THE SPATIAL ECOLOGY OF THE EASTERN BOX TURTLE (*TERRAPENE CAROLINA CAROLINA*) IN A FRAGMENTED LANDSCAPE IN SOUTHEAST TENNESSEE, HAMILTON COUNTY

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ABSTRACT

The landscape throughout the range of the Eastern Box turtle (*Terrapene carolina carolina*) has been altered significantly since the late 1880s by habitat fragmentation. In this study, the spatial ecology and seasonal movement patterns of the Eastern Box turtle in contrasting habitat types are investigated. Eastern Box turtles had home ranges that averaged (mean = 3.77 ± 2.82 ha, minimum convex polygon) in size among individuals. There was no significant difference between mean annual home range size and movement patterns between males and females; however, home range size for male turtles was larger during the summer versus spring activity season ($P = 0.02$). At the landscape level, turtles used eastern deciduous forest and successional habitats and they selected sites with ample ground and canopy cover. These findings can be used to better conserve the habitats and populations of the Eastern Box turtle.

DEDICATION

This work is dedicated to all my friends and family who have shown support and patience throughout this project. Thank you all!

ACKNOWLEDGEMENTS

I am extremely grateful to every individual that helped me with data collection and data analysis. I would like to thank Dr. Thomas Wilson for his support and endless amount of patience throughout this experience. Thank you for believing in my ability to complete this project. I also would like to thank my committee members, Dr. Brad Reynolds and Dr. David Aborn.

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I would like to thank my mother, Connie Hixson, for her encouragement and support throughout my graduate career. I thank the Tennessee Herpetological Society and Dr. Thomas Wilson for funding this research. All research was conducted in accordance with Tennessee Wildlife Resources Agency permit #3082, and the University of Tennessee at Chattanooga's Institutional Animal Care and Use Committee (IACUC 13-04). Lastly, I would like to thank my wife, Kari Dillard, for always supporting me and never once complaining when I was away from home and collecting field data.

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CHAPTER 1

INTRODUCTION

Spatial Ecology

Recent research suggests that reptiles are declining worldwide due to habitat alteration and anthropogenic impacts related to the pet trade (Budischak et al. 2006). The increase in habitat isolation or fragmentation can affect the persistence of individuals and populations of animals (Morrison et al. 1992). Habitat alteration can have serious impacts on how animals utilize and move within the landscape, particularly a species that may utilize interior portions of the habitat more than the edge portion. In addition, urban landscapes are typically altered and this determines the availability of habitats, and ultimately, what resources and habitats are accessible to animals (Morrison et al. 1992). Habitat fragmentation can alter the quality of habitat and reduce important resources needed for population viability (Morrison et al. 1992).

Eastern box turtles (*Terrapene carolina carolina*) use different habitats at different times throughout the year (Stickel 1950 and Congello 1978). Reagan (1974) suggested that during the spring they are found in habitats with an open canopy allowing turtles to bask and thermoregulate during cooler temperatures that are common during the spring season. Conversely, as the temperatures increase during the summer season, they move into mesophytic forests where canopy cover increases (Doroff and Keith 1990). Additionally, mesophytic forests are critical for providing overwintering habitats for turtles (Madden 1975). Fragmentation can also change microclimates by altering temperatures and moisture conditions, forcing certain species to increase movement patterns to find suitable habitat and resources needed for survival (Morrison et al. 1992). In turn, this can also increase exploitation as animals come in contact with humans on a more frequent basis. Seasonal habitat use and movement for box turtles is poorly understood and there is much to learn about this species to aid in conservation and management efforts.

The study of animal movements is essential in ecological research. Many factors may influence the home range size of the Eastern Box turtle. For example, microclimate temperature (Dodd 2001), food availability (Compton et al., 2002), and habitat structure (Roe and Georges, 2008) can influence the movement patterns and home-range size and terrestrial activity in a semi-aquatic turtle. Home ranges of box turtles are usually associated with several resources such as food, suitable overwintering habitat, thermoregulation sites, and mates (Vitt and Caldwell 2009). Habitat diversity can also influence the movement patterns of box turtles (Stickel 1989). As hypothesized by Stickel, turtles living in areas of high vegetation diversity, with high habitat quality and structure, have smaller home ranges and do not have to move as much to find resources as they would if the habitat was less diverse or productive (Stickel 1989).

The home range of the eastern box turtle tends to remain constant over time for both males and females, although there may be shifts in habitat use from one activity season to the next (Stickel, 1950, 1989; Yahner, 1974; Madden, 1975; Strang, 1983). Yahner (1974) captured individual turtles in the same locations that they were found in five years prior. Male and female home ranges generally are similar in size but can vary by terrain and topology (Dodd 2001). In some studies, males have a larger home range; in other studies, females have larger home ranges than males (Dodd 2001). Stickel (1950) suggested that turtles with small home-range sizes tended to reside in high quality habitats and did not have to travel far to obtain resources. Similarly, Schoener (1981) suggests that this behavior occurs in squamate species as well.

Although it is unknown if Eastern Box Turtles are currently showing signs of serious decline in the southeast region of Tennessee, obtaining spatial ecology data on movements of box turtles in this region can be extremely valuable due to the impacts of several anthropogenic factors that are presently ongoing and those that could possibly occur in the future. This project can provide much needed insight as to how turtles use this fragmented landscape and can provide an effective planning tool to possibly protect core habitats. The objective of this study is to gain a better understanding of the spatial ecology and seasonal movement patterns of the box turtle in contrasting habitat types. I also examined habitat use in radio-tagged box turtles. Because habitat use can vary in fragmented landscapes, I tested for selection at the landscape scale and microhabitat scale. Specifically, I addressed the following questions: 1) Do general movement patterns vary among turtles within a fragmented landscape; 2) Does home range size and habitat use differ between male and female turtles; and, 3) Are biophysical structures within the microhabitat, such as forest canopy gaps and open canopy habitats preferentially used compared to shaded forest locations?

Study Organism

According to Buhlman et al. (2008), there are four subspecies of the common box turtle found throughout the southeast United States including the Eastern Box turtle (*Terrapene carolina carolina*), Florida Box turtle (*Terrapene carolina bauri*), Gulf Coast Box turtle (*Terrapene carolina major*), and Three-toed Box turtle (*Terrapene carolina triungus*). The Eastern Box turtle exhibits the greatest geographic range among all of these turtle subpecies (Buhlman et al. 2008). The Eastern Box turtle ranges from extreme southern Maine to southern Georgia and then westward to the Great Lakes region and central Illinois (Ernst and Lovich 2009). Two subspecies are recognized in Tennessee: Eastern Box Turtle and Three-toed Box Turtle (*Terrapene carolina triunguis*). The three-toed box turtle is found only in the extreme southwestern portion of the state and is not known to overlap range with Eastern Box turtle (Buhlman et al. 2008), while the Eastern Box turtle occurs throughout the rest of Tennessee (Conant and Collins 1998). Due to reported population declines throughout its range, the eastern box turtle has been listed as “vulnerable” by the International Union for the Conservation of Nature and Natural Resources (IUCN; van Dijk, 2010). According to the IUCN, the status of “vulnerable” implies that a taxon is not critically endangered or endangered but is at a high risk of extinction in the wild in the short-term future. This particular status classification of “vulnerable” is most often associated with habitat loss and destruction (IUCN; van Dijk, 2010). Although this semi-aquatic turtle is common across the state of Tennessee, previous research suggests that populations are declining across its range (Donaldson and Echternacht 2005)

The Eastern Box turtle is a moderate-sized turtle, typically reaching 165 mm in carapace length (Dodd 2001). The most visible morphological feature of the box turtle is the bony, box shaped shell allowing the turtle to adopt a wide variety of defensive behaviors to adapt to their environments (Buhlman et al. 2008). Its shell is covered by a thin layer of a keratinized substance and protects the underlying bone. All box turtles have a bilobed, hinged plastron that allows it to close its shell almost completely and provides protection from meso predators (Buhlman et al. 2008). The bones in a box turtle's shell are coalesced, which provides added protection from predators (Dodd 2001). Adult box turtles are sexually dimorphic where the irises are brown and red for females and males, respectively (Buhlman et al. 2008). Conversely, the plastron of males tends to be concave while the female is flat to convex (Dodd 2001).

The Eastern Box turtle matures between 8 to 10 years of age, but males can mature as early as 6 years of age (Ernst et al. 1994). In a study in eastern Tennessee, Dolbeer (1971) observed courtship and mating twelve times during an activity season; with eight of the observations being in September. Nesting behavior seems to be unpredictable and may vary based on seasonal conditions. Eastern Box turtles typically nest from early May to the middle of July, depending on environmental conditions and microclimates (Dodd 2001). Clutch size varies from one to seven eggs. Eastern Box turtles like other similar species have environmental sex determination of the progeny. Ernst et al. (1994) reported that an internal nest temperature of 22.5-27.0°C clutches produce a predominance of males and those incubated at 28.5°C produced almost all females.

Eastern Box turtles are predominately denizens of mesic hardwood forests, and there is no single habitat affinity specific to Eastern Box turtles soley (Ernst et al. 1994). Even though Eastern Box turtles can be found in a wide range of habitats, they appear to select habitats based on thermoregulatory needs in an effort to minimize water loss and avoid critical thermal maximum temperatures (Fredericksen 2014). Additionally, it is well known that the Eastern Box turtle will utilize aquatic habitats during periods of hot weather or drought (Donaldson and Echternacht 2005). Spatial ecology studies that encompass patterns of habitat selection are critical to understanding the life-history and ecology of species and can elucidate patterns of survivorship, reproduction, and population viability (Flitz and Mullin 2006). Environmental variables such as temperature, humidity, and biophysical structure may play an important role in the selection of habitats (Reagan 1974). Habitat fragmentation and other anthropogenic impacts may increase mortality of turtles (Fredericksen 2014).

Conservation Status

Box turtle habitat has been encroached upon over the last several decades; therefore, as a consequence populations are thought to have declined throughout its range (Donaldson and Echternacht 2005). Stickel (1978) reported a reduction in box turtle population size of 50% over a 30 year period at a site in Maryland. Habitat loss due to urban sprawl, industrial development, and overcollecting for the pet trade are thought to be the primary factors leading to these population declines (Dodd 2001). Road mortality is another potentially important factor affecting box turtle populations as the traffic volumes on road networks is expanding (Gibbs and Shriver 2002). Due to these aforementioned factors, all North American box turtles are currently listed on CITES (Convention on International Trade in Endangered Species) Appendix II, which aids in regulating international trade (Donaldson and Echternacht 2005).

Box turtles are a long-lived organism; therefore, it may be difficult to detect population declines that are not immediately evident. Other box turtle life history characteristics, such as delayed sexual maturity and low juvenile and egg survivorship, may put them at an increased risk and may not allow populations to recover quickly (Klemens 2000). Additionally, few nests go undetected by predators as Flitz and Mullin (2006) reported nest depredation rates of 87.5% within the first 72 hours of eggs being deposited in fragmented landscapes. Juvenile survivorship and low adult mortality is critical to preserving stable populations (Gibbons and Avery 1990, Hall et al. 1999, Klemens 2000).

Methods & Materials

Study Site

This study was conducted on study site LT7 located in Hamilton County, Tennessee, approximately 19 km northeast of Chattanooga (35°02’44.4978”N, 85°18’34.866”W; Figure 1). This site differs in vegetation cover and infrastructure throughout the property (Figure 2; 3). The study site is bisected by a powerline right-of-way (ROW) that divides the study area into northern and southern sections.

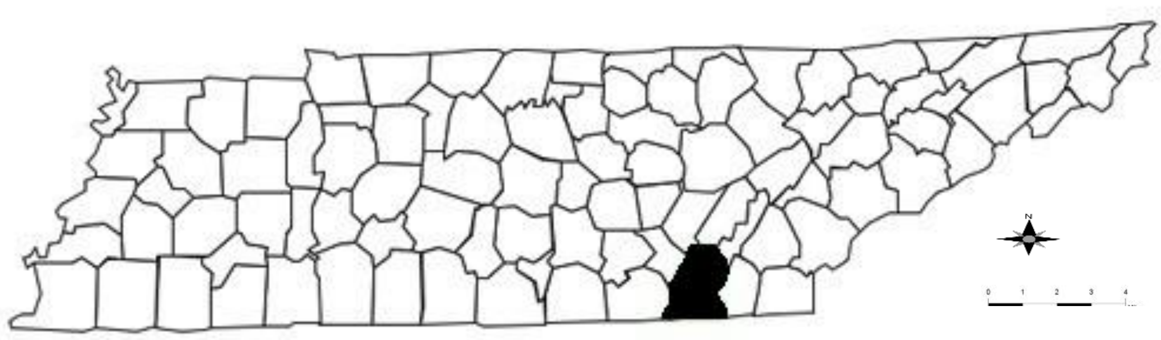

Figure 1 Tennessee county map: the study site is located in Hamilton County (highlighted in black) and is situated in southeast Tennessee

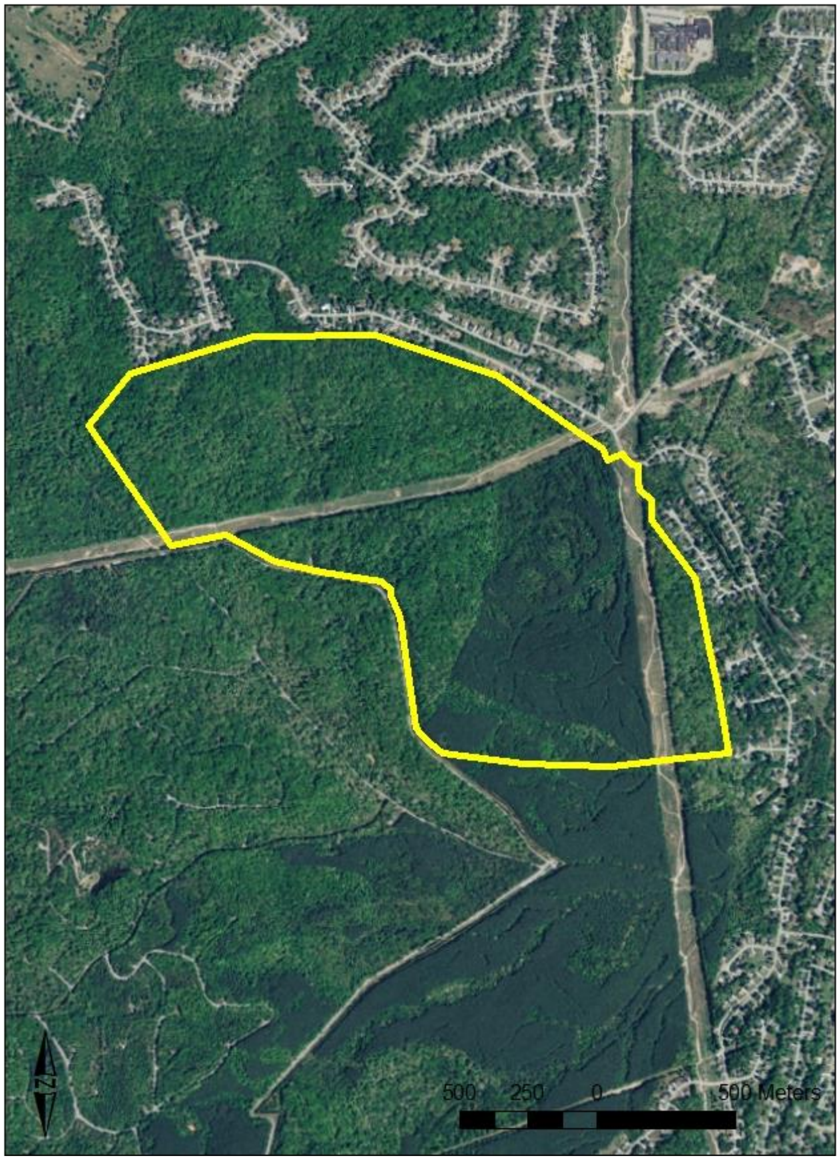

Figure 2 Aerial photo of the study site created using ArcGIS. Note the dense housing development to the north and northeast of the study site

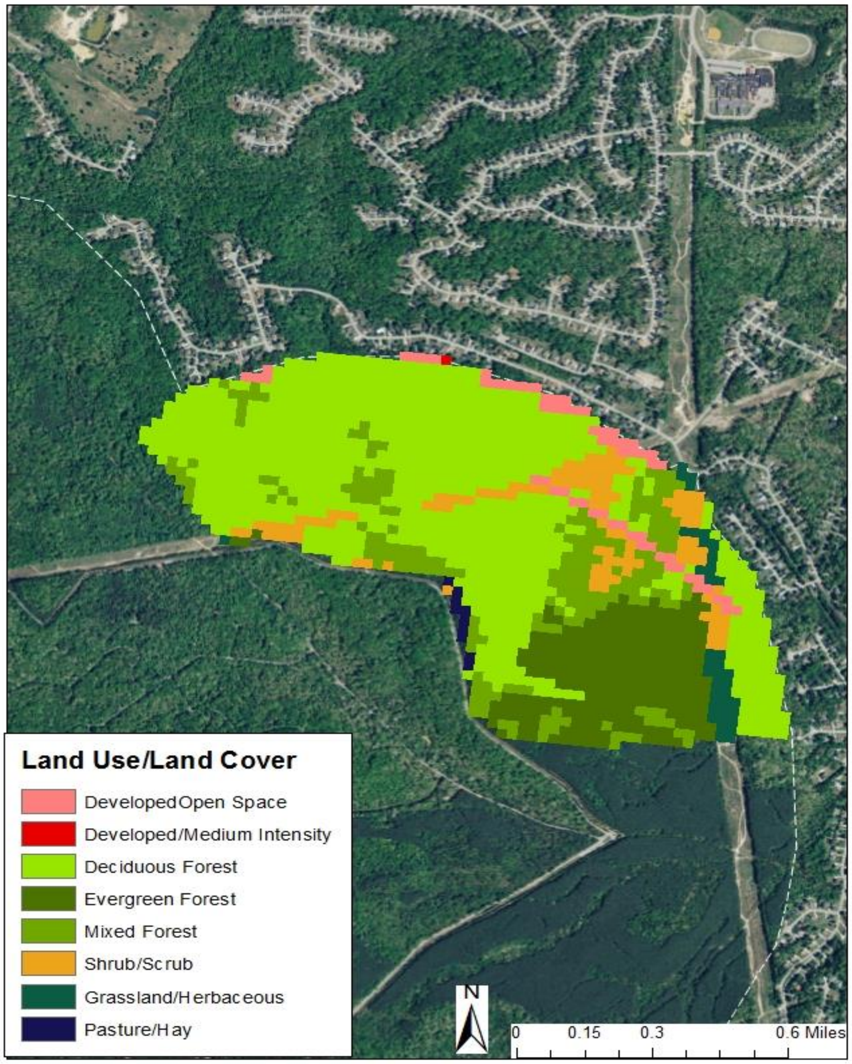

Figure 3 Study site land classification map according to National Land Cover Database 2011

The northern section of the study site is 88 ha of upland deciduous forest containing few areas of secondary growth and the dominant vegetation cover consisting of oak (*Quercus*), hickory (*Carya*), and sourwood (*Oxydendrum*) species. Hamilton County government (TN) owns and manages this parcel. The use of all terrain vehicles on this portion of the study site is similar to that on the southern site. This portion of the study site is bordered by subdivisions to the north and east. Horse riding trails are being planned which may lead to further fragmentation within this habitat and introduce invasive species even though members of the university and community are opposed to such actions. Additionally, there is significant industrial development due to the presence of Amazon and Volkswagen to the southwest of the study site.

The southern section (LT7) of the study site consists of 98 ha and is owned by The University of Tennessee at Chattanooga and is utilized by faculty and students as a biological field station. This area consists of large tracts of planted pine forests (*Pinus strobus*) with shrub thickets of Chinese privet (*Ligustrum sinense*), sumac (*Rhus glabra*), Carolina buckthorn (*Rhamnus caroliniana*), and muscadine (*Muscadinia rotundifolia*) with hickory *sp*. (Carya) interspersed occurring in the understory. A small tract of upland deciduous forest is also located within the property and totals 19 ha. The upland deciduous forest parcel is dominated by oak (*Quercus*), hickory (*Carya*), and sassafras (*Sassafras*) species. A ROW parallels this portion of the study area and lies to the west. This ROW has a high degree of human disturbance and is used frequently by people trespassing with off-road vehicles (ORV). All types of ORV trails are found throughout the study site but do not seem to be used as often as the trails located within the areas of ROW. The vegetation occupying these ROWs consist of early successional species such as broomsedges (*Andropogon*), mountain mint (*Pycnanthemum*), various *Aster* species, and bramble (*Rubus L*). The Enterprise South Nature Park lies to the south of the study site and has a service road that is used by park visitors and personnel. A chain link perimeter fence separates the study site from the service road and adjacent park.

Radiotelemetry and Data Collection

Radio telemetry was utilized to investigate the home range sizes, daily movement patterns, and habitat preferences of Eastern Box turtles across the landscape. Visual encounter surveys were conducted from August 2013 to October 2013. This method along with the use of wildlife detection dogs was employed throughout this study in an effort to locate turtles that would be deemed suitable for being tagged with a radio transmitter. These wildlife dogs were utilized to improve capture rate efficiency and increase sample size for the project.

Upon finding a turtle that was suitable for radio tagging, morphological measurements such as carapace length, width, shell height, pectoral scute width, anterior plastron length and width, and posterior plastron length and width were measured straight line with dial calipers to the nearest 0.1 mm. Suitability was determined by assessing the overall health and body mass of the turtle as adult box turtles can carry the additional weight of the transmitter package without inhibiting movement patterns versus juvenile box turtles. Total body mass was also measured using Pesola spring scales to the nearest gram (g). The gender of each turtle was determined using secondary sex characteristics of the turtle, such as iris color, plastron concavity, and tail morphology. If these characteristics were unable to be determined, the turtle was not affixed with a transmitter and released at its original capture site. A file was used to notch the marginal scutes by providing a unique identification number for each turtle (Cagle, 1939).

Transmitters were affixed to the rear carapace, towards the right side to avoid interference with copulation (Figure 4). Each turtle was fitted with a 12 g transmitter package (L.L. Electronics Mahomet, IL) that never exceeded 6% of the mass of any turtle. The transmitters were affixed to the carapace of the turtle using PC-11 epoxy. The adhesive was allowed to dry for 20-30 minutes before releasing the turtle at its original capture location. A total of 17 adult box turtles (6F and 11M) were affixed with a radio transmitter and tracked across the study site from August 2013 to November 2015. Attempts were made to radio track equal numbers of male and female turtles for this study but captures were biased towards males.

Figure 4 Transmitters were attached on the right side of the carapace, towards the rear of the animal

Each turtle was tracked 2-3 times per week during the activity season (April - October) and bi-monthly during the winter (November – March). When possible, tracking times were conducted on an alternating schedule (mornings and afternoons) to reduce potential bias of temporal effects (White and Garrot 1990). Turtles were tracked using a FLS-200 receiver (L.L. Electronics Mahomet, IL) and a three-element folding Yagi antenna (AF Electronics, White Heath, IL). Each time a turtle was tracked, GPS coordinates were recorded of the location in Universal Transverse Mercator (NAD83 map datum). This data was then exported into ESRI® ArcMap™ 10.3 for analysis using a Garmin Venture HC handheld GPS unit (Garmin

International, Inc., Olathe, KS). The total distance moved from the last known relocation was also recorded straight line distance. To measure the straight line distance moved between each relocation, I used a Leica Rangemaster CRF 1000-R Laser Rangefinder (Leica Camera AG, Wetzlar, Germany). Barometric pressure, cloud cover, carapace temperature, substrate temperature, air temperature, wind chill, relative humidity, heat index, dew point, and wind speed was measured at each radiotelemetric observation. Both carapace temperature and substrate temperature were measured directly at the turtle's location using an Extech 42540 hand-held infrared digital thermometer (FLIR Commercial Systems Inc., Nashua, NH) with a basic accuracy of ± 2°C. All other abiotic measurements were taken using a Kestrel Meter 3000 (Kestrel Meters, Birmingham, MI) handheld unit and all measurements were taken at or near the turtle to allow for a more accurate assessment.

Biophysical habitat measurements were also taken at each radiotelemetric observation including: maximum vegetation height, the most dominant species of vegetation cover present, spherical densitometer readings to estimate overstory density, and woody debris was estimated. Habitat was measured both at the turtle's location and at a nearby random location, based on a random bearing/degree and a random distance ranging from 1-100 m. This allowed resource use versus availability to be measured simultaneously elucidating a more comprehensive understanding of habitat selection via paired logistic regression (Compton et al. 2002).

Of the 17 turtles radio-tagged, 11 were used in the analysis. Six turtles were excluded from statistical analysis as they did not contain enough data to meet the assumptions of statistical tests.

Data Analysis

Home Range Size and Movement

Using the GPS coordinates collected at each relocation, I estimated both 100% minimum convex polygons (MCP) for all radio-tagged turtles. The 100% MCP was used to capture the maximum area used by each turtle, while 95% and 50% kernel density estimates (KDEs) were used to estimate core activity areas used by turtles that excluded outliers. To address potential biases caused by variation in the duration of the monitoring period and number of independent observations per individual, incremental area analysis (IAA) was used to evaluate increases in home-range size by the number of observations for each individual (Dreslik et al. 2003). If the number of relocations asymptoted, the sample size was large enough to sufficiently estimate home range size (Dreslik et al. 2003).

Linear regression was used to determine whether home range size varied with turtle body size (body mass) or carapace length. When comparing seasonal activity among turtles, I defined spring as 20 March to 20 June, summer as 21 June to 22 September, and fall/winter as 23 September to 19 March. Fall and winter seasons were combined due to no turtle activity or movements during the winter season. I compared MCP home range size by gender and season by using a two-way analysis of variance (two-way ANOVA) (Miller et al. 2012). To satisfy the assumptions for conducting this parametric statistical test, the data were natural-log transformed to first test for normality of the dependent variable, identify any outliers, and conduct a Levene's test of equality of error variances across groups. Any spatial outlier locations were used to examine gender and season effects on home range size as these may be biologically important to turtles; therefore, I used 100% MCP data analysis (Miller et al. 2012). I also tested for significant differences between the home range size of male and female turtles using a Mann-Whitney *U* test (Wasko and Sasa 2009). Because of small sample sizes and unequal variances, nonparametric statistics were used to assess movement and home range size data. Mean straight-line distance was calculated for each turtle to determine home range size and general movement characteristics (Strang 1983). If the turtle did not move between two subsequent radio tracking events, data for that relocation was not included in movement analysis. The significance level for statistical tests was set at $\alpha = 0.05$. All statistical analysis were conducted in Microsoft Excel 2007 (Microsoft Corporation, Washington, D.C.), SPSS 23.0 (SPSS Inc., Chicago, IL, USA), and SAS 2016 (Statistical Analysis Software, Cary, NC, USA).

Habitat Use and Selection

Land cover analysis was conducted with ArcGIS and ArcMap 10.3.1 (2016) to elucidate habitat selection by box turtles. All turtle locations were imported into ArcMap with 2011 National Land Cover Dataset (NLCD 30 meter resolution) to examine turtle use of the multiple macrohabitat classes within the study site. I recorded habitat use at two scales: (1) landscape level habitat type and (2) microhabitat within the preferred habitat type at each turtle relocation (Jennings 2007). I used compositional analysis to examine habitat selection at the landscape scale and tested for non-random use of habitats (Aebischer et al. 1993). Compositional analysis classifies habitat type by the proportion of habitats used by an animal relative to the proportion of habitats available (Miller et al. 2012). The land cover classes used in this study included developed/ open space, developed/medium intensity, deciduous forest, evergreen forest, mixed forest, shrub/scrub, grassland/herbaceous, and pasture/hay. Developed/medium intensity consisted of roads and subdivisions. Shrub/scrub and pasture/hay included ROW areas that contain primarily early successional species. Proportions of land cover types available were quantified with aerial photographs using ArcMap and through ground-truthing. The most abundant cover type was deciduous forest, comprising 55% of the study site. The proportions of the remaining macrohabitat types were as follows: evergreen forest (15%), mixed forest (14%), shrub/scrub (12%), and hay/pasture (.01%). The two remaining macrohabitats that turtles seldom used during this study were developed/open space and developed/low intensity (.03%). To examine composition analysis, I calculated the proportion of all the independent turtle locations (n = 447) in each of the habitats using ArcMap 10.3.1 to determine utilized habitat to available habitat types within the study area. To determine potentially distinct preferences for habitat utilization versus habitat availability, I used a Fisher's exact test for all turtles (Miller et al. 2012). I used 100% MCP home ranges to determine potential habitat preferences. Although this method may include habitat types that are not used by turtles, it provides a more comprehensive comparison between males and females; and, it is more useful in development of adaptive conservation and management strategies (Kapfer et al. 2013).

I used a chi-square analysis to determine habitat use differences between male and female turtles (Greenspan et al. 2015; Sharpe 2015). If a statistical significance was detected in any of the tests, this significance was further tested by calculating residuals to determine in which cell the source of the significance can be found (Sharpe, 2015). A residual analysis identifies those specific cells making the greatest contribution to the chi-square test result (Sharpe 2015).

In addition to the aforementioned habitat analyses, I also examined edge habitat utilization by all turtles by ground-truthing and creating a point layer using a Trimble R1 GNSS

(Trimble, Sunnyvale, CA) receiver that defined the edge boundary in all shrub/scrub ROW habitats throughout the study area and created a 5 m buffer extending out from the forest edge. A 5 m buffer was also created extending from the forest edge into the interior portion of the forest (Currylow 2011). This point layer was imported into ArcGIS and each turtle's 100% MCP was examined to determine how many turtle relocations occurred within this edge buffer zone.

Habitat features potentially important to box turtles such as feeding and thermoregulation were also measured at the local scale by measuring canopy cover using a spherical densitometer that averaged the four cardinal readings taken at the turtles location. Additionally, other microhabitat measurements were recorded, including percent vegetation cover of woody debris, minerals (exposed individual rock), and herbaceous plant species that were present at the turtle's location within the 1 m^2 plot (Eberhardt and Thomas 1991). The 1m^2 polyvinyl chloride (PVC) frame was placed on the forest floor at the turtle's location with the body of the turtle positioned in the middle of the frame. These habitat features were measured at both the turtle's location and a randomly chosen location. I only recorded location data for random locations if the turtle moved to a new location to ensure independent sampling among all turtles (Compton et al. 2002).

Cover preferences were assessed for each turtle using each unique turtle location and comparing it to random locations (Neu et al. 1974) using use – availability. Cover types were defined as groundcover *sp*. (Muscadine, *Vitis rotundifolia* and Honeysuckle, *Lonicera periclymenum*, Catbrier, *Smilax glauca*), forbs *sp*. (Carolina buckthorn, *Rhamnus caroliniana*, blackberry, *Rubus* spp.), hardwood *sp*. (*Quercus* spp. saplings), grasses (predominantly broomsedge and bluestem, *Andropogon* spp.), successional vegetation *sp*. (*Aster* spp., *Pycnanthemum* spp.), and woody debris (naturally fallen debris). Chi-square statistics were used to test which cover types were selected for comparing turtle locations versus random locations (Greenspan et al. 2015).

To compare microhabitat selection with habitat availability, using known turtle locations compared to random locations within the defined study area, I used AIC (Akaike Information Criterion; Akaike, 1973). This information-theoretic approach assesses the relative importance of the variables included in the model statement and is selected using a stepwise testing procedure (Burnham and Anderson 2002). This statistical test is more powerful than standard logistic regression for analyzing paired data (Breslow and Day 1980, Hosmer and Lemeshow 1989). Following an initial global model analysis, seven candidate models were selected based on scoring criteria. Models with the lowest Akaike score and highest Akaike weight (w), which determines the relative likelihood of the model and the probability of that model being the best among the candidate models (Burnham and Anderson 2002). When selecting variables to include in the models, specific species were chosen rather than broad cover types to elucidate whether turtles tend to select a specific vegetation species while occupying a particular habitat (Dragon 2014).

Results

Home Range Size and Movement

Eleven eastern box turtles (8 males and 3 females) were radio tracked from 236 to 833 days and acquired a total of 526 telemetry fixes (average = 47.8 fixes/turtle). Individuals ranged in size from 213.0 to 550.0 g body mass, with a mean size of 361.55 g (SE 29.98 g; Table 1). Additionally, carapace length (CL) varied among turtles and ranged in size from 99.8 to 141.0 mm, with a mean size of 122.06 mm (SE 3.49 mm; Table 1). Incremental area analysis (IAA) results indicate that most MCP home ranges reached an asymptote (Appendix B) as most home range areas stabilized after an average of 15 temporally and spatially locations. However, two turtles (2L and 1R8L) may have been under sampled as they did not reach an asymptote with area observation curves slightly climbing at the conclusion of the study, although, both of these turtles were included in the data analysis as both turtles had more than 15 independent locations. One of the eleven turtles moved outside the study site boundary but not a great distance as data collection continued and recorded for the spatial ecology of this turtle. While outside the study area boundary, the turtle was located on four radio-tracking events in shrub/scrub habitat similar to that found within the study area. Additionally, the turtle was only slightly outside the study area boundary and would not significantly affect habitat availability or proportion available; therefore, this data was included in the analysis.

Home range size varied but showed some degree of overlap between individuals (Figure 5); however, no relationship was found between body mass or carapace length of turtles and home range size (100% MCP, $r^2 = .093$, $P > 0.05$; $r^2 = 0.247$, $P > 0.05$, respectively). The mean home-range size for all turtles ($n = 11$) was 3.77 ± 2.82 ha (range = 1.12 – 9.49) based on MCP analysis. Mean home range size for males ($n = 8$) was 4.01 ha (range = 1.12 – 9.49); for females ($n = 3$), mean home range size was 3.14 ha (range = 1.57 – 5.27). A significant difference was not detected between male and female home range size (Mann-Whitney, $U = 11$, $P = 0.84$). Home range size also varied among the three methods; 95% KDE and 50% KDE were both smaller than estimates for 100% MCP (Table 2). I did not detect a statistically significant difference between male and female turtles among kernel density estimators 95% KDE and 50% KDE (Mann-Whitney, $U = 7$, $P = 0.31$, $U = 6$, $P = 0.22$, respectively). During the six activity seasons that turtles were monitored, females moved overall greater average net distances than males, but was not statistically different (one-way ANOVA, Welch's test, $F = 0.56$, $P = 0.81$; Table 3). However; males moved farther on average per radio-tracking event than females (Table 3), although, average distance moved per radio-tracking event did not significantly differ between males (mean = 70.80 m, SE = 7.63) and females (mean = 55.02 m, SE = 7.71; Mann-Whitney $U = 6$, $P = 0.28$).

Table 1 Summary of size, gender, observation period, and number of radio-tracking events of Eastern Box Turtles radio-tracked in southeast Tennessee, USA

Turtle ID	**Body Mass (g)**	**Carapace Length (mm)**	**Gender**	**Observation period (number of days at large)**	**Number of independent observations**
1R	213.0	99.8	F	8/3/2013 to 11/16/2015 (833)	118
2R	550.0	141.0	F	8/7/2013 to 6/9/2014 (307)	36
3R	485.0	128.1	M	8/23/2013 to 10/12/2014 (416)	50
8R	426.0	131.8	M	8/30/2013 to 8/17/2014 (352)	34
10R	250.0	111.8	M	10/22/2013 to 7/10/2015 (625)	58
2L	294	118.9	M	5/9/2014 to 8/19/2014 (102)	18
3L	304.0	115.4	M	5/9/2014 to 11/24/2015 (600)	72
8L	382.0	117.8	F	5/9/2014 to 12/18/2014 (224)	36
12L	378.0	134.0	M	5/9/2014 to 12/18/2014 (236)	42
1R1L	332.0	118.1	M	5/9/2014 to 12/18/2014 (236)	44
1R8L	363.0	126.0	M	8/19/2014 to 10/24/2014 (66)	18

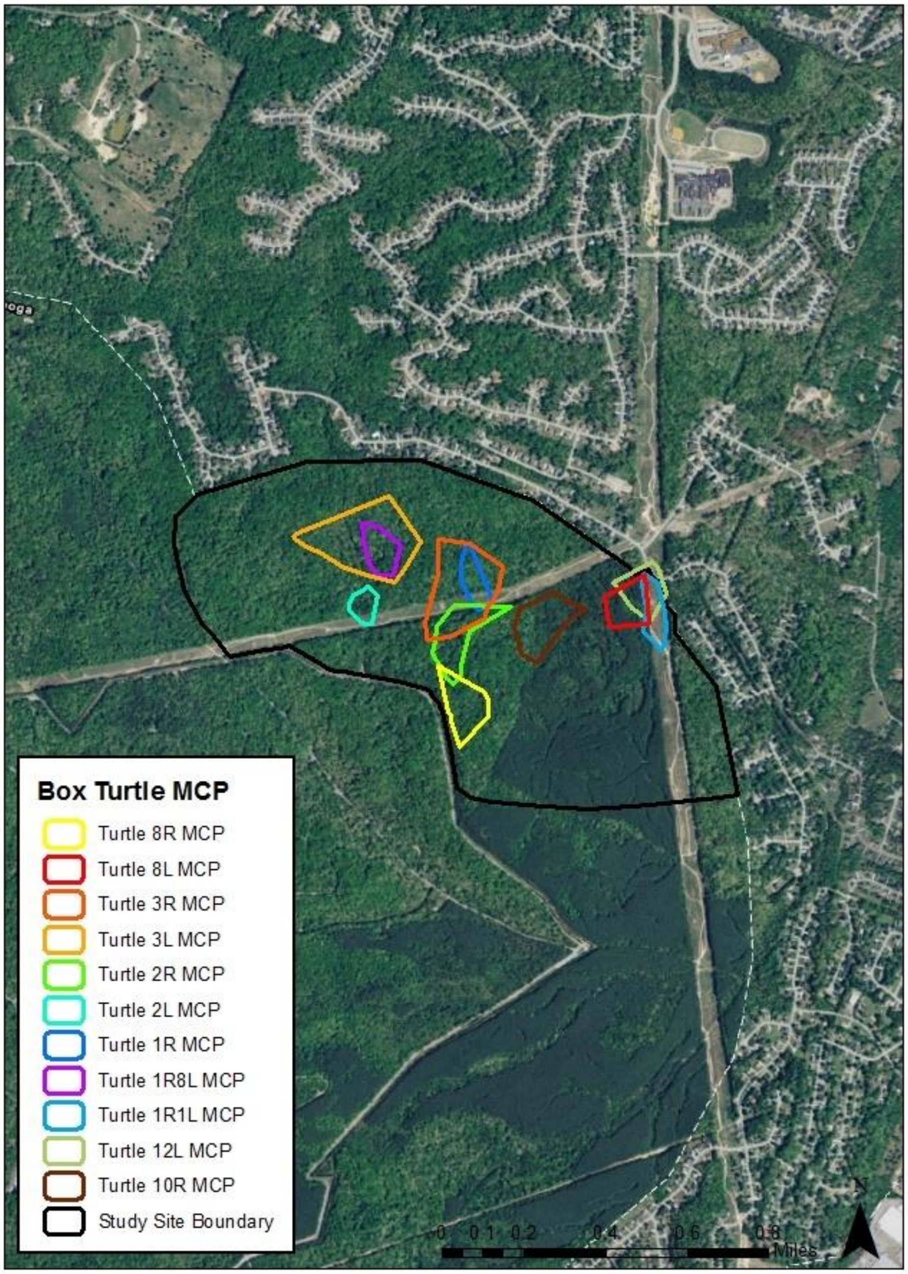

Figure 5 MCP activity area for all *Terrapene carolina carolina* at the LT7 study site. Polygons represent topological relationships of home ranges and habitat usage by box turtles

Table 2 Minimum Convex Polygon (MCP) activity areas and Kernel Density Estimates (KDE); both in hectares, for radioed turtles at the LT7 study site. Estimates did not differ significantly between genders

Turtle ID	Gender	Number of Independent Observations	Minimum Convex Polygon (MCP)	95% Kernel Density Estimate (KDE)	50% Kernel Density Estimate (KDE)
1R	F	118	1.57	1.18	0.53
2R	F	36	5.27	2.04	0.69
8L	F	36	2.57	2.13	0.77
3R	M	50	8.28	3.72	1.01
8R	M	34	2.18	1.10	0.33
10R	M	58	4.41	1.66	0.47
2L	M	18	1.12	0.35	0.16
3L	M	72	9.49	4.43	1.28
12L	M	42	2.67	1.12	0.16
1R1L	M	44	1.89	1.06	0.29
1R8L	M	18	2.04	0.82	0.32
Overall		47.82 (8.48)	3.77 (0.85)	1.78 (0.38)	0.55 (0.11)

Table 3 Eastern Box Turtle Movement Data: Distances are reported as straight-line distance meters (m) of male (n = 8) and female (n = 3) turtles at the LT7 study site

	Net Distance Moved			Average Distance Moved / Radio-tracking Event		
	Avg. (SE)	**Median**	**Range**	**Avg. (SE)**	**Median**	**Range**
Males	2,474.50 (506.82)	1,890.5	785 – 4355	70.80 (7.63)	76.89	43.10 – 101.26
Females	2,725.0 (933.79)	2,182.0	1449 - 4544	55.02 (7.71)	48.3	46.37 – 70.39

Eastern Box turtle home range size (100% MCP) varied by season ($F_{0.05, 5, 30} = 6.057$; $P = 0.007$). A Tukey-Kramer post hoc test confirmed that the mean annual home range size did not differ significantly between males and females ($F_{0.05, 1, 25} = 0.486$; $P = 0.492$), but home range size did differ among seasons ($F_{0.05, 2, 25} = 3.698$; $P = 0.039$). Although home range size differed among seasons, it did not differ between gender across seasons ($F_{0.05, 2, 25} = 0.358$; $P = 0.703$). Seasonal home range size was significantly larger in the spring and summer for all turtles ($P = 0.004$) when compared to the fall/winter. Although the Tukey Kramer post-analysis did not reveal a significant difference in home range size between genders within seasons (M_{Sp} vs. F_{Sp} , $P = 0.88$; M_{Su} vs. F_{Su}, $P = 0.84$; $M_{F/W}$ vs. $F_{F/W}$, $P = 0.45$) or between seasons for females (F_{Sp} vs. F_{Su}, $P = 0.08$; F_{Sp} vs. $F_{F/W}$, $P = 0.80$; and F_{Su} vs. $F_{F/W}$, $P = 0.17$), male home range size was larger in the summer than the spring (M_{Su} vs. M_{Sp}, $P = 0.02$; Figure 6) but not significantly different from the fall/winter season (M_{Su} vs. $M_{F/W}$, $P = 0.12$; Figure 6). The only differences in home range size between genders across seasons was between males in the spring and females in the summer (M_{Sp} vs. F_{Su}, $P = 0.02$; Figure 6); mean home range size of females in the summer was greater than that of males in the spring.

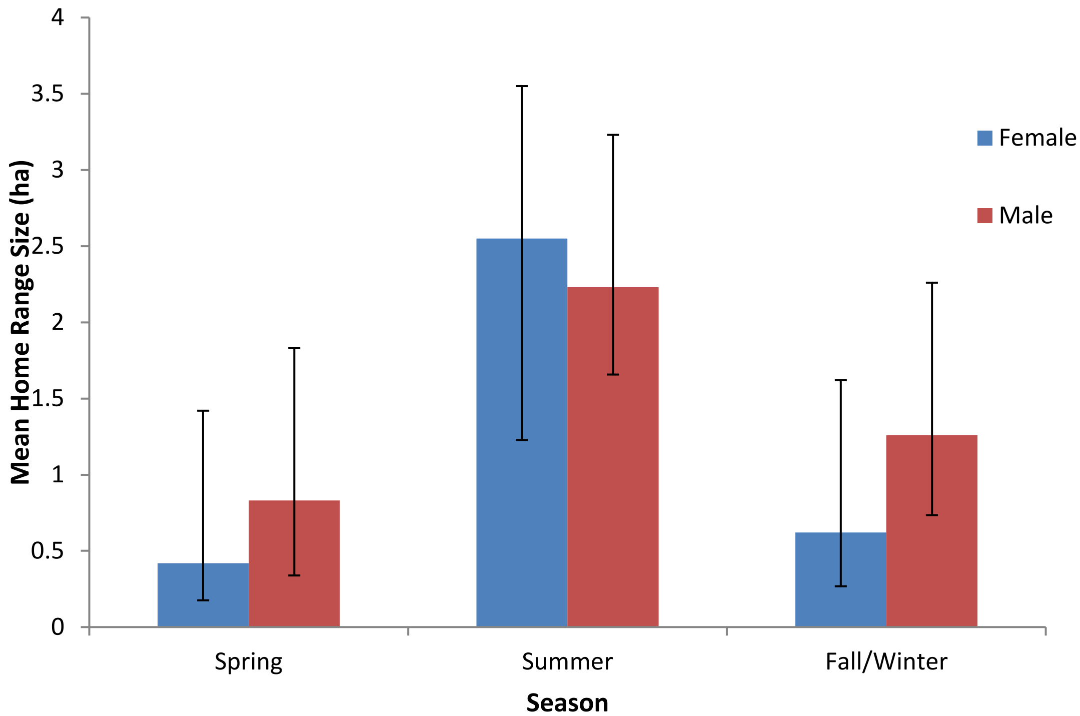

Figure 6 Seasonal home range (100% minimum convex polygon; MCP) of male (n= 8) and female (n = 3) Eastern Box Turtles, southeast Tennessee, 2013 – 2015

Habitat Use and Selection

Box turtles used various habitats throughout this study. A total of seven habitat types were available to all turtles as defined by the 2011 National Land Cover Data (Figure 7); although, none were used more extensively than mature deciduous forest (52% of all turtle locations were in this habitat). This was followed in decreasing order by shrub/scrub (29%), mixed forest (17%), hay/pasture (2%). The habitat types developed open space, developed low intensity, and evergreen forest were available to all turtles; although, no turtle observations occurred in these habitat categories (Figure 7). Both male and female box turtles were found in edge habitat only .05% of the total radio-tracked locations.

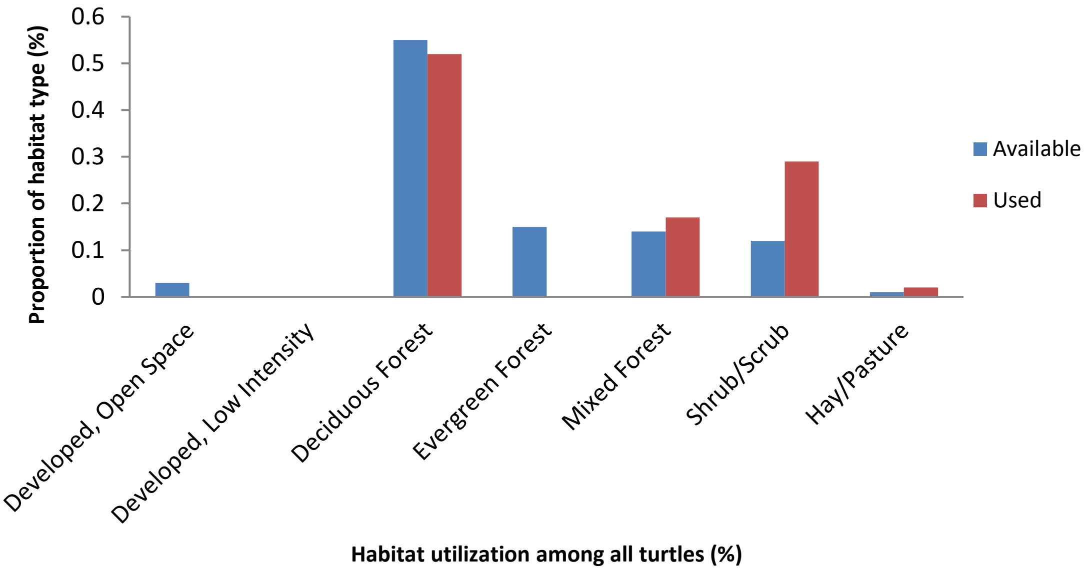

Figure 7 Number of relocations in proportion to habitat type according to National Land Cover Database 2011. Box turtles were found in deciduous forest more often relative to other habitat types

The habitat classifications used for analysis at the landscape scale were deciduous forest, shrub/scrub, and mixed forest, hay/pasture, developed open space, and developed low intensity. Although several of these habitats were very seldom used or not used at all, they were included in the analysis as they were all available to the turtles. Turtles used mixed forest and shrub/scrub in excess of their availability and deciduous forest was underused based on availability (Figure 7). Developed, open space, developed low intensity, evergreen forest, and hay/pasture were largely avoided. Habitat selection did not statistically differ between female and male turtles across all seasons (Fisher's exact test, $P = 0.55$).

Radio-tracked turtles used predominantly shrub/scrub, deciduous forest, and mixed forest habitats across all activity seasons (Figure 8). When examining all turtles across all seasons, habitat use did not differ significantly from random ($X^2 = 1.17$, 2 df, $P = 0.56$). However; when examining seasonal habitat use between genders, female and male turtles differed from each other in specific seasons. Female and male box turtles both used mixed forest habitats, but this pattern was more pronounced in males in the spring season than females ($X^2 = 7.72$, 2 df, $P = 0.02$). Females differed from males in the usage of mixed forest habitats during the summer season as males also used this habitat more often than females ($X^2 = 11.30$, 2 df, $P = 0.004$). Additionally, during the fall season, female turtles used shrub/scrub habitat far less than expected during the fall versus male turtles ($X^2 = 11.01$, 2 df, P = 0.004). Turtles increased use of deciduous hardwood forests in the fall/winter season, while decreasing shrub/scrub usage (Figure 8). Shrub/scrub usage increased in the summer season (Figure 8). Conversely, mixed forest habitat increased during the spring season (Figure 8).

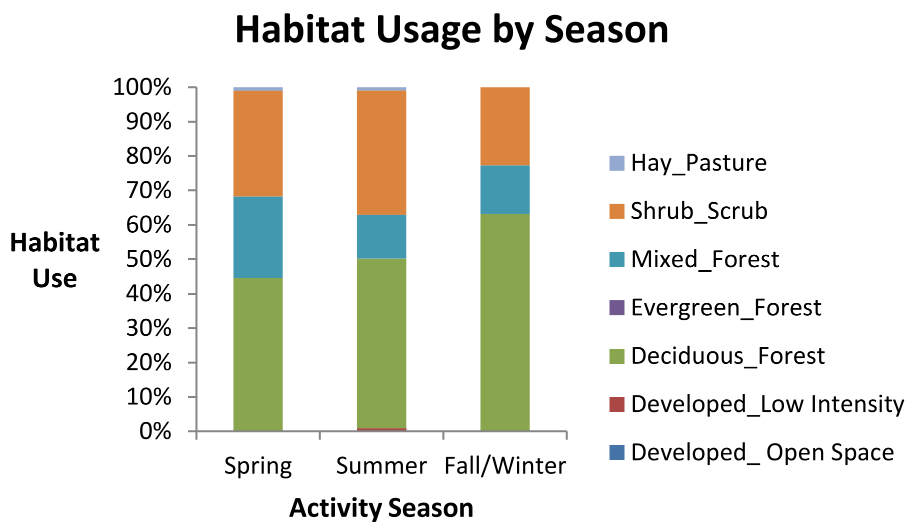

Figure 8 Habitat composition for Eastern Box turtles by season in southeast Tennessee, 2013-2015

At the microhabitat level, I found that turtles typically selected sites with more woody debris, and a greater number of early successional species than occurred at random sites and less hardwood species than occurred at random sites (Table 4). Additionally, turtles tended to select sites with more canopy cover than those of random sites (Table 4). There were no significant differences in percent groundcover, forbs, or grasses between turtle locations versus random locations.

Table 4 Percent cover at Eastern Box Turtle relocation points and random points, Hamilton County, Tennessee, 2013 – 2015

	Woody Debris	**Ground Cover *sp.***	**Succesional *sp.***	**Hardwood *sp.***	**Forbs *sp.***	**Grass *sp.***	**Canopy Cover**
Turtle Location	82.2	74.8	39.4	53.6	47.0	23.8	92.9
Random Location	74.8	71.4	26.8	68.6	42.2	20.4	89.3
McNemar (χ^2)	$P = 0.003$	$P = 0.22$	$P = 0.002$	$P = 0.001$	$P = 0.14$	$P = 0.21$	$P = 0.03$

Following initial global model analysis, seven candidate model sets were selected. When constructing the initial models, I used specific species of forbs and groundcover that were present at turtle locations in an attempt to tease out what species may be selected for versus another species. The model that indicated the highest likelihood of probability and garnered the majority of the weight included woody debris + muscadine *sp*. (groundcover) + Carolina Buckthorn (forbs) + successional *sp*. + bramble (forbs) + mineral + west cardinal direction (w = 0.40; Table 5). No other model had significant support or an Δ AIC value < 2. Although this model is weakly supported, when selecting microhabitat within the home range, box turtles were most likely to select locations with woody debris in combination with shrub cover and successional *sp*. and also preferred more groundcover with minerals present when compared to random locations.

Table 5 Eastern Box Turtle Habitat Selection Assessed Using AIC. Seven candidate models, consisting of microhabitat variables, were selected from the initial global model analyses. Only one model was supported well enough for inference (Δ AIC < 2)

Model	AIC[1]	Δ_i[2]	W_i[3]	Evidence Ratio[4]
Woody Debris + Muscadine + Carolina Buckthorn + Successional sp. + Bramble + Mineral + West	1326.445	0	0.399166	-
Woody Debris + Bramble + Successional sp. + West + Mineral + Catbrier	1330.247	3.802	0.059643	6.692581
Woody Debris +Bramble + Successional sp. + West + Mineral	1334.155	7.71	0.008452	47.2286
Woody Debris + Bramble + Successional sp. + West	1342.617	16.172	0.000123	3248.666
Woody Debris + Bramble + West	1351.178	24.733	1.7E-06	234802.4
Woody Debris + Bramble	1359.283	32.838	2.95E-08	13510796
Bramble	1370.930	44.485	8.74E-11	4.57E+09

1: Akaike Information Criteria, with the lowest value indicating the best of the candidate models.
2: Delta AIC equals the difference between a select model and the best model.
3: Akaike weight equals the probability that a model is the best among the candidate models.
4: Evidence ratio indicates a model's relative strength or the extent to which it is better than another model.

Discussion

These data are the first detailed information regarding spatial ecology of the Eastern Box turtle in southeastern Tennessee. These findings support the characterization of the Eastern Box turtle as a reptile that exhibits extensive use of mature hardwood forests. This was somewhat expected given the importance of this habitat for temperature regulation and biophysical structures. Additionally, 50% of all turtle locations observed during the summer seasons were found in the mature hardwood forest. This highlights the importance of this habitat, not only during the warmest months of the year, but also as an important overwintering site as 73% of turtles selected hibernaculum sites in this habitat. Interestingly, Eastern Box turtles also frequented shrub/scrub ROW habitat and mixed forests more often than expected, although only a small portion of the study area, likely because of the canopy cover these habitats provided and possibly increased foraging opportunities.

Turtles often spent days at the same location before moving and remained hidden in thick leaf litter or vegetation. In terms of movements, some general patterns emerged: female turtles moved greater net distances than male turtles in the duration of the study (Table 3); although, male turtles moved greater distances between radio-tracking events on average (Table 3). One female in particular was observed making an abrupt long distance movement to mature hardwoods in early June (217 m over two days in one direction and 210 m over three days on the return) to the same location in both years of the study before returning to and spending the remainder of the summer season in shrub/scrub ROW habitat. Eastern Box turtles in Tennessee mate from April to October and nesting occurs from May to September (Ernst et al. 1994); thus, it is possible that these extended movements over a short period of time were related to nesting and mate searching (Stickel 1950). Male turtles may move greater average distances than females during the activity season as they are known to mate with more than one female (Williams and Parker 1987).

Home range size differed among Eastern Box Turtles in a heterogenous landscape in southeast Tennessee, but these findings vary with those reported in the literature. Stickel (1989) reported slightly smaller home range sizes for box turtles in Maryland (1.20 ha for males and 1.13 ha for females, respectively). An average MCP size of 4.327 ha for males and 1.30 ha for females was estimated for box turtles in North Carolina (Kapfer et al. 2013) and is similar to that found in this study with the exception of female home ranges, which is much smaller than the

MCP for females in this study. In contrast to the findings here, a study in northeast Tennessee by Donaldson and Echternacht (2005), with very similar study site characteristics and sample sizes, reported an overall MCP home range of 1.88 ha for box turtles. In addition, Davis (1981) estimated an average MCP size of 0.38 for Eastern Box Turtles, which is also much smaller than the findings in this study. The difference in female home range size may be a reflection of the small sample size in both this study and the North Carolina study (n = 6; Kapfer et al., 2013). Home range size varied by season in this study and is more than likely related to the seasonal shifts in temperature and possibly resource availability such as food, hibernaculum site, finding mates, nesting, etc., and would be expected behavior in box turtles. Individual variability may also play a role in these differences among home range sizes as several turtles in this study seemingly displayed individual variability. Two male turtles in this study had small home ranges and rarely traveled out of a particular habitat patch while other males varied widely in movement and habitat use. Seibert and Belzer (2015) studied individual behavior and movement patterns in Eastern Box turtles in Pennsylvania and indicated that many box turtles within their study area demonstrated transient and unpredictable movement behavior. The cause of this behavior is unclear; although, the gene flow strategy is thought to contribute to this individual transient behavior (Schwartz et al. 1984). Such transient behavior is believed to be the agent for gene flow between isolated populations (Schwartz et al. 1984). Another explanation for the slightly larger MCPs estimated in this study than those reported in the literature may be associated with habitat quality. In addition, studies over a longer temporal scale may provide better explanations for these variations in home range sizes.

Minimum convex polygons and Kernel Density Estimates are methods commonly used in box turtle home range studies (Madden 1975; Donaldson and Echternacht 2005; Hester et al.

2008). MCP home range estimators include all GPS locations and also any outliers in the data set which may not represent part of a turtle's home range. The fixed kernel density home range estimator is a nonparametric estimator of an animal's home range using a probabilistic distribution of spatial use (White and Garrott 1990). This estimator is most often the least biased home range estimator (Worton 1989; Seaman and Powell 1996). Most home range estimators require that the input data (i.e. locations) be statistically independent both temporally and spatially (White and Garrott 1990). This kernel density utilization distribution is an effective method for obtaining an animal's core habitat and can provide spatial distribution information for conservation planning. Kernel-based methods often produce home range sizes larger than that of the minimum convex polygon (Wasko and Sasa 2009; Fitzgerald et al. 2002), as they may generate contours that extend outside the boundaries of the polygon. However, for the 95% and 50% KDE, the estimates in this study were more conservative than the minimum convex polygon home range sizes. This may be due to the fact that unlike larger terrestrial vertebrates, box turtles move very little on a daily basis, creating pockets of unused space within their home range. The kernel density estimates for the study were similar to that reported in the literature (Donaldson and Echternacht 2005; Greenspan et al 2015). Due to sample sizes of turtle locations below 30 for three of the turtles in this study, careful interpretation is suggested of the kernel density estimates reported in my findings.

Habitat use of the turtles in this study coincided with previous studies that report an association for mesophytic hardwood forests (Dodd 2001; Ernst and Lovich 2009; Stickel 1950). Box turtles in this study selected habitats that contained a significant amount of canopy cover. Turtles demonstrated avoidance of developed areas that typically had reduced canopy cover and biophysical structure. During the activity season, mesophytic hardwood forests provide turtles the ability to engage in thermoregulation to maintain an optimal temperature for their activities. In contrast, several turtles in this study used shrub/scrub habitat (ROW) almost exclusively during the summer months; although, canopy cover measurements were similar to those found in hardwood forests. It is also interesting to note that turtles decreased their use of shrub/scrub habitat during the fall/winter season as foliage started to decrease significantly in this habitat. Overall, box turtles selected areas of habitat with dense vegetation and a significant amount of canopy cover. These findings suggest that certain habitats are utilized during different times of the activity season and are dictated by the amount of canopy cover that is present within that habitat. Undoubtedly, the shrub/scrub habitat offered an optimal amount of canopy cover to facilitate thermoregulation and also provide a larger variety of food resources during the summer season when foliage production was at peak conditions. Increased development or human usage of this habitat could also pose a threat to turtles. During the summer season, these ROW areas are mowed and selectively sprayed with herbicides and exposes box turtles to yet another anthropogenic disturbance.

An interesting finding in this study was the lack of habitat utilization of evergreen forest. No turtles were located in this habitat during the course of the study. One male turtle that was removed from all subsequent analyses was radio-tracked in this habitat but could not be confirmed to be using it beyond the transient movement that was recorded. Even though this turtle was not included in the analysis, assumptions cannot be made that turtles do not use evergreen forest or pine plantation tracts for some aspects of its life history. Greenspan et al. (2015) conducted a study in south Georgia examining Eastern Box turtle habitat selection in a Longleaf Pine (*Pinus palustris*) reserve. The findings in this research indicate that box turtles use this habitat extensively, in addition to pine-hardwood mixed habitat. In contrast, box turtles in southwestern Virginia were found in an Eastern White Pine plantation only 7% out of 305 total turtle locations (Fredericksen 2014). These findings illustrate that Eastern Box Turtle behavior can vary across geographic regions with respect to habitat selection.

Most of the biophysical or structural microhabitat features examined in this study are apparently important to box turtles, as shown by greater than expected use of canopy cover, woody debris, successional vegetation, and groundcover on the forest floor. Canopy cover may be one of the most influencing factors when box turtles select habitats. Canopy cover associated with deciduous forests provide turtles with a buffer from harsh conditions and provides turtles with ideal overwintering sites (Currylow et al. 2013). Extremely cold temperatures are known to cause mortality in hardy, freeze tolerant taxa such as salamanders and frogs, as well as box turtles (Carpenter 1957; Metcalf and Metcalf 1979; Storey and Storey 1986). This habitat selection is critical to survival for poikilotherms whose body temperature is regulated by the physical environment. Leaf litter and woody debris (e.g., logs and branches) provides protection and thermoregulating properties for box turtles and is another important component to this habitat (Dodd 2001). Deciduous forests also maintain higher humidity than surrounding open areas (Reagan 1974).

Eastern Box Turtles were adept at moving through their fragmented environment and at times making large movements across the landscape. The strategy used by box turtles to deal with minimizing dessication, locating mates, and foraging may influence not only their temporal activity patterns but also their spatial patterns. The somewhat larger home range sizes exhibited by turtles in this study suggests that resources are spread out more across this landscape and require longer travel distances to find suitable nesting sites, optimal foraging, and mates. Given that box turtles are often closely associated with mesophytic forests, I expected box turtles to have smaller home range sizes due to the amount of this habitat available to them within the study site. Even though this analysis indicated a strong avoidance of forests dominated by pine species, turtles do use these habitats for transient movements and core habitat usage (Greenspan et al. 2015).

Conservation Implications

The results of this study offer insight into the ecology of a widespread species at a study site that has received little attention. Box turtle movement and habitat selection can vary widely among study systems over small spatial scales such as this one. Home range size is an important animal trait and has important implications for wide-ranging species, as it can be a predictor of extinction risk (Woodroffe and Ginsberg 1998). Habitat loss may affect wide-ranging species in particular; hence, home range size and space use is a critical component to understanding how Eastern Box Turtles interact with their environment (Woodroffe and Ginsberg 1998). At this particular study site, Eastern Box turtles seasonal habitat use may have implications for their conservation. Their extensive usage of shrub/scrub ROW habitat during the summer season may make them more vulnerable to injury or death from large mowers, pesticide exposure, and ORV traffic this time of the year. Although only one turtle crossed the road in the subdivision during this study, Gibbs and Steen (2005) found an increasing trend of road mortality among both freshwater and terrestrial turtles in the United States. A large proportion of the turtles radio-tracked in this study were male turtles. This could be due to chance but Hall et al. (1999) found similar high proportions of males in a long-term box turtle study in Maryland which suggests that female mortality is higher than males in the nesting season when females are more apt to making long distance movements. This is a significant conservation concern for Eastern Box turtles and is a scenario that is more likely to increase in fragmented landscapes when compared to remote locations (Budischak et al. 2006). Future conservation must continue to examine the influence that human population growth and habitat loss have on the habitat selection and the spatial ecology of box turtles.

Management Implications

The results of this study are limited by the exclusive study of adult box turtles, small sample size, and short duration of this study compared to the relatively long life span of recruited box turtles. However, these data can be used to create a habitat conservation strategy for box turtles on the properties managed by the University of Tennessee at Chattanooga and Hamilton County Government. Mature hardwood forests are important factors for conserving box turtles as the largest proportion of locations in this study were found in this habitat as well as over-wintering sites which are critical for over-wintering and long term survival of box turtles. In the present study, large portions of this study site will soon be bisected by recreational horse riding trails and the removal of mesophytic forest habitat can potentially threaten this forest ecosystem and the long term population viability of this vulnerable species which is protected under Tennessee State Law and CITES Appendix II.

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APPENDIX A

IACUC APPROVAL NOTICE

THE UNIVERSITY of TENNESSEE UT CHATTANOOGA

Institutional Animal Care and Use Committee
Dept. 4915
615 McCallie Avenue
Chattanooga, TN 37403-2598
Phone: (423) 425-5867
Fax: (423) 425-4052
iacucpro@utc.edu
http://www.utc.edu/iacuc

MEMORANDUM

TO: Dr. Thomas Wilson

FROM: Alexa McClellan, Assistant Director of Research Integrity
Dr. Margaret Kovach, Interim IACUC Chair

DATE: August 1, 2013

SUBJECT: **IACUC #: 13-04:** The spatial ecology of the Eastern Box Turtle in Urban and fragmented landscapes of southeast TN

The UTC Institutional Animal Care and Use Committee has reviewed and approved your application and assigned you the IACUC number listed above.

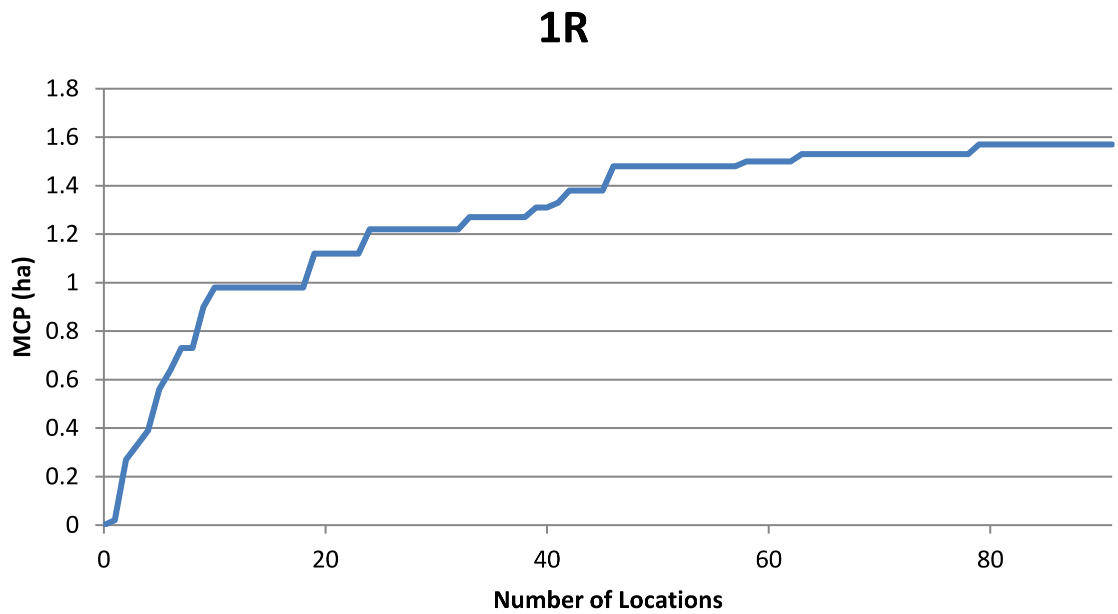
Reminder: Approved protocols must be reviewed at least annually. It is the responsibility of the principal investigator to submit an Application for Protocol Annual Continuation form to the IACUC before the anniversary date of the approved protocol. However, the Office of Research Integrity shall make every effort to send reminders 30 days prior to the anniversary date. The annual review form must be completed and submitted to the IACUC Committee before the first day of the anniversary month. New protocols must be submitted and approved every three years.

Please remember to submit a Protocol Modification Form if significant changes occur in your research design or in any instruments used in conducting the study. You should also contact the IACUC immediately if you encounter any adverse effects during your protocol.

For additional information, please consult our webpage http://www.utc.edu/iacuc or email iacucpro@utc.edu. Best wishes for a successful research project.

APPENDIX B

INCREMENTAL AREA ANALYSIS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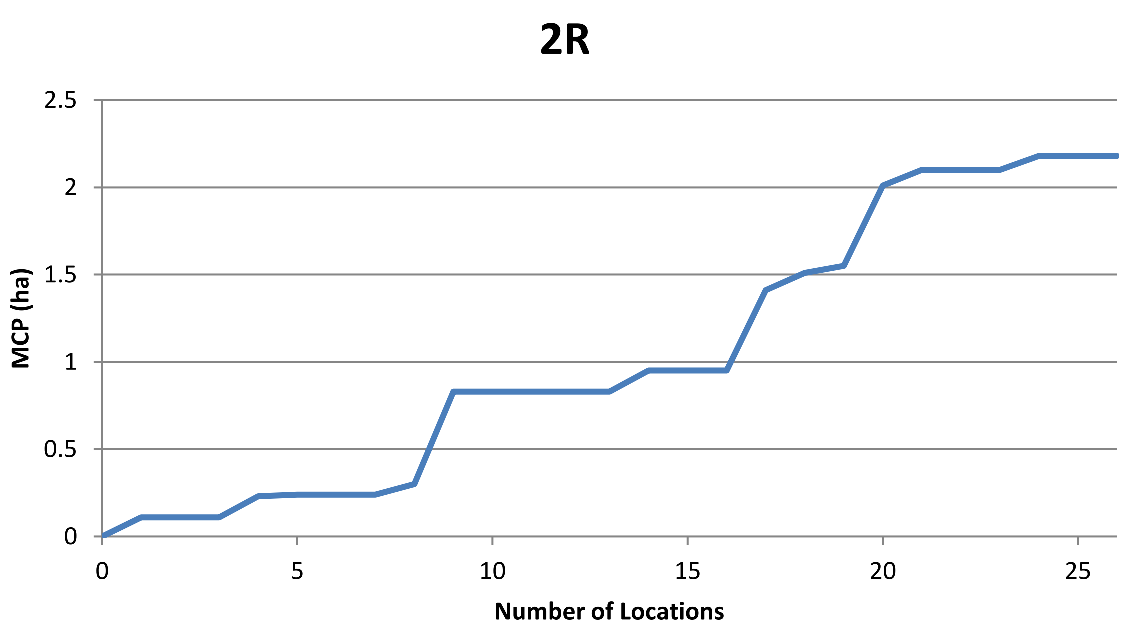
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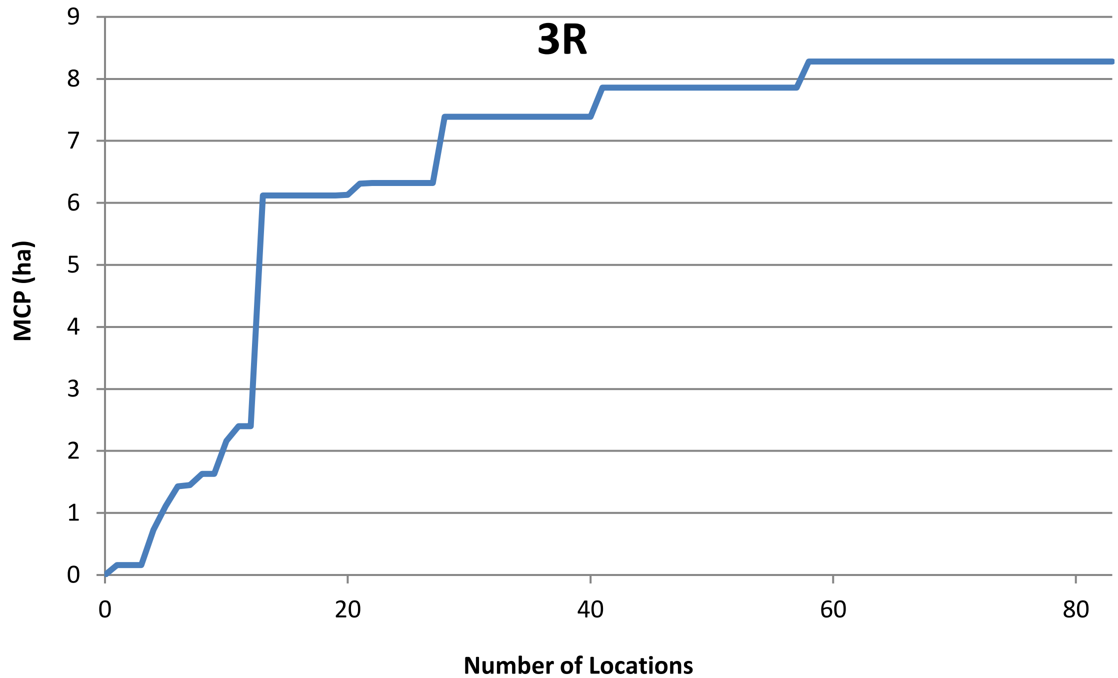
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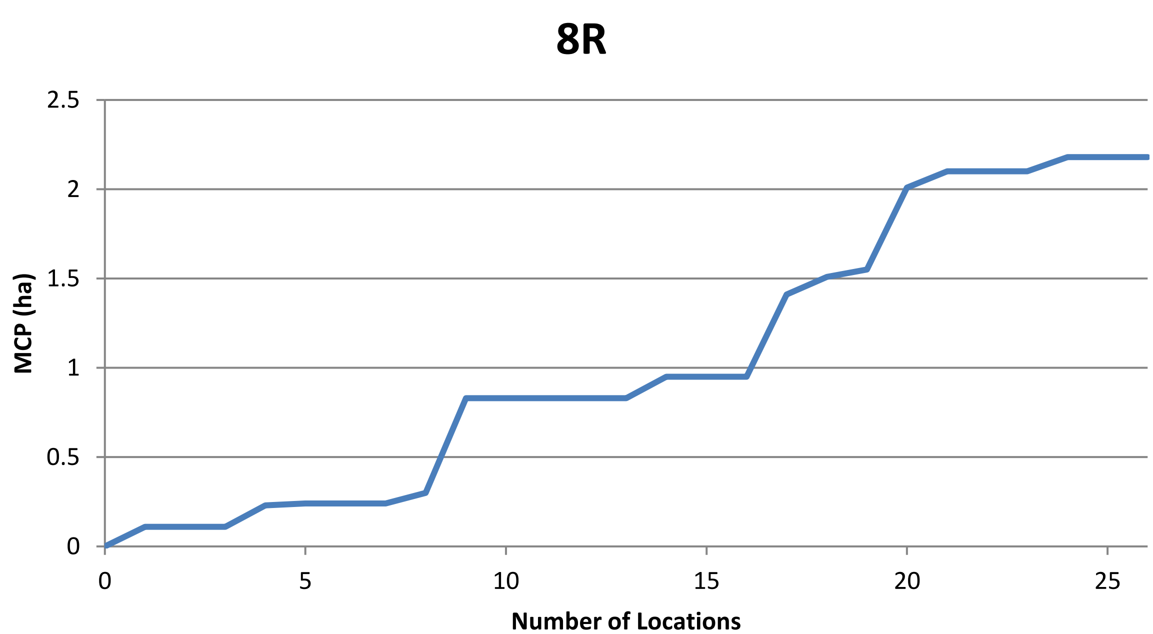
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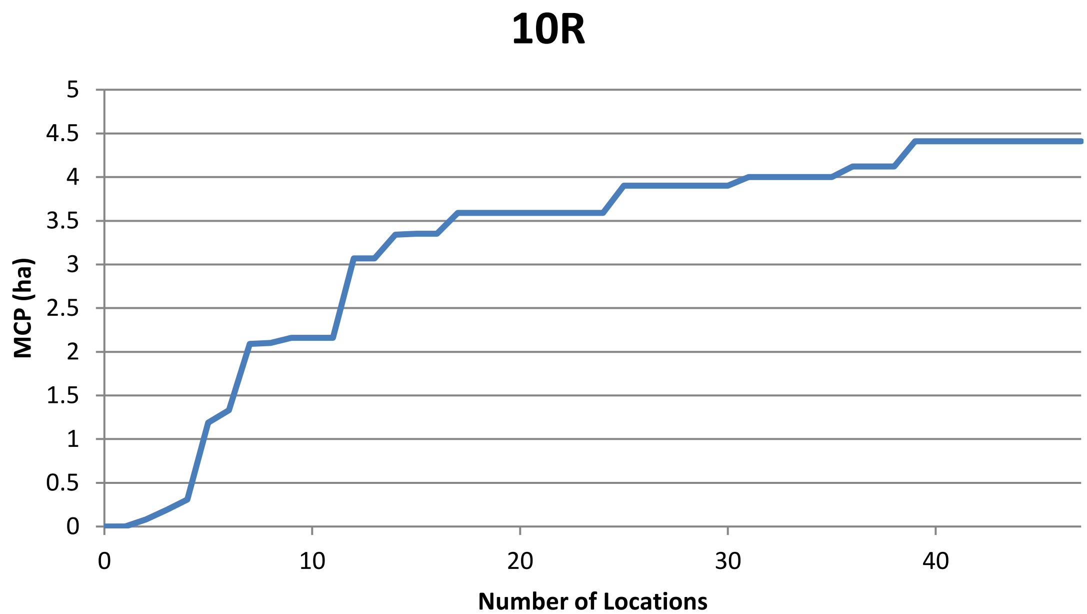
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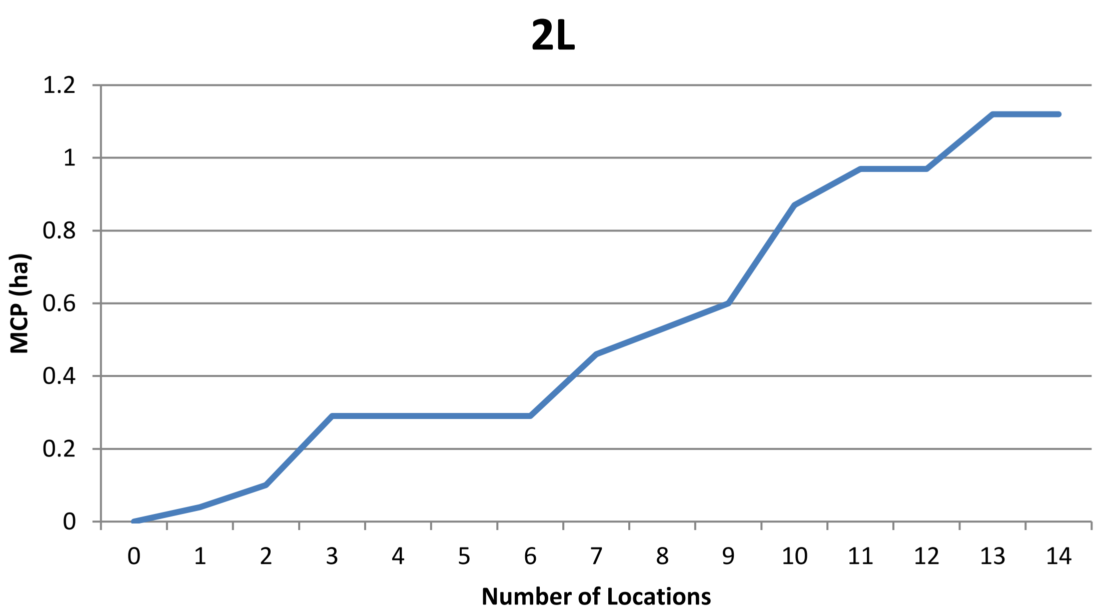
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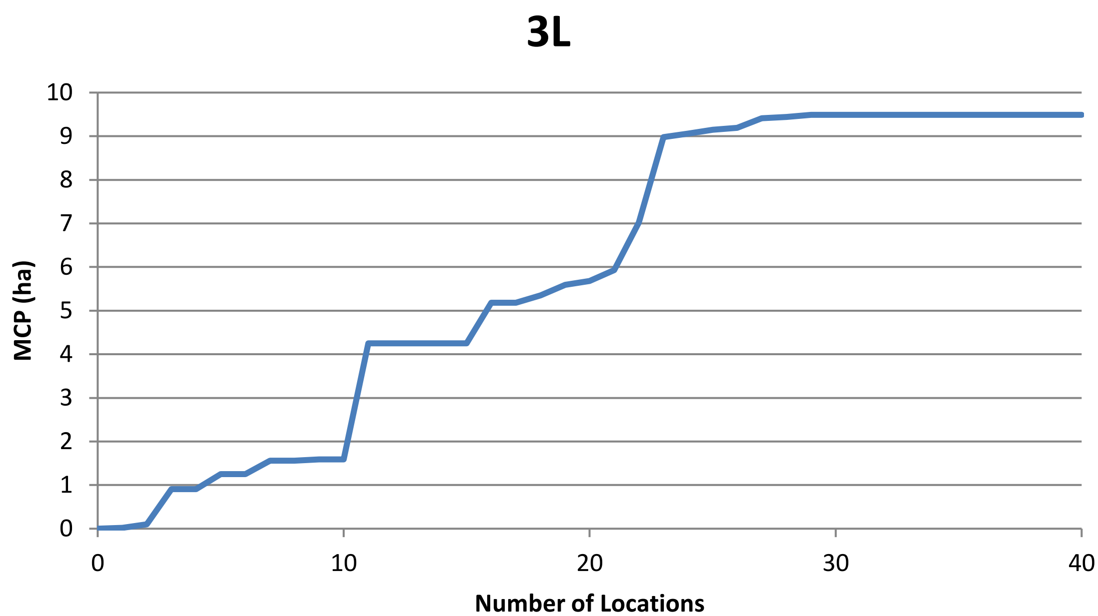
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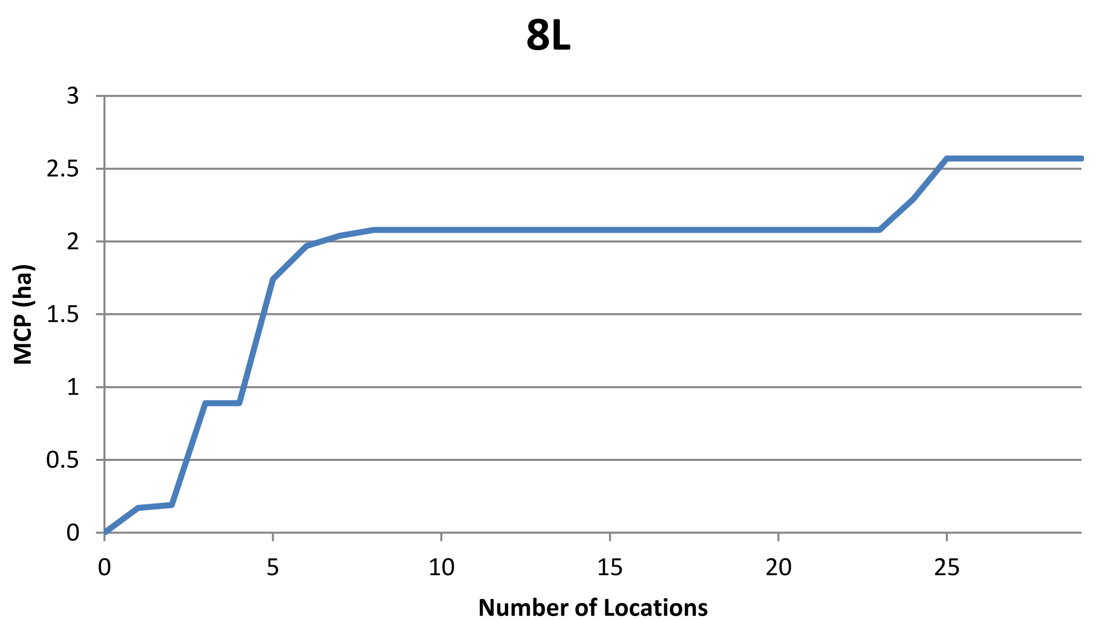
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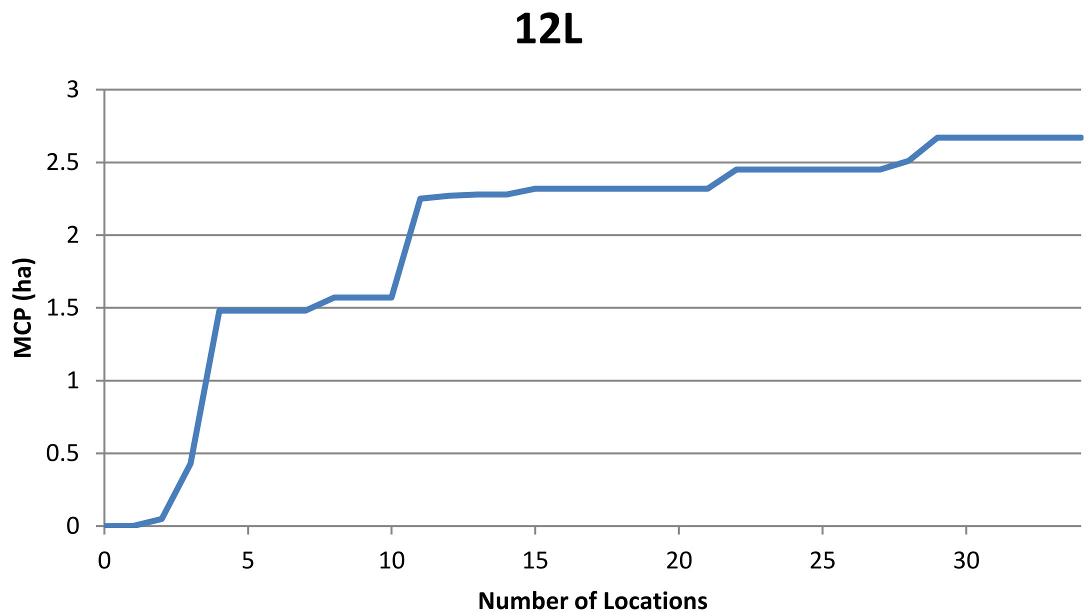
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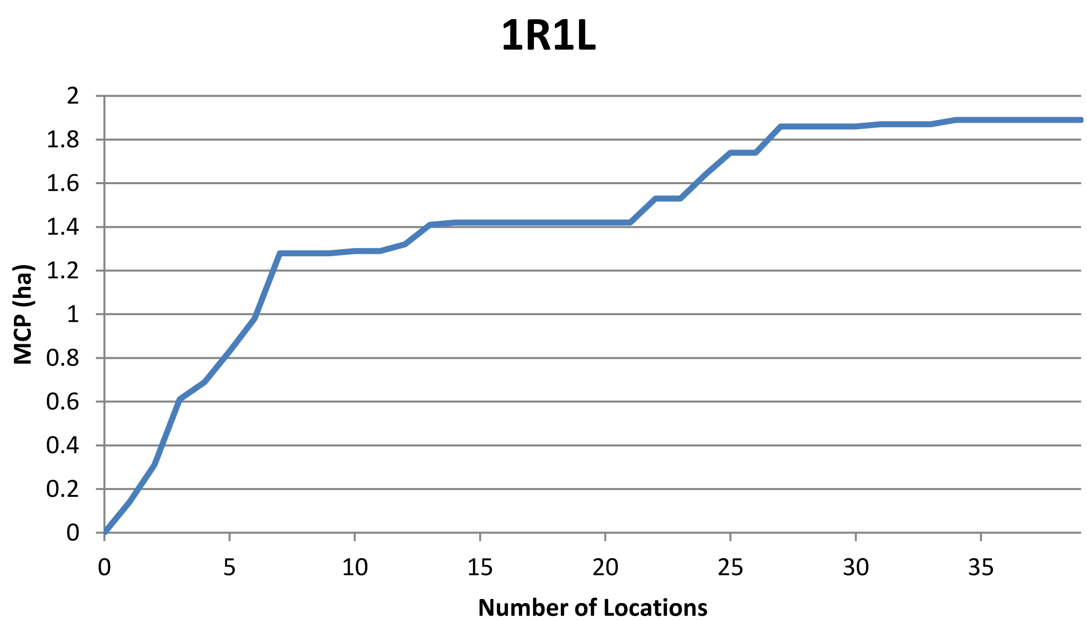
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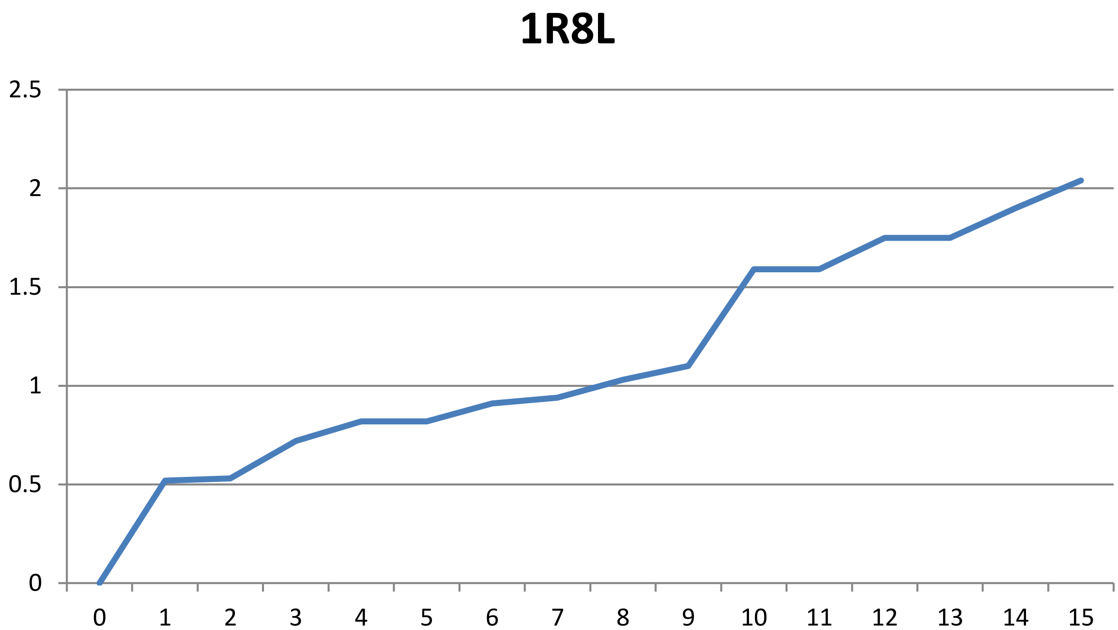
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VITA

Mark Dillard was born in Chattanooga, TN, to the parents of Jim and Connie Dillard. He is the second of two children, a younger brother to an older sister. He attended McBrien Elementary and continued to East Ridge High School in Chattanooga, TN. After graduation, he attended The University of Tennessee at Knoxville where he became interested in plant science. Mark completed a Bachelors of Science degree in December 2000 in Plant Science and Landscape Design. Mark worked for eleven years in the agricultural field before entering the Master of Science program at the University of Tennessee at Chattanooga. Mark graduated with a Master's of Science degree in Environmental Science in December 2016.