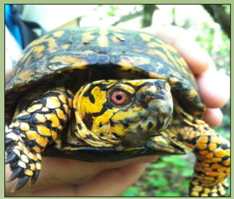
Preliminary analysis of Microhabitat use of Eastern Box Turtles (*Terrapene carolina carolina*) in northeastern Georgia.



N.L. Hyslop, and J.L. Mook

Department of Biology, University of North Georgia

Introduction

Understanding organismal use of specific resources at multiple spatial and temporal scales is vital to conservation and restoration efforts. Patterns of use may reflect arrangement of necessary landscape components including forage, mates, shelter, and potential influence of invasive species. These resources often vary spatiotemporally, especially for terrestrial ectothermic species in climatic zones with marked seasonal differences. To examine this resource use, we investigated habitat use in Eastern Box Turtles (EBT, *Terrapene carolina carolina*), a long-lived primarily terrestrial species, native to the eastern United States that has experienced population declines throughout its native range largely due to habitat loss and alterations and collection for the pet trade[1,2,3]. Despite the species' status, little long-term research has been conducted on EBT habitat use in the Piedmont region of the Southeastern United States.

Documented habitat use for EBT primarily includes upland forested and other non-aquatic habitats, with some use of streams, ponds, and wetlands[1,2,4,5,6,7,8,9]. Although broad-scale habitat use has been documented in the species, microhabitat use has not been reported frequently[10,11], nor in the north Georgia Piedmont region. Within this region, Chinese privet (*Ligustrum sinense*) is a prevalent invasive evergreen under and midstory species. The presence of invasive species is a leading cause of species extinctions globally[12], and *L. sinense* has been shown to reduce native biodiversity by displacing native vegetation and altering trophic interactions[12]. Changes to habitat conditions could negatively affect *T. carolina* persistence by interfering with food supply, thermoregulation, and cover; however, insufficient research has been conducted investigating the effects of invasive plant species on herpetofauna[14]. To further our understanding of the ecology of *T. carolina*, including the impact of *L. sinense* on the species, we conducted a long-term investigation into the habitat use of the species in the north Georgia Piedmont region.

Methods

We analyzed active season and overwintering habitat use of an EBT population monitored using radiotelemetry from 2013-2022. Our study site covered approximately 45 ha and was surrounded largely by urban development and multiple active construction areas (Fig. 1). Habitats at our site included upland areas (about 75% of study site) dominated by native hardwood-pine mixed uplands, as well as areas with canopies dominated by either privet or a privet-pine mix (primarily loblolly pine, *Pinus taeda*). Aquatic habitats (about 25% of the total site) at our study site included freshwater forested/shrub wetlands created by beaver activity along a permanent waterway in addition to multiple, unconnected seepage wetland areas dominated by either privet or native vegetation.

We opportunistically captured and marked individual EBT at our study site between 2013-2022. All captured turtles were sexed, weighed, measured, and marked with a unique notch code filed into the marginal scutes[15]. We attached radiotransmitters to the carapace (RI-2B, Holohil Systems Ltd.) using non-heating epoxy, and turtles were tracked via homing[16] 0.5-2 times per month using R-1000 telemetry receivers (Communications Specialists, Inc.). Upon locating the individual, GPS coordinates (Garmin, Etrex-20) and habitat data were taken.

We measured microhabitat vegetation and substrate composition in a 1.5m diameter circular plot around turtle locations. Within the plot, we identified plot coverage every 10cm in each cardinal direction. Understory vegetation was identified as either herbaceous, woody, or privet. Substrate composition was identified as bare ground, leaf litter, coarse woody debris (> 7 cm diameter), or water. We additionally recorded total overstory (<150 cm in height) vegetation cover and basal area. We defined active season as the time between exiting and entering brumation and differed by individual and year. In our study, of the individuals with ≥10 microhabitat plots, 58% of the turtles entered brumation by the end of November, with the remainder entering brumation by mid-December. Turtles emerged in the spring in March (24%) and April (76%). Overwintering microhabitat data were collected in the fall and winter months at the first overwintering location we detected (defined as the earliest fall or winter radiolocation where the turtle was not seen above ground again until the following spring). We assumed that the turtles only chose their overwintering location once, upon first entering brumation, and therefore we only use the microhabitat data from this first location. For all analyses, we analyzed data in Excel and ArcGIS Pro (ESRI. 2011), retaining the individual as the sampling unit.

Results

We collected microhabitat data on 46 individual turtles (26 M; 20 F) between 2013-2022 resulting in 1,806 microhabitat plots ($\bar{x}$ = 39 plots per turtle; range: 1-131) for turtles tracked between 2-93 months ($\bar{x}$ = 35 months). Active season microhabitat data was analyzed for turtles that had ≥10 novel microhabitat plots recorded (*n* = 33 turtles; 17 M and 16 F) resulting in 1,471 individual microhabitat plots ($\bar{x}$ = 43 plots per turtle; range: 18-107) between 2013-2022. Active season microhabitat plots were dominated, on average, by leaf litter (59%) and understory vegetation (33%; Table 1) and we did not detect monthly or sex differences in use of any plot variable.

All turtles tacked during the active season had at least one plot that contained privet ($\bar{x}$ = 6.5 plots; range 1-42), with between 1-31% total microhabitat plot coverage (Fig. 2). In privet dominated macrohabitats, average privet coverage at microhabitat plots was 20% (6.1% 95% CI) versus 5.4% (2.7% 95% CI) in plots in macrohabitats not dominated by privet.

We collected overwintering microhabitat data on 37 individual turtles (22 M; 15 F) for 8 overwintering periods between 2013-2022 (2016-2017 fall/winter was not included), resulting in 114 overwintering microhabitat plots (n = 7-27 turtles per year; $\bar{x}$ = 14). Overwintering microhabitat plots contained similar amounts of privet, woody debris, and basal area to active season use (Table 1), but percentage of bare ground dropped from 4% to 0% and leaf litter increased from 59% to 84% average plot coverage (Table 1).

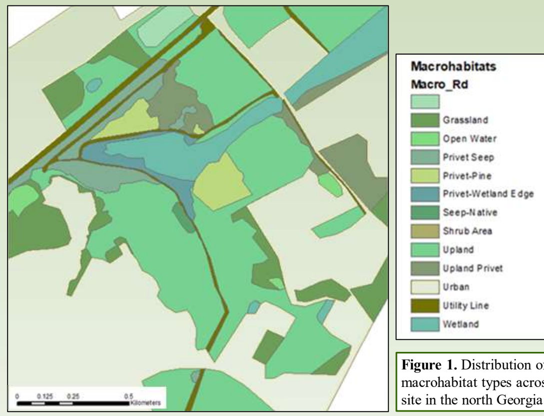


Figure 1. Distribution of macrohabitat types across our study site in the north Georgia Piedmont.

Table 1. Seasonal microhabitat characteristics associated with Eastern Box Turtle radiolocations in northeastern Georgia. Values are non-transformed proportions of cover in a 1.5-m diameter circular plot centered on turtle locations. Basal area was collected from a single point at the center of each plot. Sample size is number of individual turtles (retained as sampling unit in analysis). No differences in use between sexes was detected for any variable.

	Active Seasons			Overwintering		
Variable	**Average**	**95% CI**	**n**	**Average**	**95% CI**	**n**
Canopy	0.59	0.43-0.76	46	0.44	0.37-0.50	28
Understory Vegetation (all)	0.33	0.3-0.36	33	0.12	0.09-0.16	28
Privet	0.12	0.09-0.14	33	0.07	0.04-0.11	28
Woody vegetation	0.08	0.06-0.10	33	0.06	0.02-0.1	28
Herbaceous vegetation	0.13	0.09-0.19	33	0.02	0.0-0.4	28
Woody debris	0.04	0.03-0.05	33	0.04	0.02-0.052	28
Bare ground	0.04	0.01-0.04	33	0	-	28
Leaf litter	0.59	0.56-0.62	33	0.84	0.8-0.88	28
Basal area (ft^2/acre)	79.2	76.3-89.7	46	108.1	97.3-119.0	28

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Discussion

Over the nine years of microhabitat use data collected in this study, sampled plots around turtle locations in active season were dominated by leaf litter and understory vegetation (~92%), with the remaining comprised of woody debris, bare ground, and standing water. Microhabitat plot coverage in the active season was similar to that recorded by Rossell et al.[10], with the exception of overall understory vegetation coverage, which averaged almost twice that found in the previous study. As others have also found[10,17], we did not detect differences in microhabitat use between males and females in the active season nor during overwintering.

Overwintering plot data was generally collected in late fall and values therefore reflect the natural changes in habitats at our site as deciduous vegetation dies back during that period. Therefore, changes in use for habitat variables between active and overwintering seasons may be an artifact of changes in availability rather than in selection (Table 1). At our site, leaf litter depth varied seasonally, but the availability of areas with leaf litter did not; however, reductions in ground vegetation coverage may explain the increase in leaf litter use during overwintering.

Turtle use of areas with high amounts of privet was directly correlated with overall macrohabitat composition. Given that use of areas with privet coverage was directly corelated with macrohabitat type, our data does not support turtle selection of privet specifically, but likely of opportunistic use based on its availability. This study provides important long-term baseline data for microhabitat use in our region and illustrates the variability in microhabitat use of this species in our area.

Figure 2. Example microhabitat use in our study: a) bottomland with privet canopy; b) utility line; c) native upland forested area; d) native seepage; e) understory privet; f) bottomland in dry season.

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