge from hibernation later in the spring than adults, but we found that neonate T. carolina entered and emerged from refugia about the same time of year as did adults (Capitano 2005; Claussen et al. 1991; Klemens 1993). The minimum temperatures we recorded at the substrate surface were as much as 5°C colder than those recorded by temperature loggers buried only a few centimeters deep, which indicates that even slight burial can buffer neonate turtles from temperature extremes. Nests may be even better protection, Madden (1975) measured temperatures in 2 nests with overwintering neonates and found minimum temperatures were −1°C. Snow cover also may be a good insulator (Breitenbach et al. 1984), however, the relatively low levels of snowfall during winter 2001–2002 probably had little effect on neonate survivorship. Claussen et al. (1991) and Ernst and Lovich (2009) found that adult T. carolina buried themselves deeper underground as the winter progresses, but the neonates we observed stayed near the surface.

Although our temperature loggers recorded at the same depth and adjacent to the neonates’ winter refugia recorded temperatures as low as −7°C, we cannot determine whether the neonates froze or supercooled. Which of these 2 possibilities occurs in any particular case depends on a complex interaction of temperature profiles, availability of freezing inoculants, morphology, and behavior (Packard et al. 1999). Neonate T. carolina are unable to close their shells, and their exposed skin, nares, eyes mouths, cloacas, umbilici, and wounds may provide a location for initiation of ice formation, easily leading to whole-body freezing (Costanzo et al. 1991).

Although it is not known whether T. carolina neonates are freeze tolerant, it is reasonable to suspect that this species has a high level of freeze tolerance, based on its overwintering ecology (Dinkelaker et al. 2005; data reported here) and the fact that adults are freeze tolerant (Costanzo and Claussen 1990; Costanzo et al. 1993). If T. carolina are like T. ornata in this respect, then very likely the neonates we tracked froze. Costanzo et al. (1995) classified neonate turtles in cold climates as either 1) typically overwinter in water, 2) typically overwinter deep underground, or 3) typically overwinter shallowly underground. Species in this third category must be extremely cold adapted. Given that T.
Terrapene carolina neonates similarly overwinter on land but even closer to the surface than in their nests, we predict that they have a similar degree of cold adaptiveness.

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