POPULATION DYNAMICS OF THE EASTERN BOX TURTLE (*Terrapene Carolina carolina)* IN THE MARYVILLE COLLEGE Woods, Maryville, Tennessee

A Report of a Senior Study

by

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ABSTRACT

The Eastern Box turtle (*Terrapene Carolina carolina)* is the most common terrestrial turtle in the Eastern United States. 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, 2017). Information pertaining to existing populations of Eastern Box turtles such as sex ratios, age distribution, and home range size may have implications for their conservation and can aid in preparing management strategies. A population of box turtles is known to exist in a small (57 ha) woodlot in eastern Tennessee known as the Maryville College Woods, Maryville College Campus, Maryville, Tennessee. Searches for turtles were conducted by foot from May 2016 to August 2017, as well as the use of turtle sniffing dogs. All turtles were measured and marked to obtain morphometric data. Turtles spaced across the woods were tagged with radio transmitters and tracked to gather information about home range and activity. A total of 109 turtles were marked and used in this study, providing a gender ratio of 67M:36F. Juveniles made up 14% of the study population, suggesting a healthy, reproductively active population. There were two observed mating events, but no nesting events observed during the study. Average morphometric data resulted in an average plastron width 66.65 mm (SD=13.12 mm), average plastron length 108.86 (SD=16.13 mm), average carapace width 86.87 (SD= 17.72 mm), average carapace length 118.08 (SD=24.02 mm), average shell height 52.69 mm (SD=52.69 mm), average circumference 24.60 cm (SD=4.58 cm), and average mass 341.40 g

(SD=129.16 g) (table 2). Males had significantly larger carapace widths (p=0.0002), carapace length (p=0.003), plastron width (p=0.0006), plastron length (p=0.00002), circumference (p=0.0006), shell height (p=0.05), and mass (p=0.0005) than females. A Lincoln-Peterson mark-recapture analysis estimated that the abundance of the box turtle population in the Maryville College woods is 588 turtles; 10 turtles per hectare. Average home range size was 0.74 hectares, with no significant difference (p=0.87) in home range size of males and females. These results, which suggest a thriving, healthy, and reproductively active population, indicate a need for conservation and future study. Future research should focus on ecology of the Maryville College Woods, Maryville, Tennessee to determine what it is that has kept this population here or cause them to come here. This knowledge would provide necessary information for their ongoing conservation.

ACKNOWLEDGEMENTS

I would like to acknowledge Dr. Dave Unger for all of his knowledge, direction, and most of all support during the course of this study, Dr. Nathan Duncan and all of the Maryville College students who took the time to search for turtles, Austin, Bobby, and Adam for all of the times they helped me to track turtles, my mom for listening to all of my "turtle talk" and for supporting me and my crazy ideas, my aunt Debbie for always believing in and supporting me.

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CHAPTER I

INTRODUCTION

The Eastern box turtle (*Terrapene carolina carolina)* is native to the eastern United States and is the state reptile of North Carolina and Tennessee. It is the most common terrestrial turtle in the eastern United States, ranging from Massachusetts to Georgia and west to Michigan, Illinois, and Tennessee (Conant and Collins 1998). They occur predominately in mixed oak-pine forests, pine flatwoods, marshy meadows, maritime oak forests, hardwood swamps, agricultural areas, and occasionally enter caves (Cooper 1961, Ernst et al. 1994, Mitchell 1994, as cited in Wilson 2005). They also frequent residential areas and many habitats in the Appalachians (Wilson and Ernst 2005).

The most visible feature of the box turtle is the bony, box-shaped shell. The varied color of their shells (unique to each individual) allow for blending into the forest floor of deciduous habitats. They have a bi-lobed, hinged plastron that allow them to close their shells almost completely (Dillard 2016). In times of danger, the turtle can withdraw its head, neck, tail, and limbs completely within the shell. It does so by expelling air in the lungs to allow room for the limbs and by twisting its neck into an “s” shape to bring their head into the enclosing shell. They can create a tight seal by closing the plastron upward to fit tightly against the carapace. Unlike other turtles, the bones of box turtles fuse together and the ribs and vertebral column are fused with the bony shell. Their digestive system consists of an esophagus, stomach, duodenum, and a distensible colon, which empties into the cloaca (Parsons and Cameron 1977, as cited in Dodd 2001).

Studies of diet are fundamental to understanding the ecology of an organism, and among turtles, diet directly affects energy allocation, which in turn determines survival, growth, and reproductive rates. From a conservation standpoint, knowledge of diet is essential for assessing the potential impacts of urbanization and land-management practices on box turtle populations (Platt et al. 2009). Although there have been no reports of differences between the diets of males and females, juveniles are primarily carnivorous and become more herbivorous with age (Ernst and Barbour 1972). Olfaction appears to play some role in prey identification, as box turtles have different categories of receptor cells in their olfactory epithelium. Sight, however, still seems to be the most important sense. Box turtles have many cone cells in the retina, allowing them to see color (Dodd 2001).

Box turtles are opportunistic omnivores that consume a variety of foods including annelids, mollusks, insects, amphibians, small mammals, plants, and fungi. Differences in feeding habits may be caused by food availability within their respective habitats and likely varies geographically and temporally. Stone and Moll (2006) found that *Terrapene carolina* consumed 32% (by volume) animals and 64% plant material. Platt et al. (2009) found Gastropod remains and seeds of plants that produce fleshy fruits were the items most frequently recovered from Eastern Box turtle feces. Also found were foliage, bones, fish, insects, snake skins, *Terrapene carolina,* feces, fungus, paper, and stones. It has also been recorded in an observational study by Babcock (1919, as cited in Dodd 2001) that turtles consume toadstools, which do not seem to poison them.

Perhaps more importantly than simply eating fungi, the eastern box turtle has been shown to be a facultative fungivore that may play an important role in fungal spore dispersal (Jones 2006). Stone and Moll (2009) found that seeds from over seventeen plants were recovered from fecal samples, four of which had not been previously identified as consumed by *Terrapene.* Because seeds of these four previously unrecorded plants were undamaged in the feces, *Terrapene* could be a potential disperser of these taxa. Liu et al. (2004) reported for some species of plants, rate and percentage of germination is enhanced by digestion in turtles, but decreased in others. Along with surface foraging, box turtles also tunnel through leaf litter in pursuit of invertebrate prey.

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. The home range is described as the total area inhabited by an animal for normal activities, gathering food, mating, and caring for young (Powell 2012). Habitat loss may affect wide-ranging species. Hence, home range size and space is a critical component to understanding how Eastern box turtles interact with their environment (Woodroffe and Ginsberg 1998). Although box turtles are often described as territorial, a study by Stickel (1950) reported that the home ranges of box turtles in their study overlapped. That said, they are known to maintain a specific home range (Stickel 1989, Yahner 1974). Home ranges differ among animals of different species, among individuals within a species, and even within individuals over time (Powell 2012). Inter-populational differences in home range size are believed to reflect population density and habitat quality (Stickel 1950). According to Stickel's (1950) work, box turtle density increases and home range size decreases as habitat quality increases. The distances traveled within and outside of the turtles' home ranges can vary with sex, the weather, and seasons (Iglay 2007).

Previous studies have varying results on home range sizes for box turtles, many of which are based upon relocation and homing instincts of the turtles. Hester (2008) reported that relocated turtles had larger home ranges, moved greater average distances per day, and moved greater distances from their release points than did resident turtles. These alterations in home range size are meaningful to box turtles because they normally retain the same home ranges throughout their life. Cook (2004) studied a population of box turtles which exhibited home ranges of 9.77ha, which was considerably larger than populations to which he compared, which exhibited home ranges of 6.96ha and 1.20ha. It appears that females extend their home ranges by leaving bottomland forest to nest in drier and warmer upland sites (Stickel 1989). This is in contrast to a report by Madden (1975; as cited in Cook 2004) that concluded that most females nested at sites within their home range.

Box turtles appear to select microhabitats based on the need for thermoregulation, minimization of water loss, and reproduction. Overall habitat selection may also vary depending on age, sex, and the relative availability of habitat types. As ectotherms, they must maintain homeostasis by seeking cover when temperatures are high and basking when temperatures are low. Eastern box turtles maintain a body temperature of 29-38°C (Fredericksen 2014). During the winter months, they enter a state of torpor, burying themselves in shallow depressions or "forms", consisting of leaves, debris, or cavities in the soil (Rossell et. al. 2006). In spring and summer, Eastern box turtles spend most of their time in forests with an occasional venture into open habitats to search for food, basking, and nesting (Dodd 2001).

Adult box turtles are sexually dimorphic. Males have red irises and a concave plastron which allows them to mount females. Females have brown irises and flat to a slightly concave plastron (Dodd 2001). Sexual maturity is reached between 8 to 10 years of age (Ernst et. al. 1994). Dolbeer (1971) observed eastern box turtles at the University of Tennessee, Knoxville, Tennessee mating twelve times during an activity season with eight of the observations occurring in September. Although nesting behavior has shown to be somewhat unpredictable, research suggests that box turtles typically nest from early May to the middle of July (Dodd 2001). Clutch size varies from one to seven eggs and sex is temperature dependent. In laboratory conditions, where eggs were incubated at a constant temperature, 73% of eggs produced males at 22.5°C, 96% of eggs produced males at 25°C, and 81% of eggs produced males at 27°C. No males were produced at 30°C (Ewert and Nelson 1991; as cited in Dodd 2001). Very little is known about *T. carolina* neonates, but it is believed that they emerge from the nest in the early fall after oviposition (Ernst 2009). Juvenile box turtles may use forest with dense canopy and understory and high moisture content and dense leaf litter more than adults, so forested areas are particularly sensitive to juvenile recruitment (Jennings 2007).

Juvenile box turtles are particularly vulnerable to predators, due not only to their small size, but also because their shells are not as strongly ossified as an adult box turtle. This makes their shell soft and flexible; perfect for predators such as snakes, which can swallow hatchlings whole. Even more vulnerable than a juvenile box turtle is the egg. Eastern box turtle nests have little defense and are only as protected only as much as the female turtle conceals it. The scents that remain after deposition as well as the disturbed soil, attract predators to the newly dug nest (Dodd 2001).

In addition to their shell, box turtles defend themselves by biting and with foul smelling urine and fecal matter. Some turtles may even attempt to run and some even hiss. These behaviors, however, do little to thwart off predators. The most common predators of box turtles are birds, snakes, and small mammals (Dodd 2001).

Species with long generation times such as the Eastern box turtles suffer higher mortality in an urbanized environment than in forested habitat and show a response lag to habitat loss which delays detection of population decline (Dodd 2001). 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, 2017). According to the IUCN, the status of "vulnerable" implies that a taxon is not endangered but is at a high risk of extinction in the wild in the short-term future. They are also listed on CITES (Convention on International Trade in Endangered Species) Appendix II, which aids in regulating international trade (Donaldson and Echternacht 2005).

The unprecedented scale of natural areas converted for human use is the primary driver of biodiversity loss. Terrestrial species of turtles are particularly sensitive to habitat fragmentation (Converse 2005). Habitat loss due to urbanization, agriculture, logging, and road construction are all long- term threats to the declining species. Box turtles (*Terrapene carolina carolina)* have previously been considered common and thus a low conservation priority. Because of their specialized mobility and habitat specificity, many box turtle populations in eastern North America exist in isolated forest fragments in urban and suburban areas (Nazdrowicz et al. 2008) and can become functionally extinct (Dodd 2001). Human-induced disturbances may threaten the viability of many turtle populations. Dodd (2001) stated that the loss and alteration of box turtle habitat is the single greatest threat to their continued existence.

Because of their seasonal migrations as a part of their natural history, reptiles and amphibians are particularly vulnerable to roads. The mortality rate in the southern United States that is due to roads is greater than 5% annually, and exceeds sustainable levels (Steen and Gibbs 2004). Williams and Parker (1987) recorded a 50% reduction in estimated population size of Eastern box turtles over a 13-year period due to habitat destruction and over-collecting. Stickel (1978) reported a similar reduction after a 30-year period and Hall et al. (1999) found a decline of >75% over a 40-year period.

Box turtles also a face a threat from parasites, specifically the ectoparasites leeches, mites, ticks, and flies. Endoparasites such as Trematodes, Cestodes, and Nematodes are also of concern. In a study by Mays (1960), 74% of these parasites were found in the alimentary tract. Infestation by any of these parasites leads to infection, organ failure, and death.

Although fibropapillomas in sea turtles and respiratory diseases in wild tortoises has been reported on, little is known about diseases in wild box turtles. Respiratory diseases have been seen in free-ranging *Terrapene.* Evans (1983; as cited in Dodd 2001) reported chronic bacterial pneumonia in free-ranging *T. carolina*. Other conditions that box turtles face include botfly infestations, sebaceous-like swellings on the neck, and pathological conditions due to abnormal functioning of the reproductive system. In response to infection, box turtles elevate their body temperature like mammals do. However, because they are ectotherms, box turtles bask in direct sunlight to raise their body temperature 4-5°C (Dodd 2001).

Chemical contaminants are a serious threat to box turtles but are rarely considered when examining conservation efforts. Even though chemical exposure may not lead to direct mortality, chronic exposure depresses the animals immune system. Even this small change in homeostasis can lead to death, one without symptoms, that is often reported as unexplained. An even larger threat is compounding the number of chemicals the animal is exposed to as well as their byproducts as they breakdown (Dodd 2001). Although it may seem that box turtles would be in immediate danger of chemical threat due to their position relative to the ground and sedentary life-style, Stickel (1951) found no significant difference in sex ratios, growth, or abundance of eastern box turtles in DDT-treated and untreated sites over a four-year period.

Conservation and management planning of any species cannot take place without proper scientific investigation (Ratti and Garton 1994). Therefore, a basic understanding of a wildlife populations basic population dynamics (gender ratios, abundance, genetic diversity, home range, diet, size, fecundity, mortality, etc.) is necessary for proper conservation of box turtle populations. The factors affecting adult survival rates in relatively intact populations and habitats, for example, is important to determine what factors influence population dynamics apart from direct human-induced interaction (Converse 2005). Understanding the effects of demographic declines on diversity and structure in box turtles is a necessary precursor to developing management strategies for their protection (Kimble 2014). Species in decline have, by definition, undergone demographic reductions, which may confound our ability to differentiate between natural and anthropogenically induced changes in genetic parameters. For example, when studying long-lived species, such as box turtles, investigators must distinguish between predisturbance and anthropogenically induced genetic patterns to inform management strategies (Kimble et. al. 2014).

Developing a conservation plan for the eastern box turtle is difficult to develop because of the complexities of studying a species that is cryptic, long lived (>100 years in the wild), and whose generations overlap (Kimble 2014). But because of their limited range, long lifespan, and ease of capture, the same individuals can be studied year after year. In addition, delayed sexual maturity and low juvenile and egg survivorship may put them at an increased risk and may not allow populations to recover quickly (Klemons 2000). Few nests go undetected by predators. Flitz and Mullins (2006) reported nest predation 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; as cited in Dillard 2016).

Spatial ecology studies that encompass patterns of habitat selection are critical to understanding the life-history and ecology of species and an elucidate patterns of survivorship, reproduction, and population viability (Flitz and Mullin 2006). Nazdrowicz et. al. (2009; as cited in Colson 2009) recommended that agricultural fields adjacent to forests be planted with crops that are not mowed or if they are, mowed at a height of > 15 cm. Relocation is not a suitable option for conservation of the box turtle because displaced turtles will attempt to return to their original home range because of a homing mechanism. This displacement exposes the turtle to a life-threatening, inhospitable matrix (Hester et. al. 2008). Habitat plans within a fragmented landscape should include forested habitat patches adjoining cleared areas for protection so box turtles do not move into less desirable urban areas (Iglay et. al 2007). Long term conservation will require protection of metapopulations and ecosystems and the creation of open-space reservations that correspond to ecosystem function and realities (Klemens 2000).

To study box turtles, researchers often use different methods to track them. Three techniques available to track them include mark-recapture, thread-trailing, and radio telemetry. Mark-recapture is the least labor intensive. The animals are marked and upon recapture, distance is measured between capture points. This method does not tell the researcher much about the animals' habitat choices or regular activities, but simply and estimate of home range using a minimum convex polygon analysis. The second method, thread-trailing is the oldest technique for tracking the turtles. Researchers attach a thread of spool to the shell of the turtle and a thread is laid down as it travels. While this method is gives detailed information about how turtles use their habitats, it does not allow tracking for great lengths of time. Thread trailing can be very useful for juveniles, however, since they are often too small for radio transmitters and which can hinder growth when placed on the shell. The third method for tracking box turtles, radio telemetry, has many benefits. A radio transmitter is placed on the turtle's carapace in a location which does not hinder daily activities, growth, or mating. This method provides much longer periods of tracking movements than does thread trailing and allows researchers to study more turtles at one time (Dodd 2001).

It is currently unknown if Eastern box turtles are showing signs of serious decline in eastern 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 (Dillard 2016).

This study took place in a small (57ha) woodlot on the campus of Maryville College, Maryville, Tennessee, USA. The mixed mesophytic forest in this study is surrounded by homes, business, and a college campus which creates an isolated habitat for this box turtle population. To our knowledge, no research has yet been done on the turtles within our study area, so any information gathered will represent important baseline data for all future studies. The objectives of this study are therefore to: 1) measure box turtle abundance, gender ratio, age structure, individual size, fecundity, and mortality rates by hand capture of individuals, 2) to determine home range sizes of turtles fixed with a radio telemetry beacon, 3) determine areas of turtle habitat preference in terms of habitat type, and 4) create GIS maps of the box turtle population within our study area. By putting these factors together, the researchers hope to not only understand the population of box turtles which reside within the study area, but also create a place that they will remain for many years. The null hypotheses of this study are that 1) there will be an equal number of males and females, 2) there will be an equal number of adults and juveniles, 3) there will be no difference in home range sizes amongst turtles, 4) there will be no preference in habitat type, and 5) there will be no preference for English Ivy.

CHAPTER II

MATERIALS AND METHODS

The Maryville College woods, Maryville, TN (figure 1) were divided into sections based on pre-existing trails. Groups of researchers and volunteers walked transects looking for turtles on and in vegetation. Once a turtle was located, its GPS (Global Positioning System) location was marked (GPS, Garmin 72H, 1200 151st St. Olathe, KS 66062-3426), it was assigned a number which was written on a piece of tape and placed on its shell for identification in the lab. Turtle activity at the time of capture (feeding, mating, moving, resting, etc.) was recorded.

Turtles were weighed (grams; Mettler, Toledo, OH) and measured. All body measurements were in millimeters except circumference, which was measured in centimeters. The measurements included carapace length and width, plastron length and width, shell height, and shell circumference. All body measurements were done using a digital caliper (Mitutoyo Corporation, Kawaski, Japan) with the exception of circumference, which was done using a nylon metric tape (Forestry Suppliers Incorporated, Jackson MS). Also noted was whether the shell was open or closed at the time of processing as it influenced measurements. The turtle was given a number based on the order in which it was found. The number was marked on shell scoots via a triangular file following a numbering system developed at Maryville College, Maryville, TN (Dr. Dave Unger, Associate Professor of Biology, Maryville College, personal communication) (Figure 1). Selected turtles received a transmitter (Advanced Telemetry Systems, 4701 1st Ave. N Isanti, MN.) and it was attached with epoxy (Gorilla Glue, Cincinnati, OH) on the shell. The turtle was then released at the precise location it was found.

From May 2016 to December 2016 and April 2017 to October 2017, turtles with transmitters were located once per week, and from January 2017- March 2017, turtles with transmitters were located once every two weeks via radio telemetry (Telonics TR-4 Reciever and RA-23 Antenna, Telemetry-Electronics Consultants, 932 E. Impala Ave. Mesa, AZ 85204-6699). Prior to locating each turtle, the date, time, and weather (Weather Channel Application, 300 Interstate N Pkwy, Atlanta GA 30339) were recorded in a Rite in the Rain notebook (JL Darling LLC, 2614 Pacific Hwy East, Tacoma, WA 98424-1017). When each turtle was located, its GPS location, macro habitat, micro habitat, and activity were recorded.

Averages and standard deviations were calculated for all morphometric data, as well as t-tests to determine significance for male and female morphometric data (Microsoft Excel, Microsoft Corporation, Redmond, WA). A chi square was used for ivy use. Turtles marked prior to July 2017 were compared against new turtles captured over a two day period in July

2017 using turtle sniffing dogs. These results were used in a Lincoln-Peterson mark-recapture study to estimate population density. The Lincoln-Peterson mark-recapture analysis (Lincoln 1930) were calculated in Microsoft Excel (Microsoft Corporation, Redmond, WA).

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Home range size was calculated with ArcMap 10.4 (ESRI, 380 New York Street), via minimum convex polygon. Additionally, all maps were created using ArcMap 10.4 including home range maps, found locations maps, and telemetry maps.

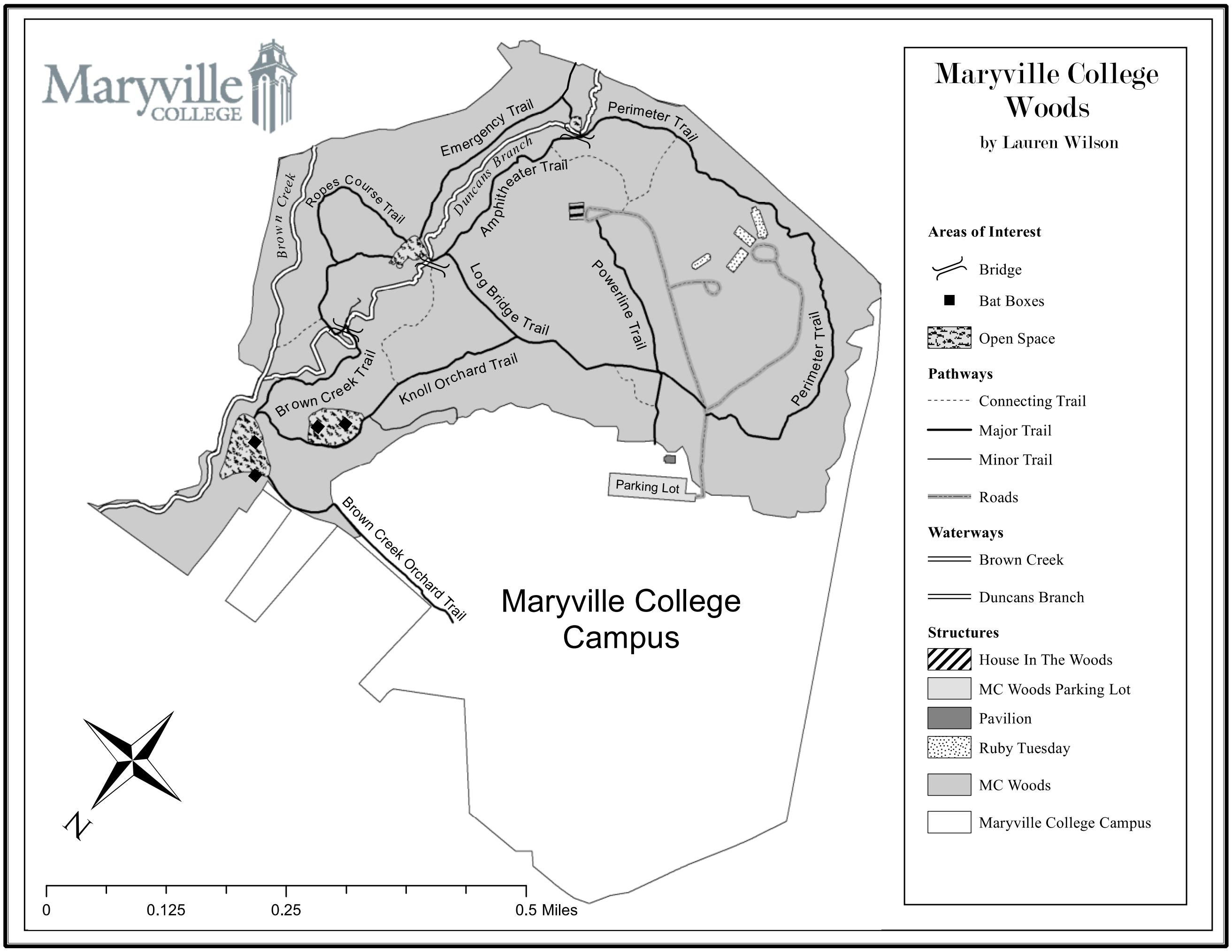

Figure 1. GIS map of the Maryville College Campus, Maryville, Tennessee. The study location is indicated by “MC Woods”. Cartographer: Lauren Wilson.

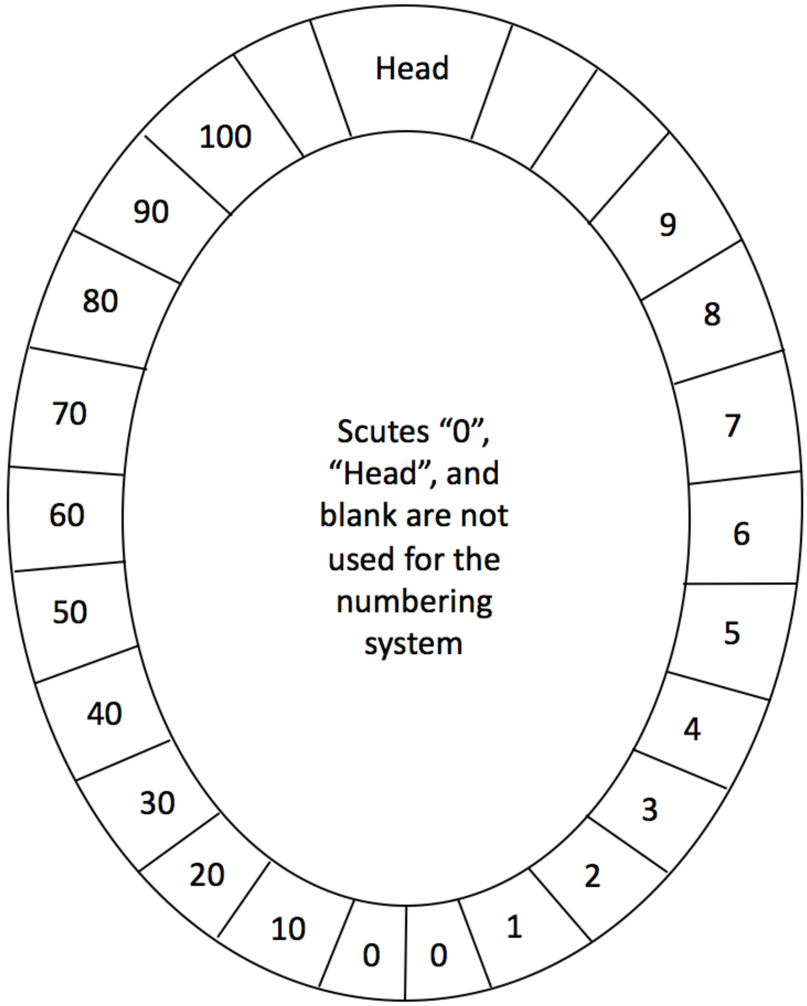

Figure 2. Numbering system created by Dr. Dave Unger (Associate Professor of Biology, Maryville College, personal communication) for Eastern Box turtles in the Maryville College Woods, Maryville, Tennessee. Turtles were marked in sequential order starting with turtle “00”.

CHAPTER III

RESULTS

A total of 109 turtles were captured and measured (figure 3). Of the turtles studied, 67 were male, 36 were female, 6 were of unknown sex, 94 were adults, and 15 were juveniles (table 1). Average morphometric data resulted in an average plastron width 66.65 mm (SD=13.12 mm), average plastron length 108.86 (SD=16.13 mm), average carapace width 86.87 (SD= 17.72 mm), average carapace length 118.08 (SD=24.02 mm), average shell height 52.69 mm (SD=52.69 mm), average circumference 24.60 cm (SD=4.58 cm), and average mass 341.40 g (SD=129.16 g) (table 2). Males had significantly larger carapace widths (p=0.0002), carapace length (p=0.003), plastron width (p=0.0006), plastron length (p=0.00002), circumference (p=0.0006), shell height (p=0.05), and mass (p=0.0005) than females. A Lincoln-Peterson mark-recapture analysis estimated that the abundance of the box turtle population in the Maryville College woods is 588 turtles. This estimate equates to density of 10 turtles per hectare.

Additionally, turtles tracked via radio telemetry (n=17) had an average home-range size of 0.74 hectares (figure 4). Females had an average home-range size of 0.70 hectares while males had an average home-range size of 0.77 hectares. There was no significant difference (p=0.87) in female and male home-range size (table 3). In thirty-percent of tracking events, turtles were found to be in open areas, 19% in English Ivy, 37% under something such as leaves, sticks, or logs, 7% buried under ground, 5% in high grass, and 5%

in washouts (figure 2). Turtles were found less often in English ivy than would be expected by chance (P<0.0001).

Five turtles were lost during the duration of the study. Two turtles became undetectable either due to a malfunctioning transmitter or by leaving the study area, three turtles were found dead (two of unknown causes and one was run over by a lawnmower), and one transmitter was found that had detached from the turtle's shell.

Table 1. Age distribution and sex ratios for Eastern Box turtles (*Terrapene Carolina carolina)* (n=109) captured between September 2014 and September 2017 in the Maryville College Woods, Maryville, Tennessee.

Age Ratio		Male	67
Juveniles	15	Female	36
Adults	94	Unknown	6
Total	109	Total	109

Table 2. Average morphometric data for all Eastern Box turtles (*Terrapene Carolina carolina)*(n=109), as well as averages for male and female turtles, captured between September 2014 and September 2017 in the Maryville College Woods, Maryville, Tennessee.

	Plastron Width (mm)	Plastron Length (mm)	Carapace Width (mm)	Carapace Length (mm)	Circumference (cm)	Shell Height (mm)	Mass (g)
Average All	66.65	108.86	86.78	118.08	24.60	52.69	341.40
Standard Deviation All	13.12	16.13	17.72	24.02	4.58	8.90	129.16
Average Male	71.50	116.35	91.99	128.23	26.16	56.42	384.22
Standard Deviation Male	7.97	12.20	15.20	13.97	3.34	6.36	97.98
Average Female	60.60	101.30	80.80	105.28	22.88	52.39	291.27
Standard Deviation Female	15.43	21.84	17.90	27.05	4.83	6.96	131.99

Table 3. Home-range sizes and average home-range size for 17 Eastern Box turtles tracked in the Maryville College woods, Maryville, Tennessee from May 2016 to August 2017. Home range was calculated via minimum convex polygon using ArcMap 10.4.

Turtle #	# of Events	Home Range (ha)
10	18	0.54
16	13	0.53
17	69	0.73
18	70	0.58
19	60	0.40
20	71	1.02
29	21	0.24
32	43	0.69
37	40	1.32
38	36	1.55
51	31	0.37
52	34	3.34
57	32	0.12
58	15	0.03
64	29	0.19
100	11	0.18
Average Home Range		0.74
Standard Deviation		0.81

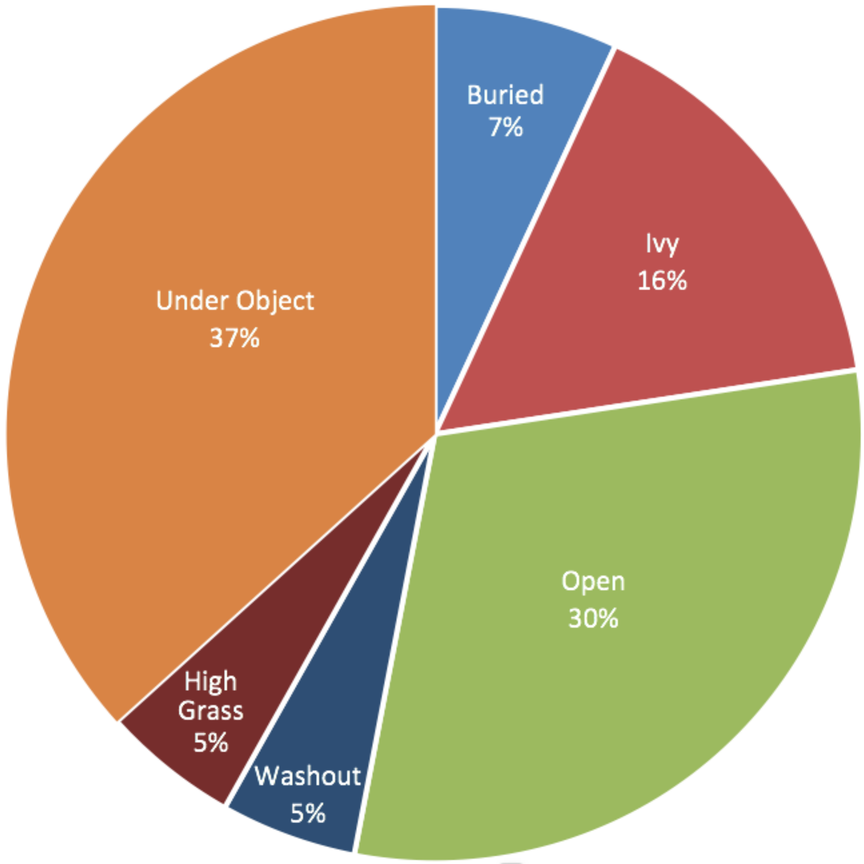

Figure 3. Microhabitat types for 17 Eastern Box turtles in the Maryville College Woods, Maryville, Tennesee that were tracked from May 2016 to August 2017. Open areas indicated that the turtles were not buried under any item on the forest floor. Objects that the turtles were found under include logs, sticks, and leaves.

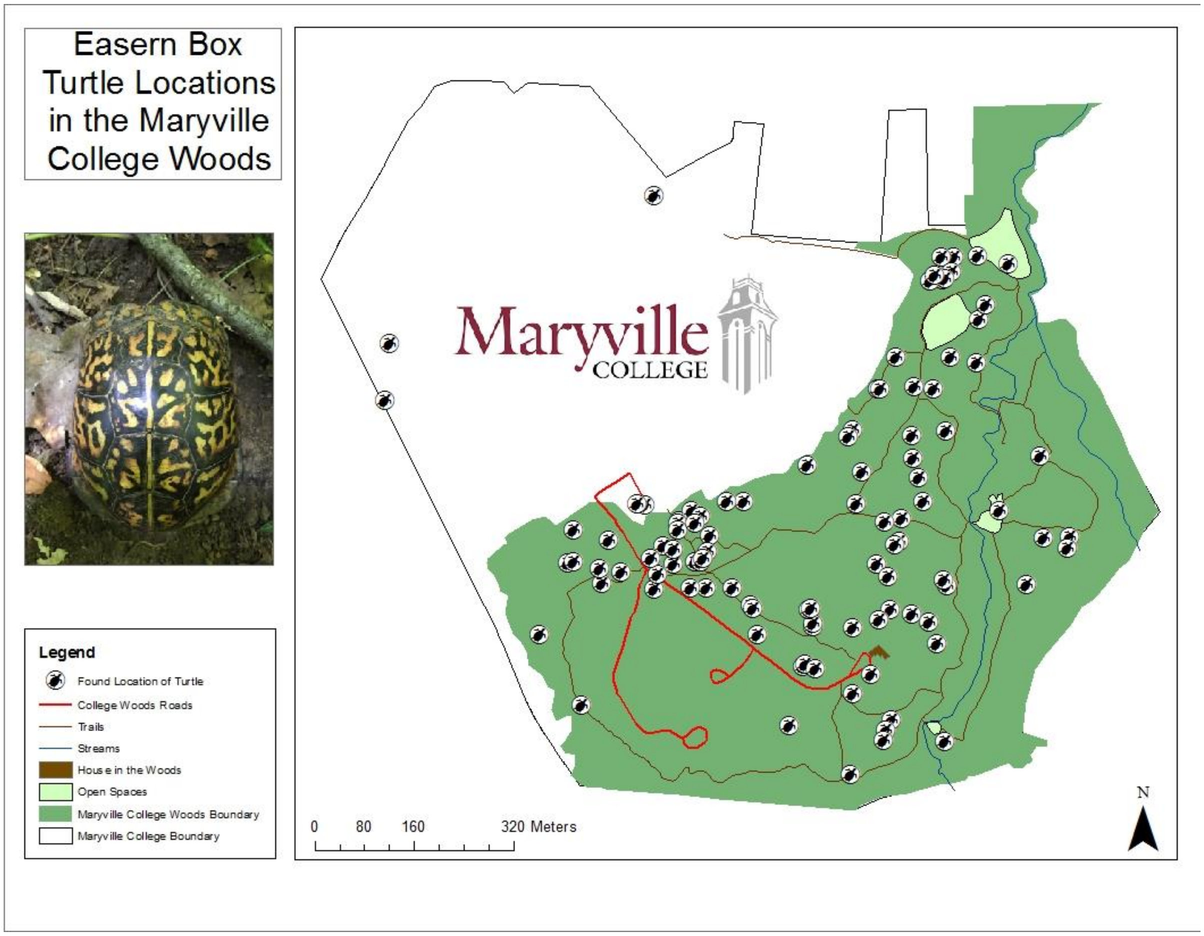

Figure 4. Found locations of all marked Eastern Box turtles (n=109) in the Maryville College Woods, Maryville, Tennessee from September 2014 to September 2017.

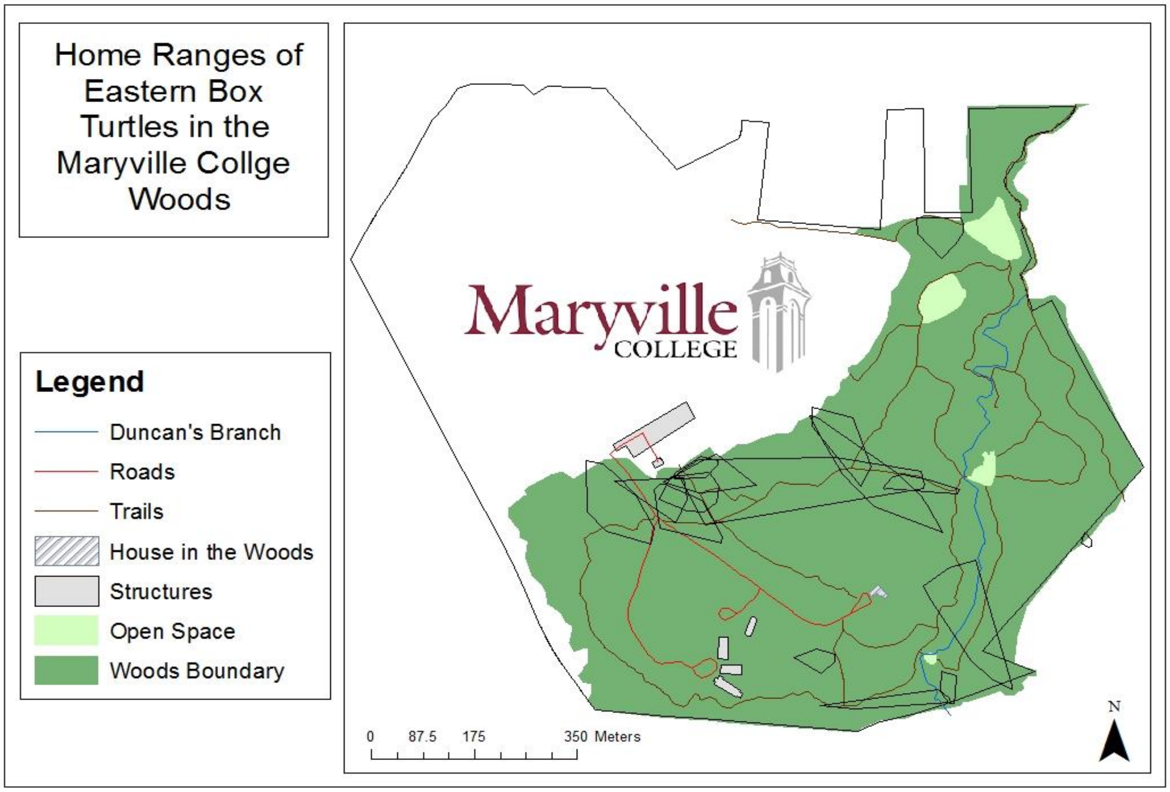

Figure 5. Home-ranges for 17 Eastern Box turtles tracked via radio telemetry in the Maryville College Woods, Maryville, Tennessee from May 2016 to August 2017. Home ranges were calculated using minimum convex polygon in ArcMap10.4.

CHAPTER IV

DISCUSSION

Many reptile populations have declined worldwide as a result of factors such as habitat loss and degradation, pollution, disease, and collection for food and the pet trade (Gibbons et al. 2000). Populations of Eastern Box turtles have declined dramatically in the last several decades. Williams and Parker (1987) recorded a 50% reduction in estimated population size over 13 years due to habitat destruction and over collecting, Stickel (1978) reported a similar reduction after 30 years, and Hall et al. (1930) reported a decline of over 75% after 40 years (as cited in Donaldson 2005). Eastern box turtle populations are threatened by urban and suburban development that may increase the likelihood of injury and mortality from vehicles, mowers, edge predators, and collection for pets. Habitat alteration can have serious impacts on how animals use and move within the landscape as well as the resources available to them. Habitat fragmentation can alter the quality of the habitat and reduce the resources a population needs to survive (Dillard 2016).

The rate at which habitat is lost may impact wildlife populations more than the actual loss or fragmentation of habitat. When the rate of landscape change exceeds the regeneration time of the species, populations may exhibit a lagged response to habitat loss. Indeed, the landscape ecological theory suggests the rate of habitat change is more critical to the viability of a population than the pattern of change. Therefore, an important topic of research is the rate at which a population is experiencing disturbance patters like road density. The rates of change vary for each habitat and cannot be extrapolated from similar structures (Colson 2009).

This was the first ever study of this population of Eastern Box turtles in the Maryville College woods of Maryville, Tennessee. This study is significant because 1) nothing is currently known about this population and 2) the college woods are isolated in a fragmented landscape. By analyzing sex ratios, age distribution, and home ranges, we can begin to create a conservation plan for the turtles which live within the Maryville College Woods.

We captured and marked 109 box turtles during the duration of this study. Fourteen percent of the marked population of turtles in the college woods were juveniles. Because turtles of all age ranges were captured, this suggests a population that is reproductively active. However, juveniles are often under-represented in habitat analyses. This may be because they use different habitats, and often habitats with higher vegetation cover that may be difficult to survey (Jennings 2007). The Maryville College woods are heavily covered with English Ivy in many areas, which could provide areas of protection for juveniles against predation. This would skew our age distribution as most searches for turtles were conducted on foot. Therefore, turtles found were those that could be located visually. Future research should investigate juvenile microhabitat use. The use of turtle sniffing dogs would provide the means necessary to find juveniles. Knowing whether juveniles prefer to use English Ivy as protection from predation is important to the conservation of this population and should be noted in any research pertaining to eradication of the invasive species of ivy.

Unlike Budischak (2006), we found more male than female turtles in the Maryville College woods. However, other studies have found that sex ratios range from even to heavily male-biased (Kiester et al. 2015 and Dillard 2016). This difference in sex ratios in the population in the Maryville College woods could affect overall reproduction rates and should be considered in future studies. Because there does not appear to be as many females as males, reproductive rates may not be as high as possible, given equal sex ratios. Our male dominated population could be an indication that local climates do not lend to even or female dominated clutches due to temperature dependent sex determination. One consideration for a male dominated population in the Maryville College woods is the high abundance of English Ivy. Clutches will be male dominated at 22-27°C. We speculate that the English ivy cover on the forest floor of the college woods may lower soil temperature and result in male dominated clutches. McKnight and Ligon (2017) noted that the heterogeneity of capture possibilities can bias both population size and sex ratio estimates. This problem can be overcome by treating males and females as separate populations and calculating a population estimate for each of them. Future research should focus on sex ratios and eliminating any bias that may result in incorrect estimates. Soil should be monitored for temperature in both areas with and without ivy to determine whether temperature could be a factor in this male-dominated population.

Also notably different than previous studies was morphometric data of males and females. Previous studies found box turtles to have an average mass of 361.0 g (Budischak 2006) and 363.0 g (Dillard 2016), while we found a slightly lower average of 341.0 g. This discretion is likely due to the inclusion of juveniles in our average measurement of mass compared to the high sample size of Busischak (2006) and exclusion of juveniles altogether in Dillard (2016). A post-hoc analysis of average weight of the population of turtles at Maryville College resulted in an average mass of 385.44 g. This result suggests that our lower average mass than other studies can be at least partially explained by the high number of juveniles present in this population.

Our findings generally agree with Budischak (2006) with regards to other morphometric aspects. They found males had significantly longer carapaces, shallower shell depths, and shorter plastron lengths than females. They found that both sexes had similar masses. However, we found males were significantly larger than females for all measurements (carapace width, carapace length, plastron width, plastron length, circumference, shell depth) as well as mass. These differences could be attributed to sample size, differences in sampling methods, or large population size. Access to resources within the Maryville College woods is also an important factor in the growth of the turtles. The college woods are largely undisturbed in regards to the forest floor and its resources. Trails provide predetermined paths for visitors into the woods and dense growth of the forest floor prevents most visitors from leaving trails. There are no known large predators within the college woods due to its isolation within a fragmented landscape. This leaves resources for small mammals and reptiles.

Larger populations may be more stable than smaller ones and are therefore more likely to have greater genetic variability. This variability provides more adaptability to environmental changes through natural selection. Future studies of this population should examine the genetic variability and relatedness of the turtles within the college woods. Knowing how the turtles within the college woods are related would provide significant insight into how they interact with one another and would allow important knowledge about how this population came to exist.

Only two mating events were witnessed during this study, both during July 2016. There were no nests identified, although there were reports from visitors to the college woods that reported seeing a female digging a nest (Dr. David Unger, Associate Professor of Biology Maryville College, Maryville, TN, personal communication). Box turtles have been reported to mate during the entire active season, but most mating events have been reported in the spring. Willey (2012) found that females used abandoned gravel pits, right-of-ways, backyards, old fields, and forest clearings as nest sites. They noted that nest sites tended to be sandy, open areas with little vegetation. The exact criteria for nesting site selection is unknown.

Congello (1978; as cited in Dodd 2001) suggested that preferred nesting sites may be in sunnier areas of an otherwise shaded forest floor. It is possible that females choose sunnier spots for nesting to increase temperature in order to increase developmental rates. An unintentional result of this nesting site selection is sex ratio determination. If shaded areas are more prevalent in a study area, as is the case in the Maryville College woods, sex ratios will be skewed. Shaded nesting sites would produce more males due to lower temperatures and sunnier nesting sites would produce more females. Females may dig nests anytime throughout the active season and box turtles are not synchronous, unlike some sea turtles. Nesting typically peaks in June, but can vary depending on environmental conditions. Box turtles may return to the same area to nest, especially if previous clutches were hatched successfully there. Females often test nesting sites prior to laying her eggs. They do so by scratching the soil surface with their back feet (Dodd 2001).

Because no nesting events were observed, it is a key element for future research within the Maryville College woods. Research should focus on timing of site selection, digging, egg deposition, and concealment. During this study, tracking events always occurred in the early morning to early afternoon hours. Tracking events were usually during dry times to protect equipment and sometimes after brief rainfall. Eastern box turtles are known to nest just before, during, or after rainfall. Congello (1987; as cited in Dodd 2001) suggested that photic stimulus or lunar cue may be involved in stimulating nesting activity. Nesting has most often been reported in the late afternoon toward dusk or after dark (Dodd 2001). Future studies of the population of box turtles in the Maryville College woods should emphasize nesting behavior. Tracking events of females should occur as often as possible, in the late afternoon, and close to periods of rainfall.

The density of this population was estimated to be 10 turtles per hectare via a Lincoln-Peterson mark-recapture study. Other populations of Eastern Box turtles have been estimated from 2.05 (Willey 2010) to 20.76 turtles/ha (Dolbeer 1968) (table 4). Density estimates for other studies not included in the table range from 0.22 ha (Davis 1981) to 187.6 ha (Currylow et al. 2013). It is important to note that estimations of population density may be calculated in different ways. Dolbeer (1968) used a multiple-mark recapture study developed by Jolly (1965; as cited in Dolbeer 1968). This method is stochastic (random) and estimates the population size at each collecting trip. This method is not completely reliable and therefore estimated population ranged all the way from 99 to 386 turtles per acre between trips. His average estimate of 20.76 turtles/ha is much higher than most other studies and methods should be taken into consideration when comparing these studies.

Our population is more dense than populations in most other studies. Comparing our population to those in table 4, only two other studies (Stickel 1950, Maryland and Wilson and Ersnt 2005, Virginia) had higher densities. Dolbeer (1968) was excluded from this comparison due to sampling and calculation methods. Average density of these studies was 6.44 turtles/ha. The population density of the turtles in the Maryville College woods is higher than average, suggesting the woods provide habitat and nutritional needs to allow for a thriving population.

Table 4. Estimated Eastern Box turtle densities in studies of previous populations. The estimated density of the Eastern Box turtle population in the Maryville College woods, Maryville, Tennessee is listed at the top of the table and was calculated using a Lincoln-Peterson mark-recapture study. Table was revised from Kiester et al. 2015.

Author(s)	Location	Date of Study	Turtles / ha
This Study	*TN*	*2016-2017*	*10*
Nazdrowicz et al. 2008	DE	2001-2003	2.22
Williams and Parker 1987	IN	1958-1984	3.70
Willey 2010	MA	2005-2008	2.05
Stickel 1950	MD	1944-1947	10.63
Hallgren-Scaffidi 1986	MD	1984-1985	8.62
Hagood 2009	MD	2005-2006	7.60
Kapfer et al. 2012	NC	2011	2.86
Chute 2007	NC	1999-2006	7.00
Madden 1975	NY	1969-1972	3.71
Dolbeer	TN	1968	20.76
Wilson and Ernst 2005	VA	2000-2002	16.00

There was no significant difference in female and male home range sizes which is consistent with other studies that state home range sizes are not significantly different

(Stickel 1950; Dolbeer 1969; Cook 2004; Baker 2009; Aall 2011, and Kapfer et al 2013). Additionally, box turtles are known to remain in the same home ranges throughout their life (Claussen et al 1991). We found a much lower average home range (0.74 ha) than previous studies (Busischak 2006; 3.77 turtles/ha, Nazdrowicz et al. 2008; 3.26 turtles/ha, Donaldson 2005; 1.88 turtles/ha) using the minimum convex polygon method. Our study area was a small, 57 hectare plot of deciduous forest. It is a fragmented landscape, surround by roads and highways, as well as commercial and residential property on all sides. It is managed by Maryville College, Maryville, Tennessee. Because this area is privately owned and maintained, it provides all nutritional, microhabitat, and reproductive needs for the box turtles in a smaller space. Due to the size of the college woods and the high estimated population density, turtles likely do not have to travel far for mating purposes. The high estimated density also suggests that the college woods are high in nutritional resources and microhabitat availability. Small home ranges may not be unusual. In a study by Schwartz and Schwartz (1974), seventy percent of the home ranges of 239 T.*c. triunguis* were less than 2 ha (as cited in Dodd 2001). Habitat quality, habitat diversity, and individual preference account for variation in home range size.

Because Eastern Box turtles are not social animals, home range size is not likely due to locations of other turtles. Dodd (2001) noted that currently, there are no studies which show a correlation between population density and home range size. In habitats where food sources are abundant, turtles will not have to travel as far for these resources. Box turtles need access to food, protection, nesting sites, and access to other turtles. In study areas where these resources are abundant, as our study suggests is true for the Maryville College woods, turtles will not have large home ranges. In addition to resources, habitat structure can affect home range size. Obstacles such as fences, buildings, roads, ponds, ravines, and tall grasses can all affect movements. The more obstacles a habitat has, the larger home range the turtles will have due to the need to navigate around them. Age may also affect home range size. juveniles have been found to increase home range size and location as they grow larger (Dodd 2001).

Although no turtles were found in water, movements tracked via radio telemetry indicated that at least one turtle crossed creeks within the college woods on more than one occasion. The Maryville College woods are bisected by Duncan's Branch and Browns Creek from south to north. To access the northeast part of the woods, turtles would have to cross the creek or remain on one side. Turtles were found on both sides of the creeks, but only one appeared to cross back and forth. This turtle was found on both the east and west sides of the creeks during different tracking events, indicating that she must have crossed more than once to access each side of the woods. There is debate among naturalists as to whether or not box turtles like water. Eastern Box turtles are capable of swimming both on the surface and underwater. In the heat of the season, they have been found congregating along the edges of ponds and streams (Dodd 2001).

Our results indicate a thriving species despite habitat fragmentation. Only three turtles were found deceased during the duration of this study, indicating a 98% survival rate. Annual survival rates for adults can range from 81%- 96%, but have been as low as 56% (Nazdrowicz et al. 2008, Currylow et al. 2010, and Verdon and Donnelly 2005). Only one of those found deceased was due to anthropogenic factors (lawn mower). Kiester et al (2015) found that sources of mortality often include predation, disease, forest fire, prescribed burns, road mortality, mowing, and winterkill. A study in Alabama showed that box turtles accounted for 85% of the turtles killed on a series of roads (Dodd et al. 1989; as cited in Budischak 2006).

Management Implications

The results of this study offer insight into the dynamics of a previously unstudied population of Eastern Box turtles. One area of interest that may be difficult to answer based on the campus location is whether this population was isolated here or whether the turtles arrived after fragmentation. More research of this population's genetic variability is needed.

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 Ginsburg 1998; as cited in Dillard 2016). This study examined home range to determine the types of habitat turtles spend their time in and if that has any implications for their survival. Specifically, we looked at English Ivy use. Our results indicated no significant preference for it. We tracked one turtle that was captured in, and consistently tracked, within ivy. Future research should look deeper into English ivy use and whether turtles use it for any specific purposes. Additionally, the use of English ivy by juveniles for hiding from predators as well as its role in temperature dependent sex determination should be considered. Turtles seemed to have a preference for open areas of the forest floor and the ability to hide under fallen trees. An appropriate balance of open forest floor as well as areas to hide, such as fallen logs and leaf litter, must be maintained. Pastures, such as the orchards within the Maryville college woods, should not be mowed during the spring and summer months when turtles are most active. These areas should only be mowed during the winter months to avoid adult turtle mortality. Open areas within the woods that offer canopy cover and well-drained soils should be maintained as such because they are ideal nesting habitat. Females often select sunlit areas for nesting in order to facilitate incubation (Frederickson 2014). Additionally, juveniles prefer dense vegetation to avoid predation. Allowing these pastures to grow would provide the necessary space for juvenile survival.

It is important to create an educational campaign among students on the Maryville College campus. Many natural science students are already aware of the box turtle project and have assisted in searching for turtles. Presentations of this study should be used to inform the remainder of the student body of the presence of this species within the college woods and what its preservation means to our community. This ongoing campaign would seek to educate students on this population of box turtles, how they can help in our growing knowledge of it, and what to do if they cross one in the college woods.

Additionally, the town of Maryville is very involved in Maryville College and often assists in projects around campus such as maintaining the orchards and campus beautification projects. Reaching out to the community about our population of box turtles for the purpose of searches could help in identifying more turtles and increase our knowledge about its existence. More important than help with the searches is outreach regarding box turtle conservation. Educating the Maryville community is extremely important. One threat to box turtles is collection for pet trade. It is important that the public be educated as to why keeping wild box turtles as pets threaten their species and harm the turtle. Public outreach could be accomplished through presentations at the college and information on the college's website.

This study was the first every study of the population of Eastern Box turtles in the Maryville College woods. There are many areas of future research that should be considered before we can begin to understand this population. Searches for turtles should continue throughout the active seasons to increase sample size. The more turtles that are marked and measured, the more information we can gather about what role the college woods and its resources provide for this species. Additionally, studies into the genetics of this population should provide information about relatedness. Knowing how the turtles interact with one another would give unprecedented insight into this population.

Although we found many juveniles during this study, more information is needed about them. A method should be developed to track juveniles. It is important to know where they go and how they use the habitat. Turtle dogs should be use to find juveniles to aid in this research as juveniles are hard to find by searches on foot. Examining sex ratios should also be considered for future research. This population, as we know it, is currently male dominated. We do not know, however, if this is due to search methods, bias, or coincidence. Soil temperature throughout the college woods should be monitored, focusing on the differences between areas with and areas without ivy to determine whether ivy could play a role in lower clutch incubation temperatures.

Lastly, monitoring nesting activity should be a top priority. Females should be tracked often, in the late afternoon to dark, and as close to rainfall as possible. Nests should be identified, monitored, and protected from predation.

APPENDICES

MARYVILLE COLLEGE INSTITUTIONAL ANIMAL CARE & USE COMMITTEE (IACUC)
Application for Use of Vertebrate Animals in Faculty Research or Teaching

Faculty at Maryville College that use vertebrate animals in teaching or research are required to complete an IACUC proposal for each project.

Provide information after each bold item
Faculty Name: Nathan Duncan and David Unger

Email Address: Nathan.duncan@maryvillecollege.edu; dave.unger@maryvillecollege.edu

Date: September 19, 2015

Species to be used: Eastern Box Turtle (*Terrapene carolina Carolina***)**

Age of animals: hatchling to 25

Number of animals in study: NA

Brief description of use (teaching or research): Long-term mark and recapture study to examine habitat preferences of Eastern Box Turtles (*Terrapene carolina Carolina***)**

Duration of use: Ongoing

Location of animals (building and room) : Sutton 119. Captives will be held for a maximum of 72 hours to attach thetransmitter.

List personnel to call if problems with animals develop:

Name	Daytime Phone	Nighttime Phone	Emergency No.
Dave Unger			
Nathan Duncan			

What will happen to the animals at the end of the use? If euthanasia is required, state the methods.

Animals will be released at their point of capture.

(Do not write below line: For MC IACUC Use)

Maryville College IACUC Approval Number: __201505__________
Date Approved: ___Sept. 19, 2015_____________
Signed:

Appendix 2: TWRA Scientific Collection Permit

TENNESSEE WILDLIFE RESOURCES AGENCY

ELLINGTON AGRICULTURAL CENTER

P.O. BOX 40747

NASHVILLE, TN 37204

Scientific Collection Permit

Permit # 3908 **Expiration Date** 3/24/2018 **Date of last annual report:**

Permittee(s)

David Unger, Nathan Duncan, Drew Crain, Jennifer Brigatti.

Organization Name

Maryville College

Address 502 East Lamar Alexander Parkway

City Maryville **State** TN **Zip Code** 37804-

Work # 865-981-8009

Email dave.unger@maryvillecollege.edu

Species to Collect:

Box Turtles (Terrapene carolina carolina). Capture and release at capture site. Attachment of radio transmitters permitted. May take swabs for disease testing.

Summary of Need:

Presence/absence surveys, population dynamics and home range study.

A map of TWRA administrative regions and counties may be obtained at...

http://www.state.tn.us/twra/pdfs/allregionsdistrictmap.pdf

Location:	Collection Method(s):	Disposition of Specimens:
Maryville College campus, Maryville, TN (Blount County)	Hand	Release at capture site

Appendix 3: TWRA Scientific Permit Amendment

TENNESSEE WILDLIFE RESOURCES AGENCY

ELLINGTON AGRICULTURAL CENTER
P. O. BOX 40747
NASHVILLE, TENNESSEE 37204

TO: David Unger, Maryville College

FROM: Russell L. Boles, Wildlife Criminal Investigator

SUBJECT: Scientific Permit #3908 Amendment

DATE: March 27, 2017

Scientific Permit # 3908 has been amended in regards to collecting in the field. The following changes shall be added to Scientific Permit #3908:

The State of Tennessee
AN EQUAL OPPORTUNITY EMPLOYER

May collect blood and tissue samples for genetic testing. Will be held future studies.

The following name shall be added to the permit:

Valerie Whitehead

In accordance to the Rules and Regulations governing Scientific Permits, a copy of this document shall be attached to the permit and be in possession of personnel collecting in the field.

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