

MOVEMENT AND HABITAT ECOLOGY OF PROTECTED SPECIES  
IN NORTH CAROLINA

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partial fulfillment of the requirements for the degree of Master of Science in Biology.

By

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## ABSTRACT

### MOVEMENT AND HABITAT ECOLOGY OF PROTECTED SPECIES IN NORTH CAROLINA

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Reptiles and amphibians are declining worldwide, especially from global climate change and habitat loss and fragmentation. Conservation efforts for imperiled species usually involve habitat protection, but are only effective if biologists and land managers have a thorough understanding of a species' habitat requirements. This prerequisite knowledge is complicated for many herpetofauna because they utilize different habitats throughout their lifetime, such as separate breeding and non-breeding habitats. Thus, multiple habitats must be studied and protected for conservation to be successful. This research aimed to better understand the habitat ecology of two protected herpetofaunal species in North Carolina to enhance future conservation. The first species, mountain chorus frogs (*Pseudacris brachyphona*), are small, terrestrial frogs, and a state species of special concern. Like many amphibians their breeding habitat has been studied, but little is known about their post-breeding habitat. Nineteen individuals from two breeding sites were tracked by radio telemetry for approximately 25 days as they left their breeding site to examine their post-breeding habitat. Breeding pools were surrounded closely by field and orchard habitats, and more distantly by forest. Frogs traveled 11.4-475.6 m from their breeding site, and no macrohabitat selection was detected among available habitats. However,

the majority of individuals from the breeding site nearest the forest entered the forest, and the farthest traveling individuals from the other breeding site did as well. Mountain chorus frogs likely continued moving after 25 days, and were selecting forest habitat. I measured percent cover of vegetation within 1 m<sup>2</sup> plots in all habitats, and forest had significantly greater leaf litter and canopy cover than did field and orchard. Likewise, there were significantly more burrows available in the forest than in other habitats. These habitat characteristics would provide greater protection from predators and desiccation in the forest, which could explain preference for forest. The second species studied, the bog turtle (*Glyptemys muhlenbergii*), is a small freshwater species, that is both federally- and state-threatened. The majority of its habitat in the southeastern United States is small wetlands in livestock pastures, dominated by emergent vegetation (rushes and sedges) and with little shrub and canopy cover. I followed the movements of six turtles using radio telemetry from May-October 2015 in a unique bog turtle wetland. This site is in Nantahala National Forest, has likely had little human disturbance for 80 years, and approximately half of the wetland is shrub/scrub habitat. Resident turtles significantly preferred shrub/scrub habitat with 68% of locations within this habitat type. I located two nests, both in emergent habitat. Shrub/scrub had significantly greater abundance of deep mud, which could explain turtles' preference for this habitat. Bog turtles frequently burrow down into mud, and deeper mud might be easier to move through. Females had greater mean daily movement rates and home ranges (8.3 m/day, 0.6064 ha) than males (5.0 m/day, 0.4458 ha), which might be due to nesting migrations to emergent habitat. Thus, bog turtles will utilize shrub/scrub habitat where available, but nest in emergent habitat, likely due to its better thermal environment for offspring development.

## INTRODUCTION

Amphibian population declines have been documented worldwide since the late 1970s (Wake 1991). A variety of causes have been linked to these declines, including exotic or invasive species, over-exploitation, habitat loss and fragmentation, global climate change, and disease, and it is likely that these causes are interacting with each other in most scenarios (Collins and Storfer 2003). Unfortunately, recent research has suggested that these declines could be worse than previously thought. Adams et al. (2013) reported that the annual mean rate of change in amphibian occupancy throughout the United States was -2.7% for species listed as least concern by the International Union for Conservation of Nature (IUCN), and -11.6% for species listed as near threatened, vulnerable, and endangered. Furthermore, many of their study sites were on federally protected land already being managed for wildlife habitat. Thus, the trend of amphibian declines is extending to species that may not currently be of conservation concern, and habitat protection alone may not be a sufficient remedy.

Similar to amphibians, reptiles are declining globally as well (Gibbons et al. 2000; Böhm et al. 2013). In their global assessment of 1500 species, Böhm et al. (2013) concluded that 19% of all reptile species are threatened, and crocodylians and turtles are disproportionately affected. Many of these species are at risk due to their slower life history rates (Gibbons et al. 2000; Reading et al. 2010), and/or small range size, habitat specialization, and high human accessibility to their habitats (Böhm et al. 2016). Also like amphibians, many at-risk reptiles currently may not be on the radar of conservation biologists. For example, approximately half of the species identified as threatened by Böhm et al. (2013) were only listed as vulnerable by the IUCN.

Habitat loss and fragmentation and global climate change are the greatest threats to all biodiversity, including herpetofauna (Sala et al. 2000; Gardner et al. 2007). Specifically, it is the

rapidity of these changes that makes them so threatening because herpetofauna may not be able to disperse or adapt quickly enough to keep up with new their environmental conditions. Reptiles and amphibians tend to be poor dispersers and philopatric (Gibbons et al. 2000; Araújo et al. 2006). Araújo et al. (2006) used the climatic conditions European reptiles and amphibians experience in their current distributions to predict their future distributions under multiple climate change scenarios. Almost all species' distributions shrank under the assumption of no dispersal, and even under the unlikely assumption of unlimited dispersal one-third of species' distributions still decreased. Likewise, juvenile dispersal of the common lizard (*Lacerta vivipara*) was significantly negatively correlated with increasing June temperatures from 1989 to 2004 (Massot et al. 2008). During that time frame the proportion of juveniles dispersing from their maternal home range decreased from approximately 60% to approximately 30%. This decrease in dispersal could lead to the breakdown of metapopulation dynamics, and an increased extinction risk. In their study of larval amphibian communities from subtropical and temperate regions, Duarte et al. (2012) concluded that the upper critical thermal limit ( $CT_{max}$ ) a species can tolerate evolved in relation to its maximum environmental temperature ( $T_{max}$ ). In addition, they concluded that as  $T_{max}$  increases, the rate at which  $CT_{max}$  increases is slower in temperate species than subtropical species. Thus, warm-breeding temperate species may face a high extinction risk because their  $CT_{max}$  cannot adapt as quickly as environmental temperatures increase.

In light of the severity of the threat habitat and environmental change poses to herpetofauna, a better understanding of these species' habitat requirements is warranted. This issue is complicated by the fact that many amphibians (Baldwin et al. 2006; Fellers and Kleeman 2007; Qi et al. 2011; Humphries and Sisson 2012) and reptiles (Congdon et al. 1987; Rovero and Chelazzi 1996; Millar and Blouin-Demers 2011) utilize different habitats during reproductive

and non-reproductive seasons. In order for conservation efforts to be effective, all habitats an organism uses throughout its lifetime must be considered (Dittmar et al. 2014). For example, Burke and Gibbons (1995) noted that the legislated buffer zone around a wetland in South Carolina only protected 44% of the nests and hibernation burrows of the turtles at that wetland.

This research aims to improve the understanding of habitat use of two protected herpetofaunal species in North Carolina. Specifically, I seek to investigate 1) the movements and post-breeding habitat selection of mountain chorus frogs (*Pseudacris brachyphona*), a species of special concern in North Carolina (NCWRC 2015); and 2) the movements and habitat use of bog turtles (*Glyptemys muhlenbergii*), a state threatened species (NCWRC 2015), in an undisturbed wetland. The knowledge gained will enhance future conservation efforts and land management decisions for both of these species in the state.

CHAPTER ONE: MOVEMENT AND POST-BREEDING HABITAT SELECTION OF THE  
MOUNTAIN CHORUS FROG (*Pseudacris brachyphona*)

**Introduction**

The mountain chorus frog (*Pseudacris brachyphona*) is a small, brown terrestrial frog found throughout the Appalachian Mountain region of the eastern United States (Dodd 2013). Its range extends southwest from southwestern Pennsylvania into north and central Alabama and northwest Mississippi (Dodd 2013). It is a member of the trilling chorus frog clade within *Pseudacris*, and is most closely related to Brimley's chorus frog (*Pseudacris brimleyi*) (Moriarty and Cannatella 2004; Lemmon et al. 2007).

Throughout their range, mountain chorus frogs (MCF) are declining. From 2001 to 2011, MCF experienced a mean decline in occupancy of 3.51% in the northeastern United States (Weir et al. 2014). Furthermore, it is listed as a species of "immediate concern" to conservation in Pennsylvania (PAGC and PAFBC 2005), is state endangered and possibly extirpated in Maryland (MDNHP 2010; E. Thompson, pers. comm.), and is a species of special concern in North Carolina, where this study took place (NCWRC 2015).

MCF were discovered in North Carolina in 1954 when individuals were heard calling from a ditch, culvert, and flooded pasture near Murphy, Cherokee County (Schwartz 1955). There was also anecdotal evidence at that time of MCF being present in the nearby town of Andrews (Schwartz 1955). Currently in the state, the species is known from only Cherokee and Clay counties (Williams 2012).

Like many amphibians, MCF occupy different habitats during their breeding and non-breeding seasons. MCF breed in a variety of shallow, ephemeral waters including pools,

flooded pastures, and roadside ditches (Green 1952; Barbour 1957; Dodd 2013), and canopy cover may be an important breeding habitat characteristic. Felix et al. (2010) observed that MCF deposited more egg masses in mesocosms placed in forest plots retaining all or 75% of their basal area than in mesocosms placed in plots retaining 25-50% of their basal area or that had been clear-cut.

While information on the breeding habitats of amphibians is fairly common, comparatively little is usually known about non-breeding habitats (Pellet et al. 2006). This trend extends to MCF as well. Early natural history studies found that this species usually occupies forests and woodlands during the non-breeding season, and observed that canopy cover and leaf litter appear to be critical features (Walker 1932; Green 1952; Barbour 1957). In fact, canopy cover distinguishes the terrestrial habitats of MCF and upland chorus frogs (*Pseudacris feriarum*) where they are sympatric. Both in Georgia (Martof and Humphries 1955) and Kentucky (Barbour 1957), in regions where both species occur, MCF are only found in heavily wooded habitats, while upland chorus frogs are only found in more open, cleared areas.

The knowledge gap in understanding the breeding and non-breeding habitat requirements of this species has important conservation implications. If an organism utilizes different habitats during different phases or aspects of its life, then wildlife management and habitat protection measures must include all of these habitats in order to be successful (Dittmar et al. 2014). My goal was to address that knowledge gap by determining the post-breeding habitat of MCF in North Carolina, and the distance individuals migrate from breeding to non-breeding habitats. This information will help wildlife agencies and land managers better protect MCF habitat where it is already of conservation concern.

## Methods

### Study Site

This study was conducted at the USDA Forest Service Beech Creek Seed Orchard near Murphy, Cherokee County, North Carolina (Figure 1) by the Beech Creek arm of Lake Hiwassee. The Seed Orchard is 250 acres, and is surrounded by intact native forest with interspersed residential housing. The Seed Orchard has large areas of cleared land containing plots of assorted trees, including oaks (*Quercus sp.*), dogwoods (*Cornus sp.*), pines (*Pinus sp.*), American chestnut (*Castanea dentata*), butternut (*Juglans cinerea*), and black cherry (*Prunus serotina*), which are grown for seed to be planted on national forests throughout the country (R. Taylor, pers. comm.). Hereafter, these plots of trees are referred to as “orchard habitat.” The fields separating the plots of trees were originally planted with fescue grass (*Festuca sp.*) in the 1960s, but now also contain orchard grass (*Dactylis glomerata* L.) and other native vegetation (R. Taylor, pers.comm.). There are also two small feed plots that are planted with corn and turnips for wild game (Figure 2). Several ephemeral ponds and puddles in which MCF breed form in the swales of these fields. Surrounding the cleared land is native temperate forest.

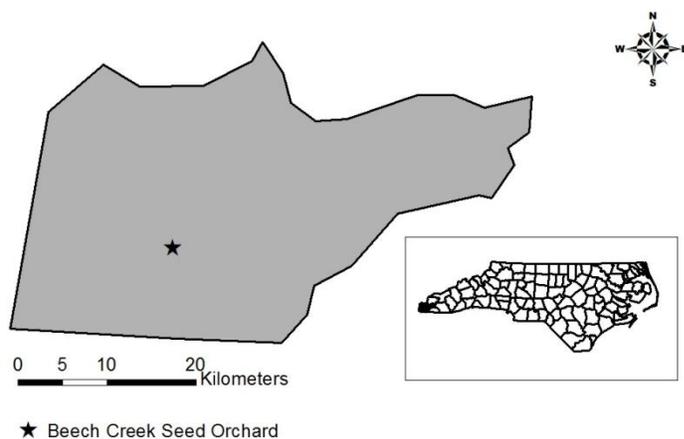


Figure 1: Location of USDA Forest Service Beech Creek Seed Orchard in Cherokee County. Inset shows Cherokee County within North Carolina.

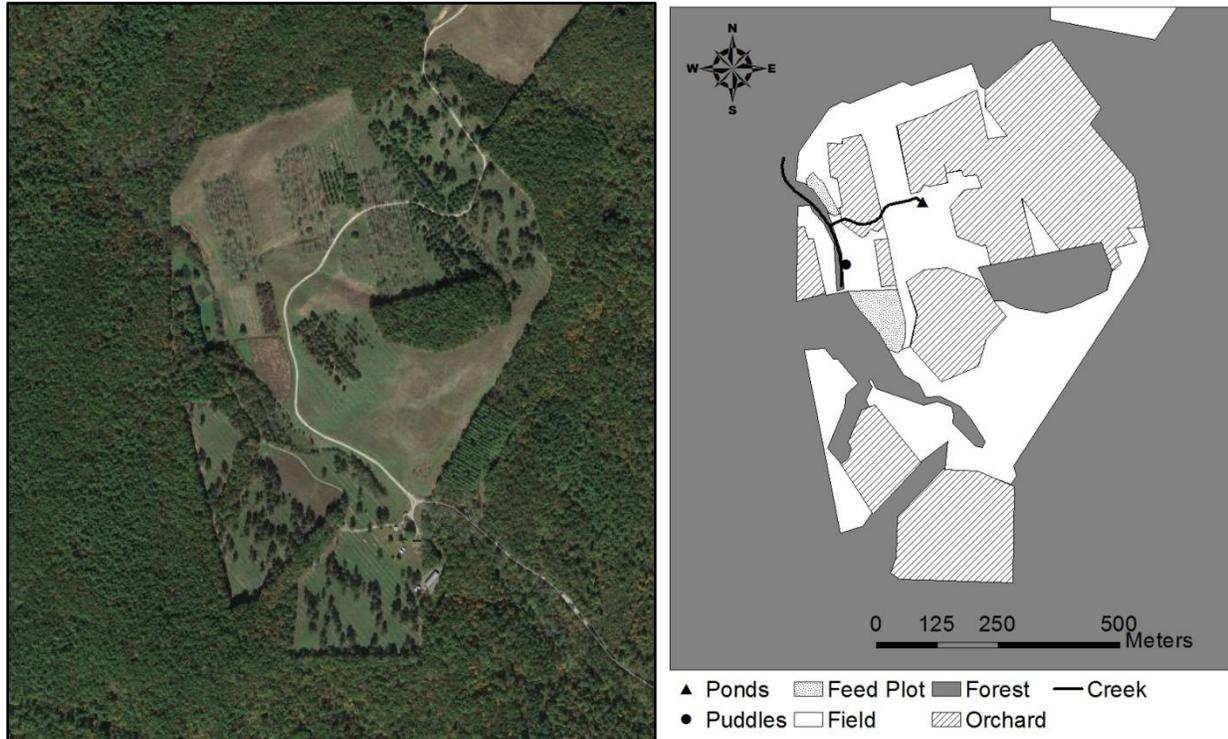


Figure 2: Aerial photograph of USDA Forest Service Beech Creek Seed Orchard (left), and map depicting the habitats present on the property and the locations of the two breeding sites used in this study (right).

## Movement

I located MCF during the breeding season, in March 2015, at night, through visual encounter and aural surveys. Drift fences were constructed of silt fencing around portions of known breeding sites in order to increase captures (Dodd and Scott 1994), but only one individual was located along a drift fence. I captured lone adults and amplexing pairs with small hand nets. All individuals were captured from either two small ponds near the beginning of a creek or a series of small puddles, hereafter referred to as “Ponds” and “Puddles” respectively (Figure 2). Amplexing pairs were left in buckets containing water and vegetation in shaded areas near the breeding sites, and allowed to complete breeding (12-60 hr). I measured snout-vent length (SVL) and mass, and determined sex by visual examination of all captured individuals.

Radio transmitters weighing 0.2 g with a projected lifespan of 35 days (A2412, Advanced Telemetry Systems, InSanti, Minnesota) were fitted to captured individuals in the field using belts constructed from elastic thread and glass beads (Muths 2003). Transmitter belts were only fitted to individuals after amplexus was completed (12-60 hr). The combined mass of the transmitter and belt did not exceed 10% of the individual's mass (Richards et al. 1994). Fifteen MCF from the Ponds (14 female, 1 male) and five MCF from the Puddles (4 female, 1 male) were fitted with radio transmitters. Females of this species are larger than males. Thus, given the mass of the transmitter belt and the 10% body mass limit, mostly females were large enough to receive a transmitter belt. All frogs were released in the water at their site of capture upon being fitted with transmitter belts.

Individuals were located three times weekly for the duration of the transmitter batteries' lifespan. When frogs were located GPS coordinates were recorded using a Garmin eTrex Vista HCx (Garmin International Inc., Olathe, Kansas), the location was flagged, and the area was briefly searched for the frog. If the frog was found, then I inspected it for evidence of irritation from the belt. If irritation was observed, then the belt was removed, and the frog was placed in a container with water and vegetation in situ in the shade. After approximately two days when the frog had healed, it was fitted with a larger transmitter belt. At the end of the tracking period I removed the belts when possible. Distances between consecutive tracked locations and straight-line distances from an individual's breeding site of origin to the final location were calculated using Google Earth Pro 7.0.3.8542 (Google, Mountain View, California).

## **Reproduction**

For amplexed pairs that finished breeding in buckets I recorded the number of egg masses and eggs produced per female.

## Habitat

**Vegetation.** The greatest distance an individual traveled from each breeding site was used as the radius to determine the circular potential area of occupation surrounding each breeding site (Williams 2012). I measured habitat parameters at each location a frog was tracked to (hereafter referred to as “tracked locations”) and at randomly selected locations between consecutive tracked locations (hereafter referred to as “pass-through locations”). One pass-through location was selected for every 50 m between consecutive tracked locations. All transects of frog movement were extrapolated to the maximum distance traveled from the original breeding site. One location was randomly selected for every 50 m in the direction the individual had last been traveling until the maximum distance was reached. These locations are referred to as “extrapolated locations.” The tracked, pass-through, and extrapolated locations of an individual frog are considered a frog transect.

The circular potential area of occupation around each breeding site was then divided into 30° arcs, and each arc was subdivided every 50 m. In order to sample all available habitat, I sampled the habitat at one randomly chosen location within every subdivision that did not contain either a tracked, pass-through, or extrapolated location. These locations are hereafter referred to as “random” locations. All random locations within the same 30° arc are considered a random transect.

At all locations, I visually estimated percent cover of bare ground, leaf litter, grass, herbaceous vegetation, vines, seedlings, shrubs, and trees within a 1 m × 1 m square quadrat. I classified leaf litter type as deciduous, conifer, or mixed, used a ruler to measure leaf litter depth, and used a spherical densiometer held at chest height (approximately 1.5 m) to estimate percent canopy cover. Vegetation sampling was conducted from 22 May–16 August, 2015.

**Soil.** At all locations I noted whether burrows were present or absent within a 1 m × 1 m square quadrat centered on the location. Burrow sampling was conducted from 22 May–16 August, 2015.

I took two samples of soil moisture measurements from locations at both the Ponds and the Puddles from 30 August–15 November 2015 using an Aquaterr Instruments M-350 soil moisture meter (Aquaterr Instruments & Automation, Costa Mesa, California). A single sample of measurements at the Puddles consisted of all five frog transects and at least three (of eleven total) random transects, and all locations were measured on the same day. A single sample of measurements at the Ponds consisted of ten (of eleven total) frog transects and at least four (of six total) random transects, and all locations were measured over two consecutive days. For both the Puddles and the Ponds all locations were sampled at least once. When sampling a location, I took five readings with the soil moisture meter, and then calculated the mean of those readings.

I took two soil cores from locations at both Puddles and Ponds from 30 August– 23 November, 2015. The cores had a diameter of 1.75 cm, and were taken to a depth of 10 cm. I cleared away any leaf litter before taking each core. I collected cores from all frog and random transects. For frog transects I gathered cores from the first, middle, and final tracked, pass-through, and extrapolated locations (nine locations total). From random transects I collected cores from the first, middle, and final random location (three locations total). I collected soil cores from fewer than three locations per location type on individual transects that were particularly short.

Soil cores were placed in plastic bags in the field, and brought back to the lab for texture analysis. I collected cores from a total of 184 locations (62 Puddles, 122 Ponds). A portion of those cores (5 Puddles, 27 Ponds) were lost due to an initial attempt to determine soil texture by

the hydrometer method. Lost cores represented all location types and all habitats. Soil texture was ultimately determined by dry sieve (Kroetsh and Wang 2008; D. Kinner, pers. comm.). Cores from the same location were combined, and dried in an oven at 105 °C for 24 hr. After drying I used a mortar and pestle to break up aggregates. I determined Munsell color for all samples using the Munsell Soil Color Book (Munsell Color 2012). Samples were sieved for 10 min using a W.S. Tyler Sieve Shaker Model RX-86 (W.S. Tyler Incorporated, Gastonia, NC). I used a #10 sieve (2.00 mm) to separate out gravel and large organic material, and a #230 sieve (0.063 mm) to separate sand from fine particles (clay and silt). I measured the mass of the sieves and bottom pan with and without each sample in order to determine the mass of each textural component for each location's sample. I determined the proportion by mass each textural class represented for each sample. I determined the bulk density of each soil sample using its total dry mass.

## **Analysis**

I conducted a chi-square analysis on each frog's final tracked location to determine macroscale habitat selection between the four major habitat types (field, forest, orchard, and wildlife feed plot) present within the potential area of occupation surrounding each breeding site. Expected values were determined using the proportion of the potential area of occupation represented by each habitat type. I used multi-response permutation procedures (MRPP) and non-metric multidimensional scaling (NMDS) to determine differences in percent cover of vegetation among macroscale habitat types (field, forest, and orchard) and among location types (tracked, pass-through, extrapolated, and random). I used indicator species analysis (ISA) to determine which vegetation classes were significantly associated with particular habitats and location types. I used pairwise G-tests with Holm's sequential Bonferroni to determine

differences in the presence of burrows among habitats and location types. I used ANOVA in which I blocked by sample number, followed by Tukey's Honestly Significant Difference (HSD) test, to determine differences in soil moisture among habitats and location types. To determine differences in soil texture among habitats and location types I used MANOVA with Pillai's trace using the proportional textural class composition of the soil cores. If the MANOVA was significant I conducted ANOVAs and Tukey's HSD tests to determine which textural classes differed among habitats and location types. I used ANOVA with Tukey's HSD test to determine differences in bulk density among habitats and location types. For all analyses I analyzed data from the Puddles and Ponds separately. I conducted MRPP, NMDS, and ISA using PC-Ord software (MjM Software Design, Gleneden Beach, Oregon). All other analyses were conducted in R software version 3.1.2 (R Core Team 2014).

## **Results**

### **Movement**

Between both breeding locations, 72 MCF were captured. Females had a mean snout-vent length (SVL) of 33 mm (SE = 0.3 mm; range 29-37 mm) and mean mass of 3.38 g (SE = 0.08 g; range 2.45-5.02 g) after dropping their eggs, whereas males had a mean SVL of 29 mm (SE = 0.3 mm; range 26-32 mm) and mean mass of 2.68 g (SE = 0.05 g; range 1.97-3.22 g; Appendix A). Radio transmitter belts were fit onto 20 individuals of sufficient size; 15 (14 female, 1 male) from the Ponds, and 5 (4 female, 1 male) from the Puddles. Given the mass of the transmitter belt and 10% body mass restriction, the majority of individuals large enough to receive a transmitter belt were female. Mean mass of tracked MCF was 3.39 g (SE = 0.06 g; range 3.00-3.95 g), and mean belt size was 0.32 g (SE = 0.003 g; range

0.31-0.34 g). Thus, the transmitter belt was 9.7% of an individual's body mass on average (SE = 0.1%; range 8.5-10.7%).

The belt from one individual from the Ponds fell off shortly after being released, so only 19 individuals were tracked. One individual was found dead in the Ponds two days after being fitted with a transmitter and released there. I found one individual dead in the field following several days without rain and presume desiccation was the cause of death. Seven frogs developed irritation and sores under their belt due to poor fit. I brought them to the lab for recovery; three recovered, four did not.

Mean distance traveled was 106.3 m (SE = 38.9 m, range 11.4–475.6 m) from the Ponds, and 123.7 m (SE = 34.9m, range 26.0–219.5 m) from the Puddles. In general, MCF were more likely to have moved after rain events when it was warm (> 10 °C) at night than when it remained dry for multiple days.

One individual from the Puddles was fitted with a new transmitter after 28 days, before the battery of the original one expired, and was tracked for an additional 12 days. During this additional time the frog moved three more times for a total distance of 55 m but remained in the same general vicinity. The average distance traveled per move was 18 m, but the straight-line distance between the starting and ending locations of this 12-day period was only 6 m. Throughout this time the frog was in the forest or in the edge of the adjacent orchard block.

## **Reproduction**

Females laid eggs in masses that were typically attached to vegetation. The number of eggs per female ranged from 227-634 and the mean was 362 eggs (SE = 41 eggs). The number of egg masses deposited per female ranged from 9-32 and the mean was 16.8 masses

(SE = 2.2 masses). The number of eggs per mass ranged from 1-113 and the mean was 22 eggs/mass (SE = 2 eggs/mass).

**Habitat**

**Vegetation.** Chi-square analysis showed individuals from the Ponds preferentially selected field habitat ( $X^2 = 9.67, df = 3, p = 0.02$ ), but this trend disappeared when mortalities due to ill-fitting belts were removed ( $X^2 = 3.91, df = 3, p = 0.27$ ; Table 2). No habitat preference at the Puddles was detected ( $X^2 = 0.47, df = 3, p = 0.92$ ; Table 1).

Table 1: Number of frogs whose final location was in each habitat. For the Ponds, the first count excluded only the individual whose belt fell off and the individual found dead two days after release. The second count (in parentheses) excludes all mortalities.

	<u>Habitat</u>		
	<b>Field</b>	<b>Forest</b>	<b>Orchard</b>
<b>Ponds</b>	8(4)	1	4(3)
<b>Puddles</b>	2	2	1

Multi-response permutation procedures (MRPP) indicated significant differences in vegetation cover among habitats for MCF from the Puddles (overall  $p < 0.01$ ); all habitats differ from each other ( $p < 0.01$  for all pairwise comparisons). However, there were no significant differences in vegetation cover among location types (i.e., tracked, pass-through, extrapolated, and random) for MCF from the Puddles (overall  $p = 0.08$ ). Non-metric multidimensional scaling (NMDS) revealed that all habitats were separated (Figure 3), but there was no separation among location types (Figure 4). Field habitat was significantly associated with greater percent cover of herbaceous vegetation and vine (Figure 5). Forest habitat was significantly associated with greater percent cover of leaf litter, seedling, shrub, and canopy (Figure 5), as well as greater leaf litter depth, and leaf litter composed mostly of deciduous and deciduous/conifer mixtures. Orchard habitat was significantly associated with greater percent cover of bare ground and grass

(Figure 5). Extrapolated locations were significantly associated with greater percent cover of herbaceous vegetation (Figure 5).

MRPP indicated significant differences in vegetation cover among habitats for MCF from the Ponds (overall  $p < 0.01$ ); all habitats differ from each other ( $p < 0.01$  for all pairwise comparisons). There were also significant differences in vegetation cover among location types (overall  $p < 0.01$ ). Tracked and pass-through locations differed significantly from extrapolated and random locations (pairwise comparisons  $p < 0.01$ ). NMDS showed that all habitats were separated (Figure 6), and there was some separation among location types (Figure 7). The points in the upper half of Figure 7 are predominantly extrapolated and random locations, whereas the points in the lower half are predominantly tracked and pass-through locations. Field habitat was significantly associated with greater percent cover of herbaceous vegetation and vine (Figure 8). Forest habitat was significantly associated with greater percent cover of leaf litter, seedling, shrub, moss and woody debris, and canopy (Figure 8), as well as greater leaf litter depth, and leaf litter composed mostly of deciduous and deciduous/conifer mixtures. Orchard habitat was significantly associated with greater percent cover of bare ground and grass (Figure 8). Tracked locations were significantly associated with greater percent cover of grass, herbaceous vegetation, and vine (Figure 8). Extrapolated locations were significantly associated with greater leaf litter depth. Random locations were associated with significantly greater percent cover of bare ground, leaf litter, moss and woody debris, and canopy cover (Figure 8), and had mostly deciduous leaf litter.

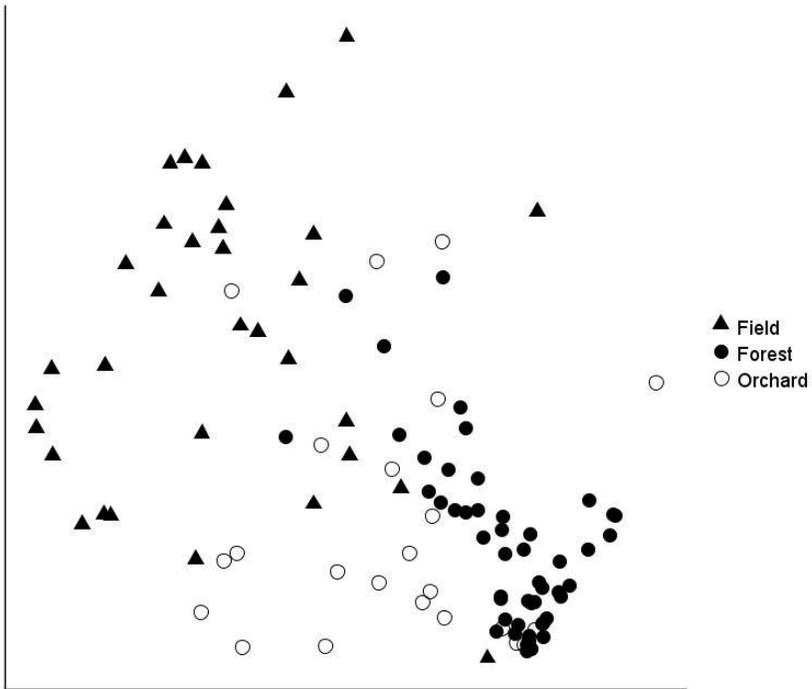


Figure 3: Non-metric multidimensional scaling (NMDS) plot of vegetation cover among MCF habitats at the Puddles.

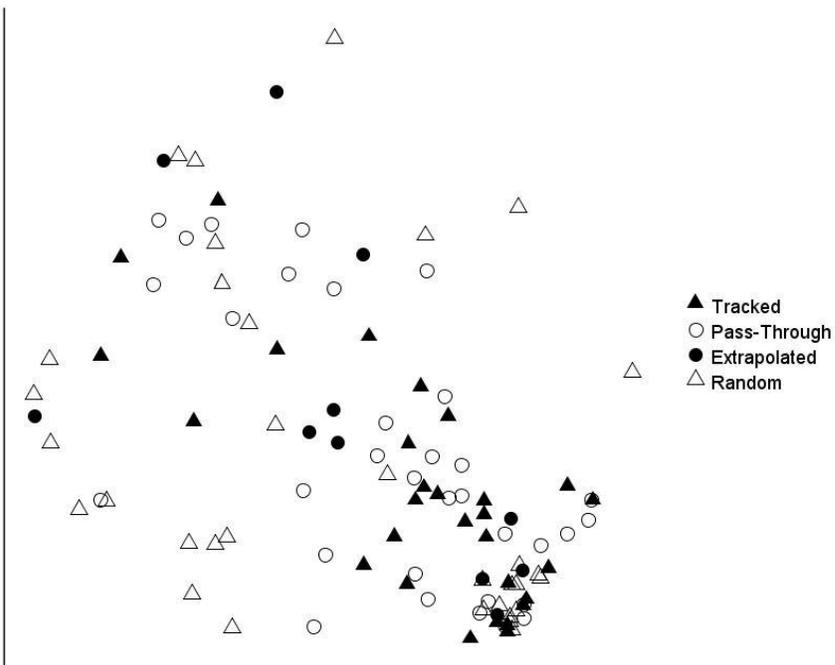


Figure 4: Non-metric multidimensional scaling (NMDS) plot of vegetation cover among MCF location types at the Puddles.

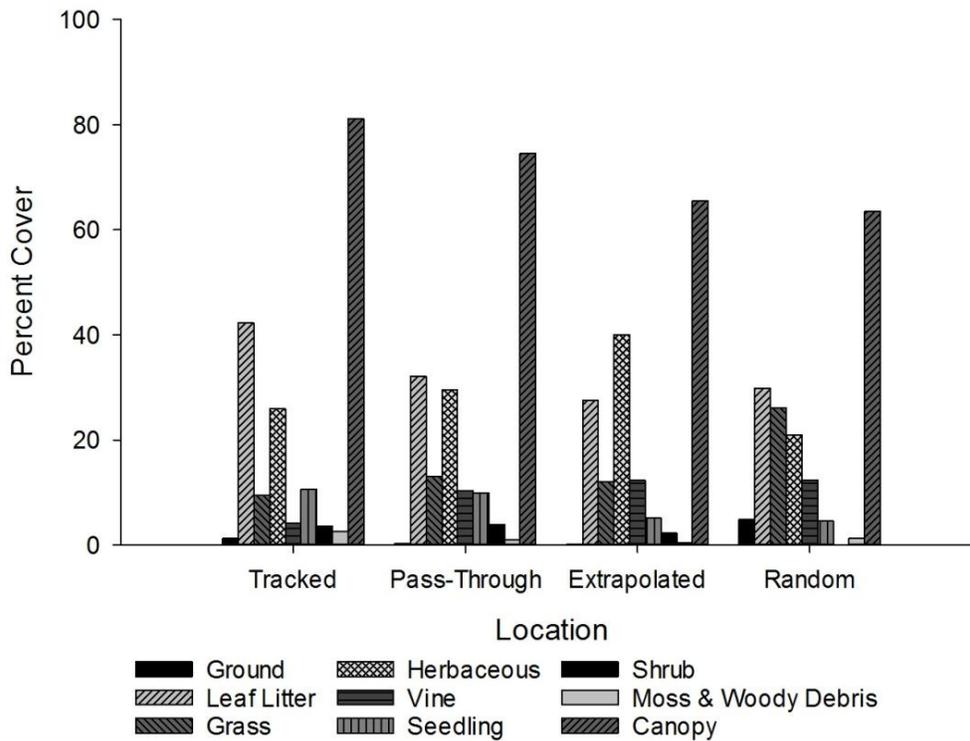
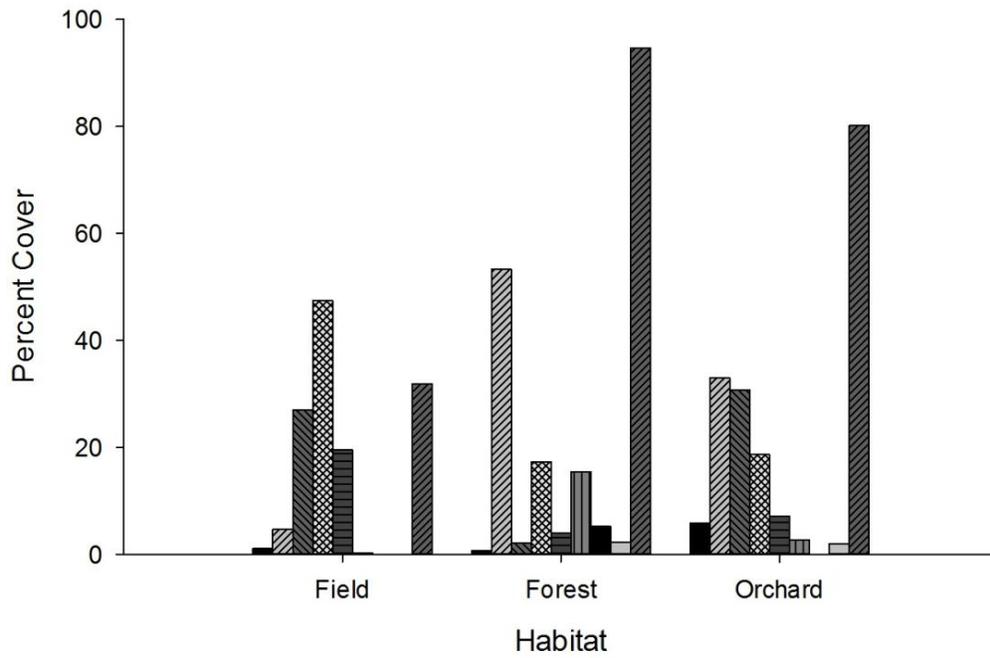


Figure 5: Percent cover of vegetation by habitat (top) and location type (bottom) for MCF locations from the Puddles.

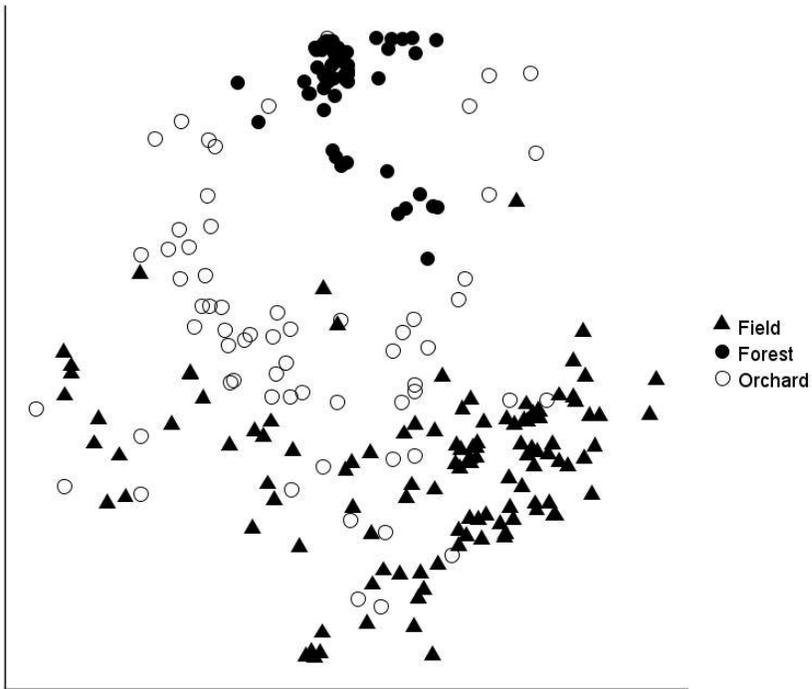


Figure 6: Non-metric multidimensional scaling (NMDS) plot of vegetation cover among MCF habitats at the Ponds.

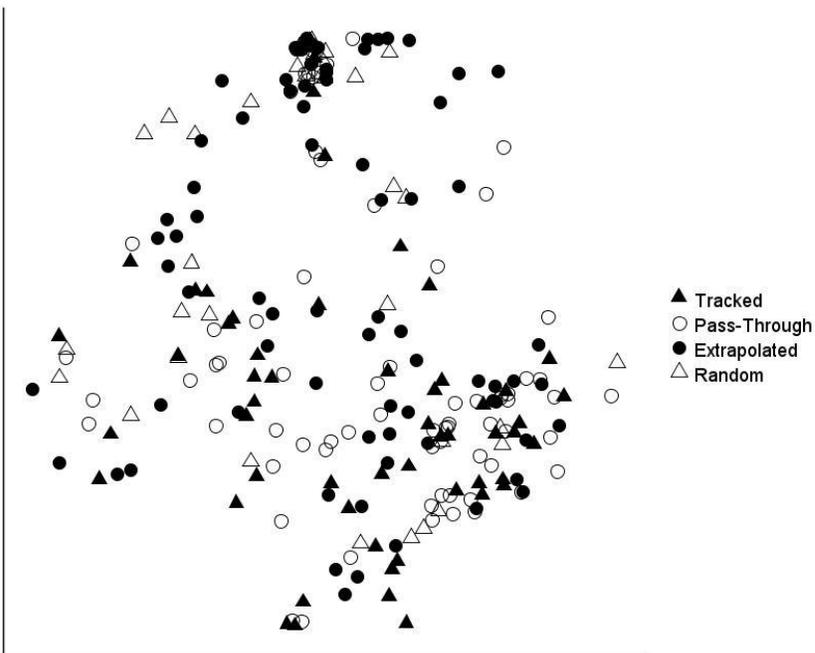


Figure 7: Non-metric multidimensional scaling (NMDS) plot of vegetation cover among MCF location types at the Ponds.

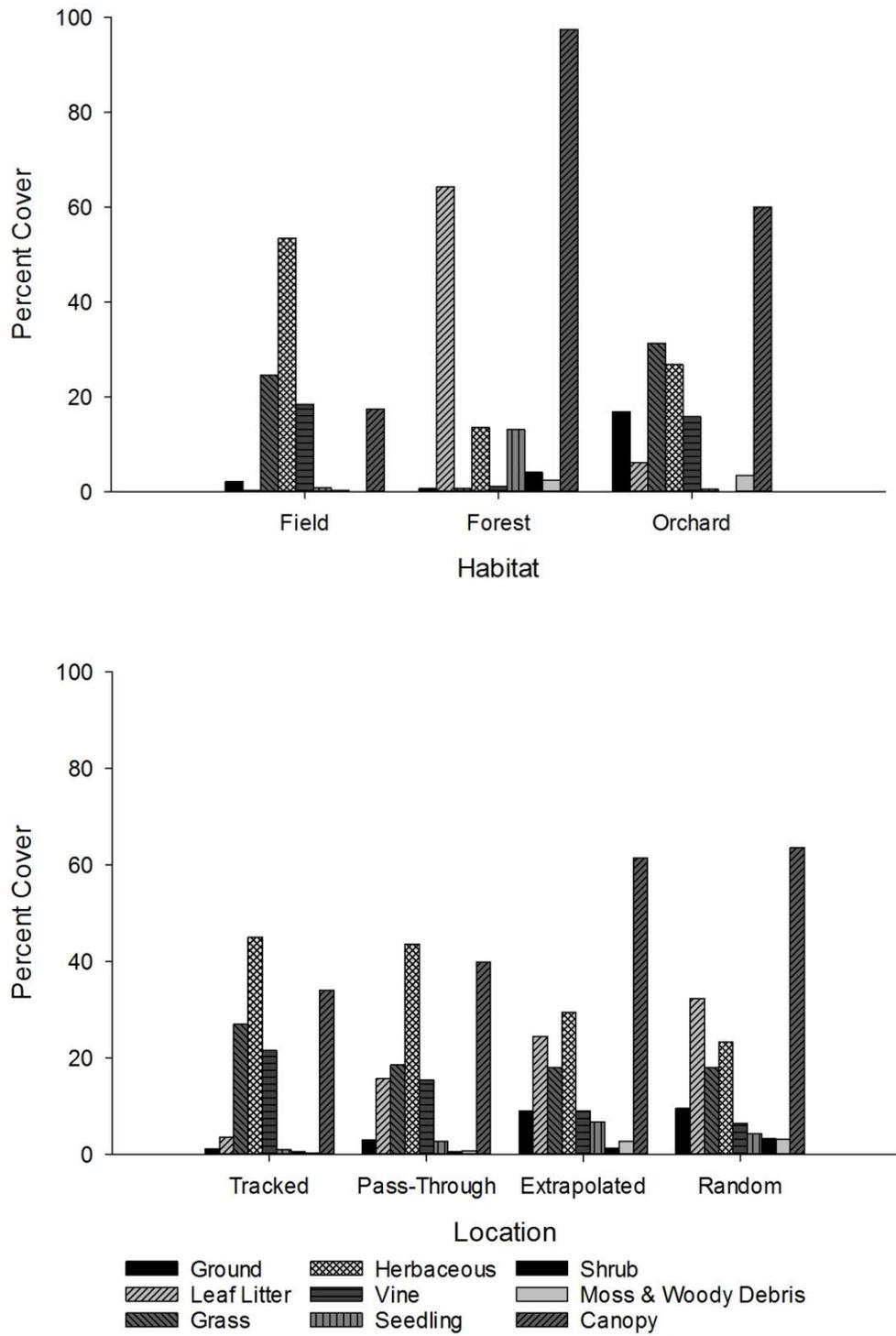


Figure 8: Percent cover of vegetation by habitat (top) and location type (bottom) for MCF locations from the Ponds.

**Soil.** At both the Puddles and the Ponds burrow presence significantly differed among all habitats (all  $p < 0.05$ ), but not among location types (Appendix B). Forest habitat had the largest percentage of samples containing burrows (56% and 42% for Puddles and Ponds respectively), followed by orchard and field habitats (Figures 9 and 10).

Soil moisture was significantly different for all habitats at the Puddles (overall  $F = 123.65$ ,  $df = 2$ ,  $p = 2.2 \times 10^{-16}$ ; all individual comparisons  $p < 0.01$ ). Field soils were the wettest and had a mean soil moisture of 66% (SE = 1.4%), followed by Orchard soils with a mean soil moisture of 59% (SE = 1.6%), and forest soils with a mean soil moisture of 38% (SE = 1.4%) (Figure 11). Soil moisture also significantly differed among location types at the Puddles (overall  $F = 2.90$ ,  $df = 3$ ,  $p = 0.036$ ) with tracked and random locations being significantly different ( $p = 0.025$ ). Soils at random locations were the wettest and had a mean value of 57% (SE = 2.8%), followed by extrapolated locations with a mean value of 53% (SE = 3.9%), pass-through locations with a mean value of 50% (SE = 2.2%), and tracked locations with a mean value of 46% (SE = 2.2%) (Figure 11).

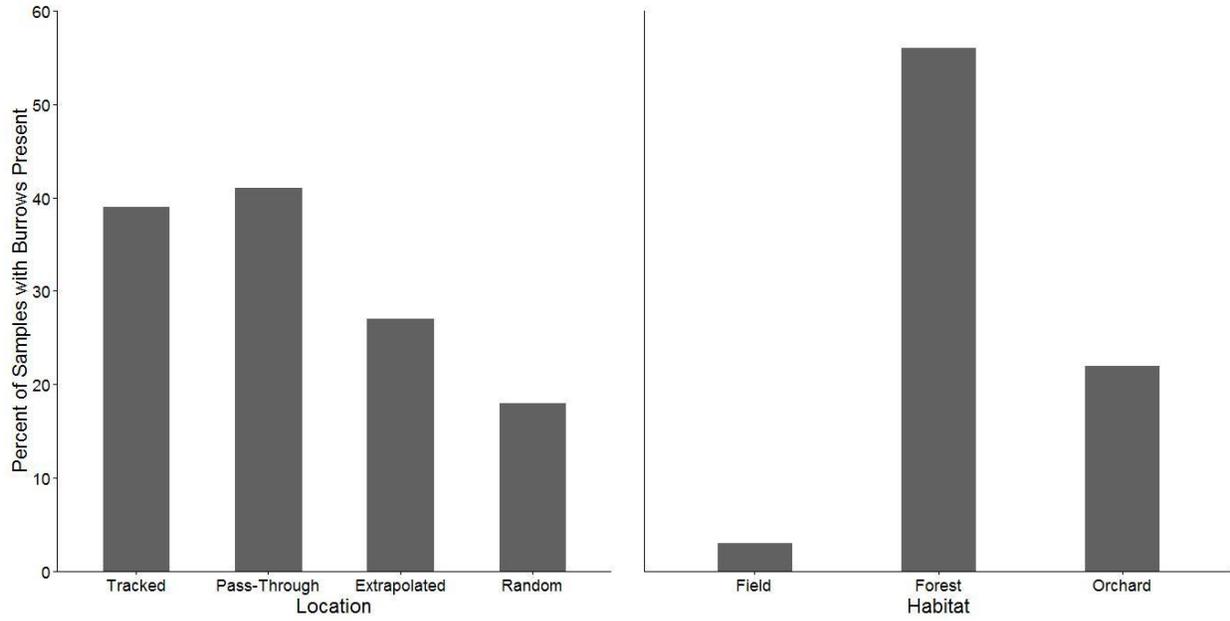


Figure 9: Percent of samples containing burrows by location type (left) and habitat (right) at the Puddles.

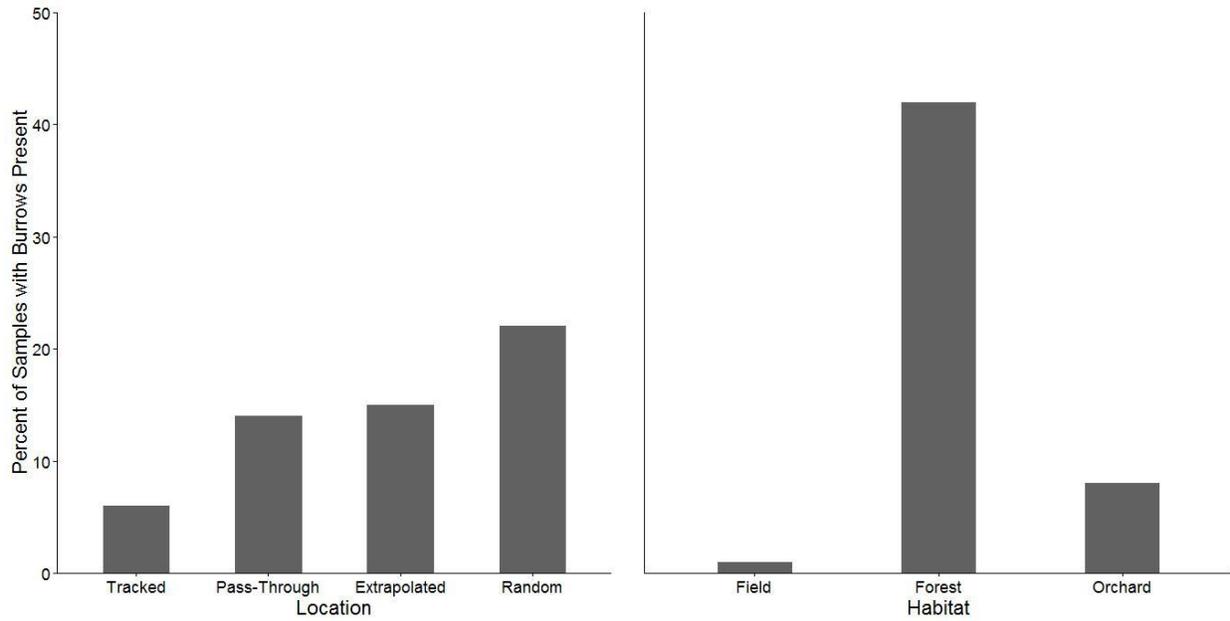


Figure 10: Percent of samples containing burrows by location type (left) and habitat (right) at the Ponds.

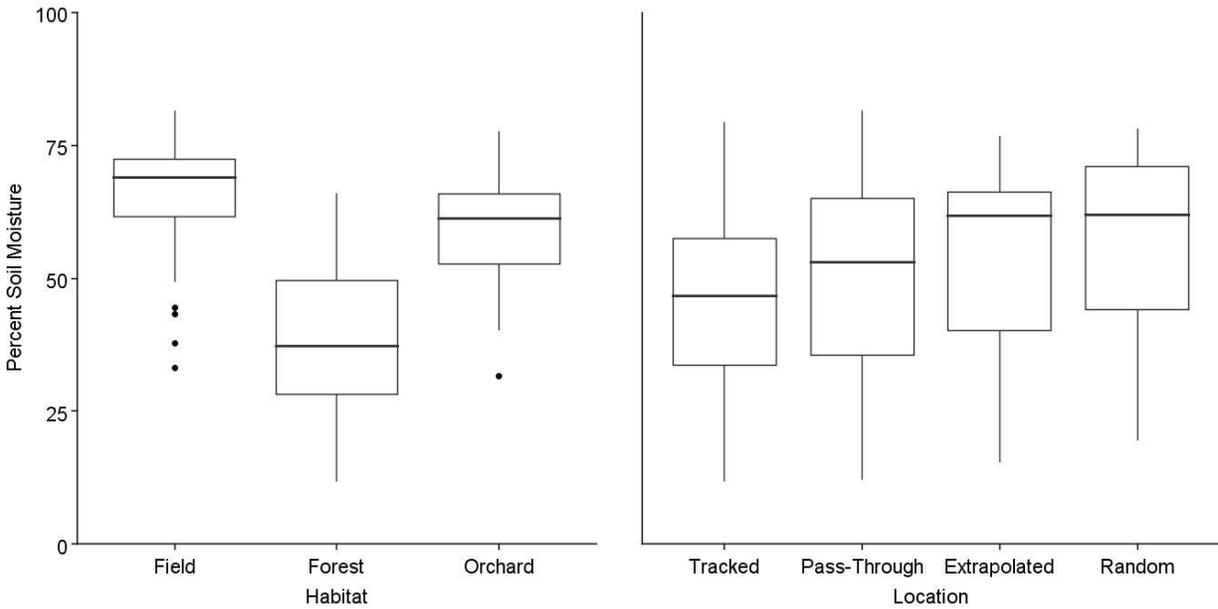


Figure 11: Box plot of soil moisture values by habitat (left) and location type (right) for the Puddles.

Similarly, soil moisture was significantly different among all habitats at the Ponds (overall  $F = 532.65$ ,  $df = 2$ ,  $p = 2.2 \times 10^{-16}$ ; all individual comparisons  $p < 0.01$ ). Field soils were the wettest and had a mean value of 71% (SE = 0.7%), followed by orchard soils with a mean value of 67% (SE = 0.9%), and forest soils with a mean value of 36% (SE = 1.0%) (Figure 12). Soil moisture also differed significantly among location types at the Ponds (overall  $F = 18.26$ ,  $df = 3$ ,  $p = 4.5 \times 10^{-11}$ ). Tracked and pass-through soils were both significantly different from extrapolated and random soils (individual comparisons  $p < 0.01$ ). Tracked soils were the wettest with a mean value of 69% (SE = 1.6%), followed by pass-through soils with a mean value of 64% (SE = 1.6%), extrapolated soils with a mean value of 55% (SE = 1.6%), and random soils with a mean value of 54% (SE = 2.2%) (Figure 12).

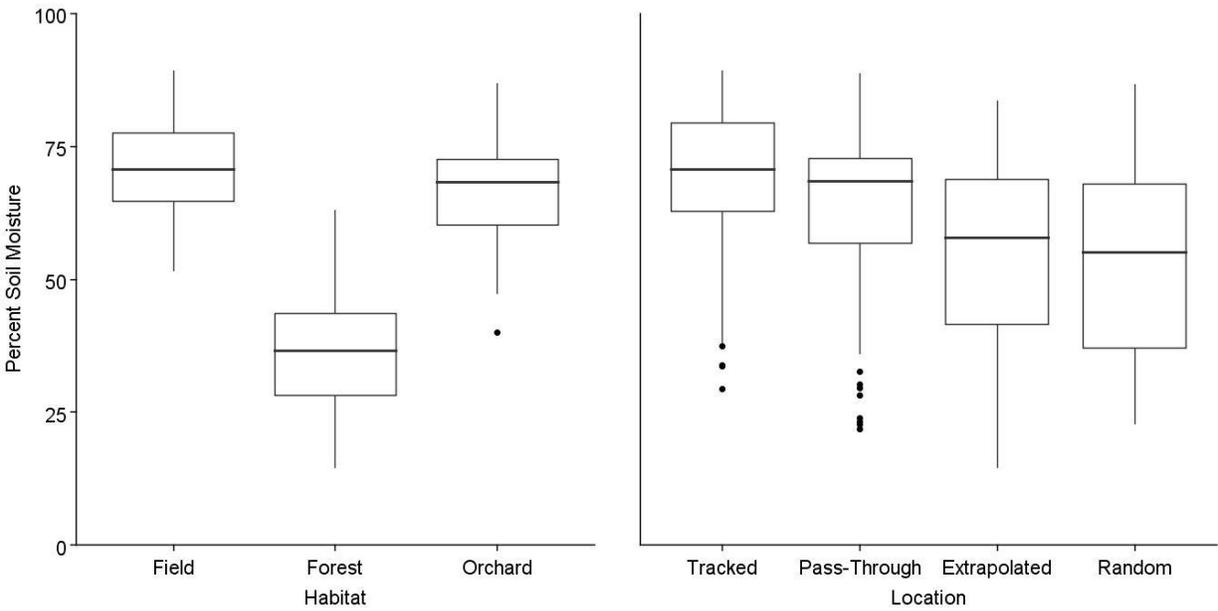


Figure 12: Box plot of soil moisture values by habitat (left) and location type (right) for the Ponds.

Overall, most soil samples were brown or red brown. At the Puddles, field habitat had the most diversity of color, and only approximately half of the samples were brown (Figure 13). Samples from the forest and orchard were both approximately 75% brown and 25% red brown (Figure 13). The majority of tracked and pass-through samples were brown with the remainder being other shades of brown or grey (Figure 13). Approximately half of samples from extrapolated locations were red brown, and the remaining half were mostly brown or yellow brown with a few yellow red samples (Figure 13). Samples from random locations contained the most diversity with approximately 60% being brown or red brown, and the remainder being yellow brown, greys, and black (Figure 13).

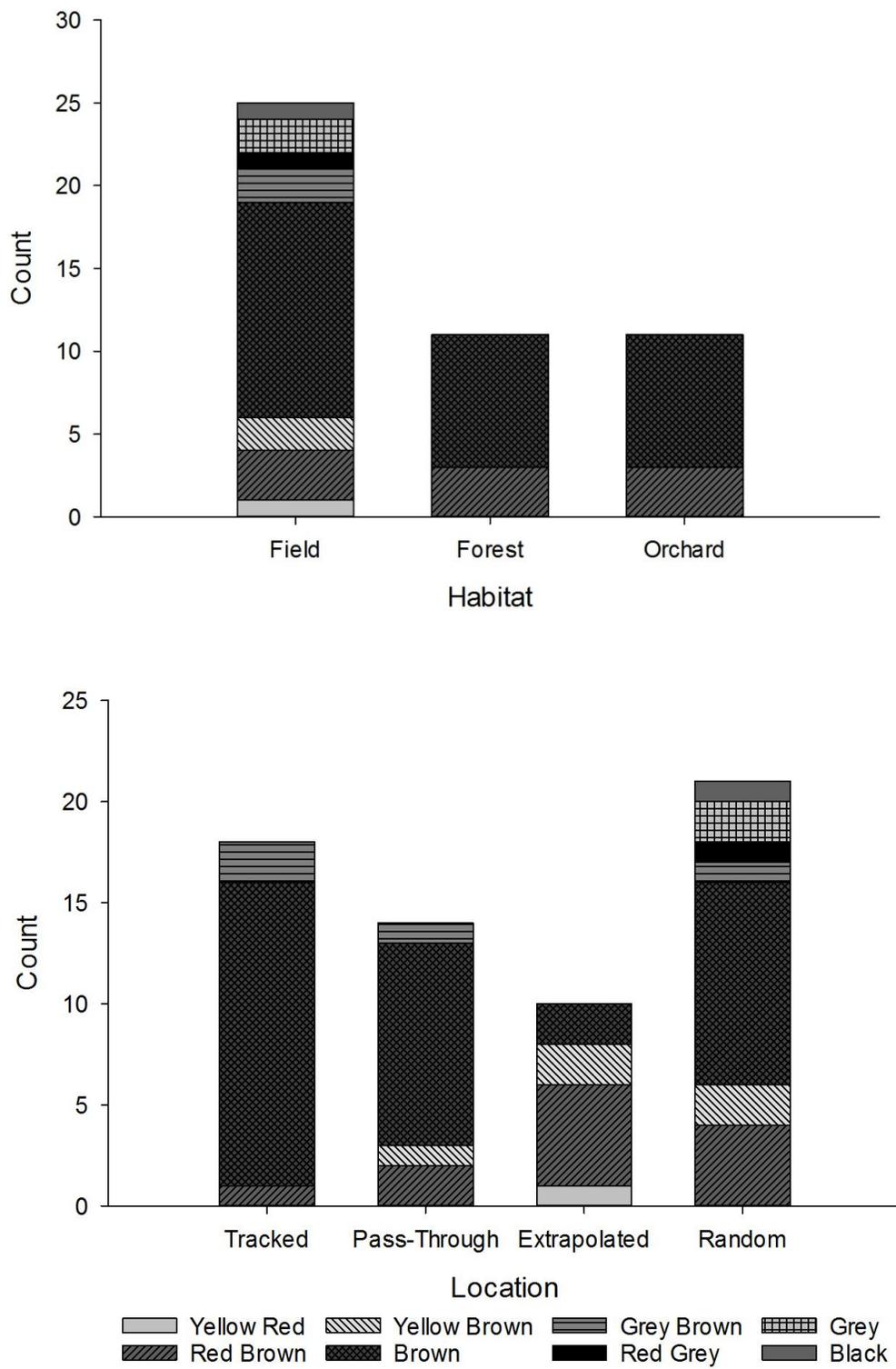


Figure 13: Counts of soil colors by habitat (top) and location type (bottom) at the Puddles.

Field habitat at the Ponds also contained the most color diversity with approximately 60% of samples being brown, and the majority of the remainder being other shades of brown and a few greys (Figure 14). Similarly, forest habitat was approximately 60% brown with the remainder being mostly other shades of brown (Figure 14). Orchard habitat was approximately 75% brown with the remainder also being other shades of brown and some greys (Figure 14). Tracked and pass-through locations were both approximately 75% brown while the remainder was a mix of shades of brown and grey (Figure 14). Extrapolated and random locations were both only approximately 50% brown, and the other half was a mixture of shades of brown and grey (Figure 14).

Soil texture differed significantly among habitats at the Puddles (Pillai's trace = 0.23, approximate  $F = 3.30$ ,  $df = 2$ ,  $p = 0.014$ ). Mean percents of gravel and large organic material, sand, and fine particles (clay and silt) were 3% (SE = 0.53%), 85% (SE = 0.77%), and 12% (SE = 0.47%) respectively for field soils, 4% (SE = 0.73%), 82% (SE = 0.55%), and 14% (SE = 0.64%) respectively for forest soils, and 5% (SE = 0.74%), 83% (SE = 0.85%), and 12% (SE = 0.89%) for orchard soils (Figure 15). Forest habitat had a significantly greater percentage of fine particles than field soil ( $p = 0.024$ ), and forest had a significantly smaller percentage of sand than field ( $p = 0.015$ ). Soil texture did not differ significantly among location types at the Puddles (Pillai's trace = 0.16, approximate  $F = 1.44$ ,  $df = 3$ ,  $p = 0.21$ ). Mean percents of gravel and large organic material, sand, and fine particles were 3% (SE = 0.58%), 84% (SE = 0.64%), and 13% (SE = 0.79%) for tracked locations, 3% (SE = 0.96%), 85% (SE = 0.79%), and 12% (SE = 0.72%) for pass-through locations, 5% (SE = 1.5%), 81% (SE = 1.6%), and 14% (SE = 1.6%) for extrapolated locations, and 4% (SE = 0.56%), 83% (SE = 0.71%), and 13% (SE = 0.56%) for random locations (Figure 15).

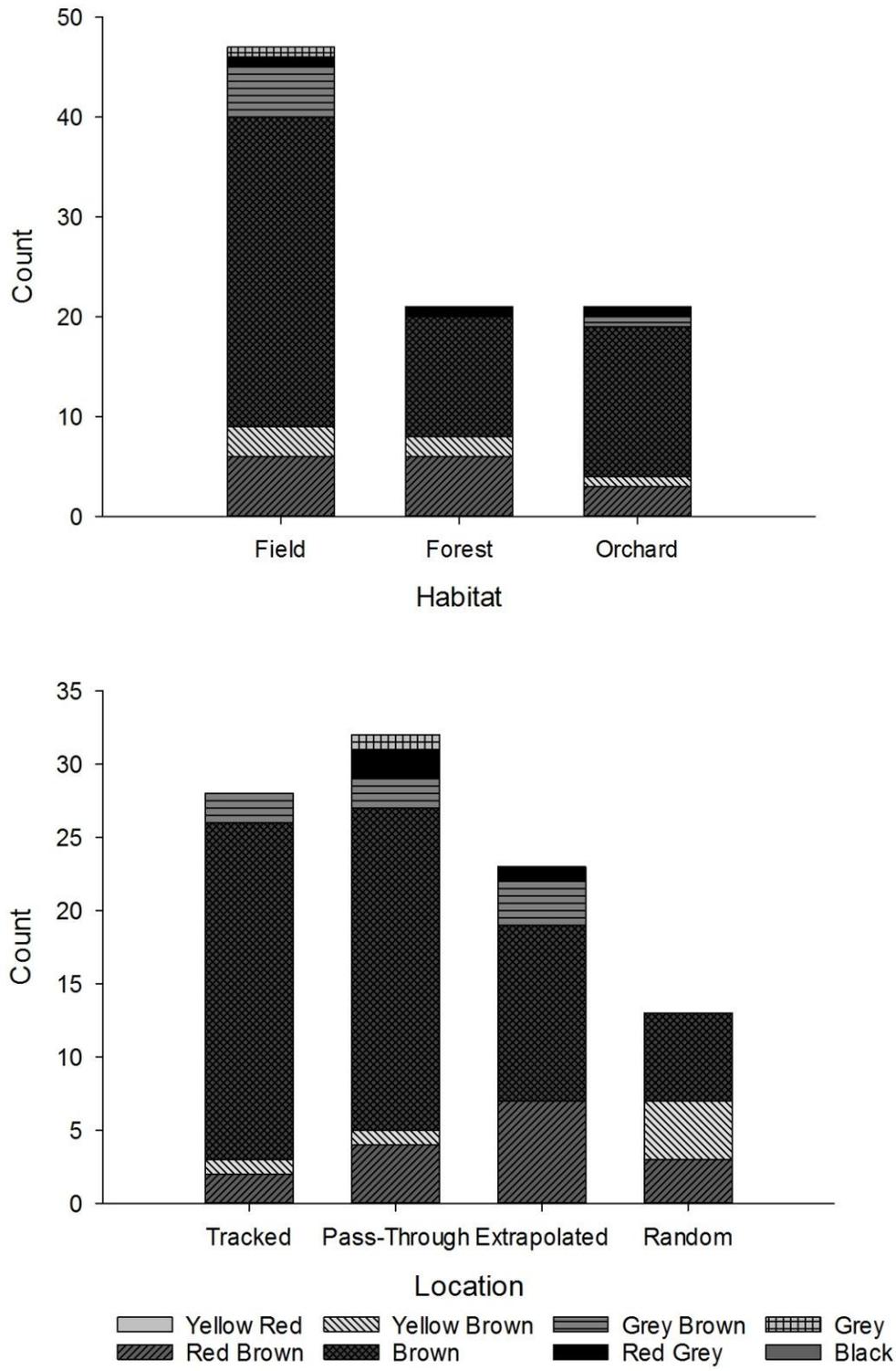


Figure 14: Counts of soil colors by habitat (top) and location type (bottom) at the Ponds.

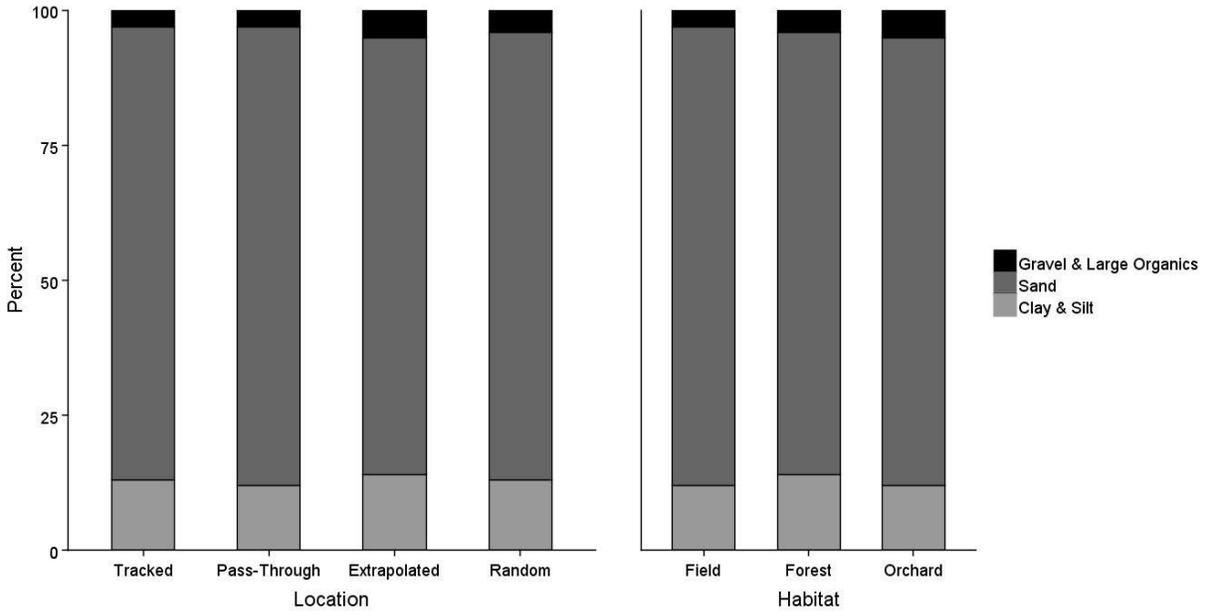


Figure 15: Soil texture by location type (left) and habitat (right) for soil samples at the Puddles.

Soil texture also significantly differed among habitats at the Ponds (Pillai's trace = 0.41, approximate  $F = 10.92$ ,  $df = 2$ ,  $p = 6.7 \times 10^{-8}$ ). Mean percentages of gravel and large organic material, sand, and fine particles were 5% (SE = 0.51%), 84% (SE = 0.48%), and 11% (SE = 0.33%) for field habitat respectively, 6% (SE = 0.80%), 78% (SE = 1.1%), and 16% (SE = 0.78%) for forest habitat, and 6% (SE = 1.1%), 80% (SE = 0.78%), and 14% (SE = 0.79%) for orchard habitat (Figure 16). All three habitats significantly differed in their percentage of fine particles with forest having the greatest and field having the smallest (each comparison  $p < 0.02$ ). Forest and orchard also both had significantly smaller percentages of sand than field soils (each comparison  $p < 0.01$ ). Significant differences in soil texture also existed among location types at the Ponds (Pillai's trace = 0.16, approximate  $F = 2.43$ ,  $df = 3$ ,  $p = 0.028$ ). Mean percentages of gravel and large organic material, sand, and fine particles were 6% (SE = 0.95%), 82% (SE = 0.78%), and 12% (SE = 0.57%) for tracked locations, 4% (SE = 0.59%), 83% (SE = 0.92%), and 13% (SE = 0.72%) for pass-through locations, 6% (SE = 0.88%), 80% (SE = 1.0%), and 14% (SE = 0.58%) for extrapolated locations, and 4% (SE = 0.77%), 81%

(SE = 1.0%), and 15% (SE = 1.1%) for random locations (Figure 16). Tracked locations had a significantly smaller percentage of fine particles than random locations ( $p = 0.012$ ).

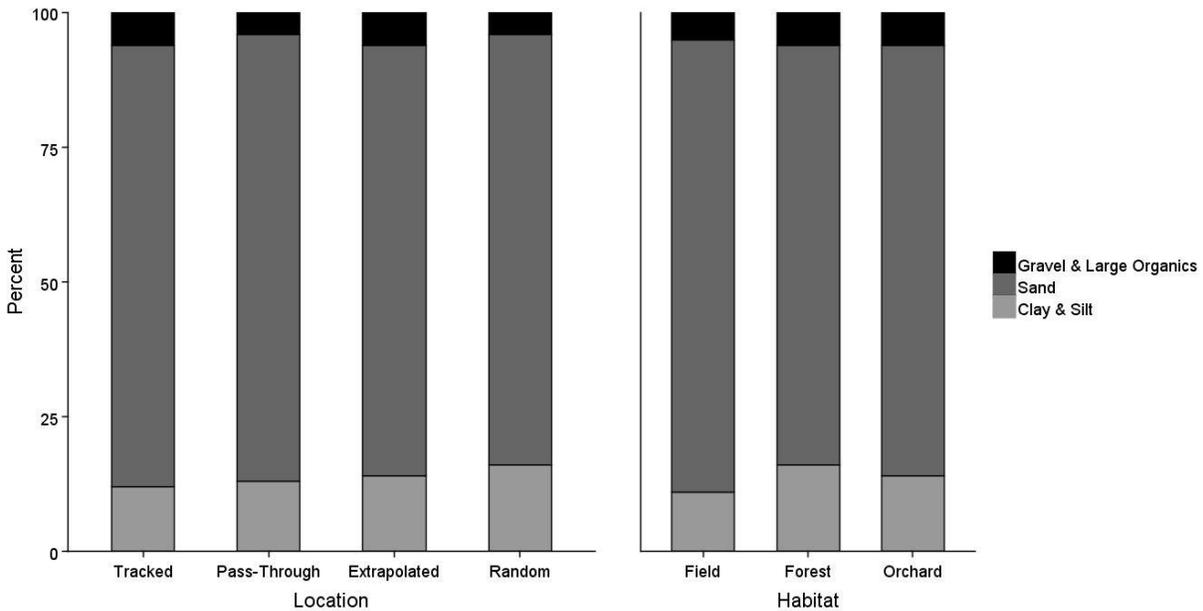


Figure 16: Soil texture by location type (left) and habitat (right) for soil samples at the Ponds.

Mean bulk density was  $1.0 \text{ g/cm}^3$  (SE =  $0.05 \text{ g/cm}^3$ ) for field soils,  $1.0 \text{ g/cm}^3$  (SE =  $0.03 \text{ g/cm}^3$ ) for forest soils, and  $1.3 \text{ g/cm}^3$  (SE =  $0.06 \text{ g/cm}^3$ ) for orchard soils at the Puddles (Figure 17). Significant differences in bulk density existed among habitats (overall  $F = 10.23$ ,  $df = 2$ ,  $p = 1.9 \times 10^{-4}$ ). Both field and forest soils had significantly lower bulk densities than orchard soils (individual comparisons  $p < 0.01$ ). Mean bulk density was  $1.0 \text{ g/cm}^3$  (SE =  $0.08 \text{ g/cm}^3$ ) at tracked locations,  $1.1 \text{ g/cm}^3$  (SE =  $0.04 \text{ g/cm}^3$ ) at pass-through locations,  $1.1 \text{ g/cm}^3$  (SE =  $0.05 \text{ g/cm}^3$ ) at extrapolated locations, and  $1.0 \text{ g/cm}^3$  (SE =  $0.06 \text{ g/cm}^3$ ) at random locations at the Puddles (Figure 17). No significant differences existed among location types (overall  $F = 0.36$ ,  $df = 3$ ,  $p = 0.78$ ).

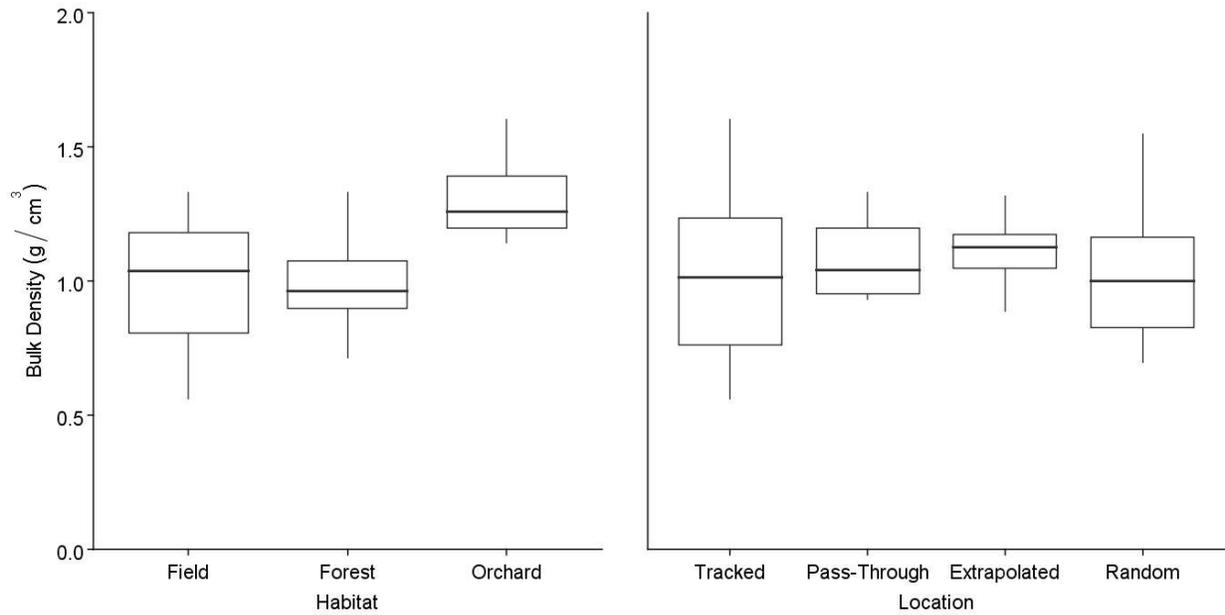


Figure 17: Bulk density ( $\text{g}/\text{cm}^3$ ) by habitat (left) and location type (right) at the Puddles.

At the Ponds mean bulk density was  $1.1 \text{ g}/\text{cm}^3$  ( $\text{SE} = 0.03 \text{ g}/\text{cm}^3$ ) for field soils,  $1.0 \text{ g}/\text{cm}^3$  ( $\text{SE} = 0.04 \text{ g}/\text{cm}^3$ ) for forest soils, and  $1.1 \text{ g}/\text{cm}^3$  ( $\text{SE} = 0.04 \text{ g}/\text{cm}^3$ ) for orchard soils (Figure 18). Bulk density differed significantly among habitats (overall  $F = 3.76$ ,  $df = 2$ ,  $p = 0.03$ ). Orchard soils had significantly greater bulk density than forest soils ( $p = 0.02$ ). Mean bulk density was  $1.1 \text{ g}/\text{cm}^3$  ( $\text{SE} = 0.03 \text{ g}/\text{cm}^3$ ) at tracked locations,  $1.0 \text{ g}/\text{cm}^3$  ( $\text{SE} = 0.03 \text{ g}/\text{cm}^3$ ) at pass-through locations,  $1.0 \text{ g}/\text{cm}^3$  ( $\text{SE} = 0.04 \text{ g}/\text{cm}^3$ ) at extrapolated locations, and  $1.2 \text{ g}/\text{cm}^3$  ( $\text{SE} = 0.06 \text{ g}/\text{cm}^3$ ) at random locations at the Ponds (Figure 18). Bulk density did not significantly vary among location types (overall  $F = 2.45$ ,  $df = 3$ ,  $p = 0.07$ ).

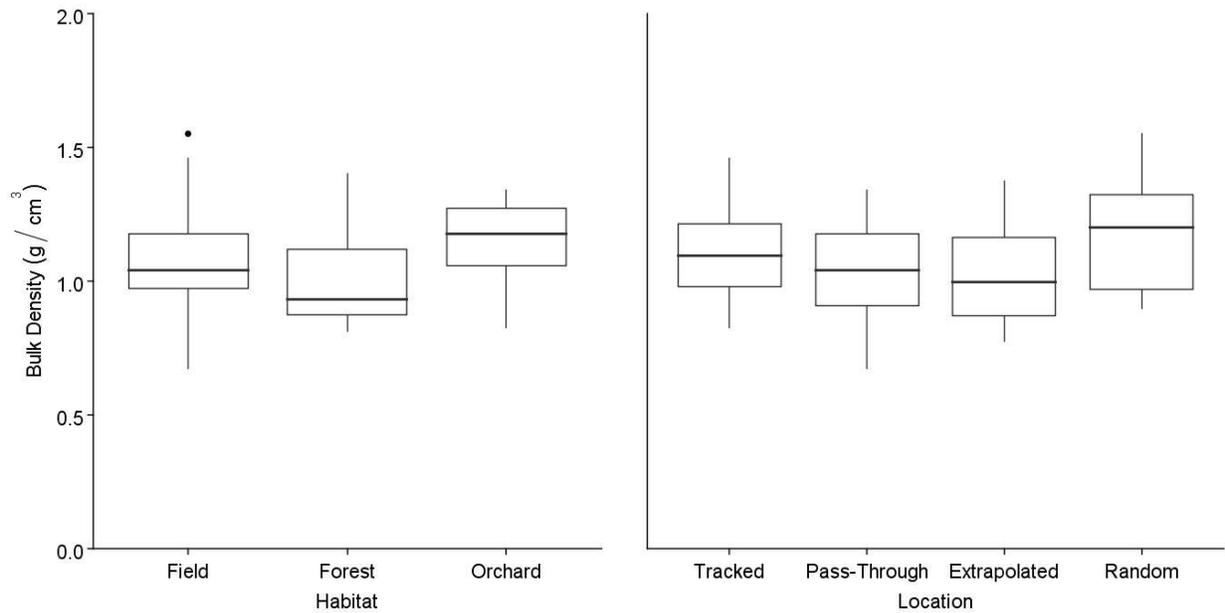


Figure 18: Bulk density ( $\text{g}/\text{cm}^3$ ) by habitat (left) and location type (right) at the Ponds.

## Discussion

### Movement

Maximum distances MCF moved from the breeding sites were 475.6 m from the Ponds and 219.5 m from the Puddles. These distances are less than those reported by Green (1952), who observed MCF moving from breeding in valley pools to pools on the adjacent hillside. The greatest movement he observed was for six individuals between pools located 610 m (“2000 ft”) apart. This greater distance could be a result of the longer time span of Green’s study. He marked individuals by toe clipping and recaptured them during visits to these breeding sites over 4-5 months. The six individuals that moved between those pools did so in 43-66 days (Green 1952) whereas individuals in this study were only tracked approximately 25 days.

Further, individuals likely continued moving after tracking ceased. For example, the one individual I fitted with a second transmitter and tracked for an additional 12 days continued to move. Likewise, the greatest movement distance Green (1952) observed for an individual between seasons was greater than the 610 m movement he observed within a single season. The

distance between one individual's final location of 1945 and first location of 1946 was 1219 m ("4000 ft").

The movements observed in this study are also similar to those reported for other anuran species, including those closely related to MCF. For example, upland chorus frogs (*Pseudacris feriarum*) are known to disperse  $\leq 200$  m from their breeding sites (Lemmon 2008). Palis and Aresco (2007) observed southern chorus frogs (*P. nigrita*) and ornate chorus frogs (*P. ornata*) on the outside of their drift fence 200 m away from their breeding pool, suggesting that the frogs came from farther away. Individuals of larger species are known to travel even farther. Heemeyer and Lannoo (2012) reported crayfish frogs (*Rana* [= *Lithobates*] *aerolatus*) in Indiana traveling a maximum of 1044 m (mean 493 m) and 1188 m (mean 377 m) from breeding sites during two years of study. California red-legged frogs (*Rana draytonii*) have been observed traveling a maximum of 390 m and 1400 m from two breeding sites respectively (Fellers and Kleeman 2007), and traveling as far as 3600 m to reach a breeding site (Bulger et al. 2003).

Given that I observed irritation in the groins of certain individuals I recommend other belt methods be employed in the future, especially for studies tracking movement for longer spans of time. This irritation was likely caused in part by not having enough beads to cover the entire thread. Unfortunately, adding more beads would have made the transmitter belt exceed the weight limit for the frogs. Until transmitters become more lightweight, I recommend belts be made from surgical tubing, which have successfully been used on California red-legged frogs (Chan-McLeod 2003) and pine barrens treefrogs (*Hyla andersonii*; N. Shepard, pers. comm.). Furthermore, the waist of each frog could be measured in order to better ensure proper fit of the belt (Chan-McLeod 2003).

## **Reproduction**

Females in this study produced 227-634 eggs/individual (mean = 362). These numbers are within the range reported for MCF in other parts of their range. Barbour and Walters (1941) reported 983-1202 eggs/female (mean = 1092) in Kentucky, Green (1938) reported 318-1479 eggs/female (mean = 646.5) in West Virginia, and Forester et al. (2003) reported 90-118 eggs/female (mean = 105) in Maryland. I observed 1-113 eggs/mass, which is a greater range than those reported in other studies (Green 1938: 10-50 eggs/mass; Barbour and Walters 1941: 28-40 eggs/mass; Forester et al. 2003: 3-50 eggs/mass). However, my mean of 22 eggs/mass was similar to the other reports (Green 1938: “most masses containing about twenty-five eggs”; Barbour and Walters 1941: 34 eggs/mass; Forester et al. 2003: 14.6 eggs/mass). The lone eggs I observed were likely originally part of masses, but came free when eggs masses were being handled and counted.

Clutch sizes reported here are also consistent with those for MCF congeners. In captivity, female Brimley’s chorus frogs (*P. brimleyi*) produced 264 and 290 eggs (Gosner and Black 1958). Upland chorus frogs (*P. feriarum*) produce 163-534 eggs/female (Dodd 2013).

## **Habitat**

Differences in vegetation and soil properties among location types were not the same at the two breeding sites. However, the differences at both sites can be explained by the differences among habitats. For example, at the Ponds tracked and pass-through locations collectively differed from extrapolated and random locations in vegetation cover. Here, tracked and pass-through locations were predominantly field and orchard, whereas extrapolated and random locations were predominantly forest. The results of the indicator species analysis support the correlation between location type and habitat. Tracked locations contained significantly greater

percent cover of grass, herbs, and vines. Percent cover of herbs and vines were also significantly greater in field habitat, and percent cover of grass was significantly greater in orchard habitat. Similarly, extrapolated locations contained significantly greater leaf litter depth, and random locations contained significantly greater percent cover of bare ground, leaf litter, moss/woody debris, and canopy. Forest habitat also contained significantly greater cover of leaf litter, moss/woody debris, and canopy, and significantly greater leaf litter depth.

The location type-habitat connection is also evident in soil properties at the Ponds. Soils at tracked and pass-through locations were significantly moister than soils at extrapolated and random locations. Tracked location soils also contained a significantly smaller percentage of fine particles (i.e., clay and silt) than random location soils. These differences are again due to tracked and pass-through locations mostly being in field and orchard habitats, and extrapolated and random locations containing the majority of locations in forest habitat. Soil moisture differed significantly among all habitats with field having the highest values and forest having the lowest. Soil texture also differed significantly among all habitats with field having the lowest percentage of fine particles and forest having the highest.

Differences in vegetation and soil properties among location types at the Puddles differed from those at the Ponds, but can still be explained by habitat. There were no significant differences in vegetation cover or soil texture among location types at the Puddles. The lack of differences is likely due to the fact that all location types were present in all habitats at the Puddles. Despite this, there were still significant differences in soil moisture among location types. Tracked location soils at the Puddles had significantly lower soil moisture values than soils at random locations. This difference is likely due to a larger proportion of tracked locations being in forest habitat, and a larger proportion of random locations being in field habitat. Forest

habitat is only to the west of the Puddles, and four of the five frogs at the Puddles made it there at some point. The fifth frog stayed immediately adjacent to the Puddles during the entire tracking period. Thus, most of the random locations were to the east of the Puddles, which was composed entirely of field and orchard habitats.

MCF were likely selecting forest habitat. Four of the five frogs at the Puddles made it to the forest. Of those four, two were in the forest when their transmitters died. One had moved out of the forest into the orchard when its transmitter died. The fourth frog was the individual fitted with a second transmitter and tracked for an additional 12 days. The final location with the first transmitter was in the forest near its edge with orchard habitat. She spent the additional 12 days hopping between the forest and adjacent orchard in the same general vicinity. Of the 15 frogs at the Ponds, only two individuals made it to the forest.

A greater proportion of individuals from the Puddles than the Ponds made it to forest likely due to proximity. The nearest forest habitat to the Puddles was 50-100 m away, whereas the nearest forest to the Ponds was 180-240 m away. The Puddles' proximity to forest also explains why the maximum distance traveled from the Puddles was less than that from the Ponds. MCF at the Puddles did not travel as far because they did not have to in order to make it to ideal habitat. This habitat selection efficiency has been observed with other species, too. For example, Bulger et al. (2003) observed that the majority of California red-legged frogs moved to the nearest suitable habitat adjacent to their breeding site after breeding.

MCF were likely selecting forest because it was better quality habitat than the field and orchard with respect to both vegetation and soil. Forest had greater canopy cover and leaf litter than the other habitats, which both contribute to high quality microhabitats for amphibians by reducing the risks of desiccation. California red-legged frogs show a similar significant

preference for forest over more open habitats (Chan-McLeod 2008). When leaving breeding sites 86% of individuals moved exclusively or nearly so in forest habitat. Conversely, 51.3% of individuals were expected to enter the 12 year-old clearcut, but only 30% of individuals did. Furthermore, the individuals that did enter clearcut habitat tended to be larger, suggesting they would be less susceptible to desiccation due to their smaller surface area-to-volume ratio (Chan-McLeod 2003). Likewise, wood frogs in Maine use leaf litter during their post-breeding migration (Baldwin et al. 2008). Even when the leaf litter was dry, it was still more humid amidst the litter than the ambient conditions.

In addition to a reduced risk of desiccation, amphibians would also likely have a reduced risk of predation in forest compared to more open habitats. In an enclosure experiment, mole salamanders (*Ambystoma talpoideum*) moved more frequently in enclosures with pine litter removed than in control enclosures (Moseley et al. 2004). This increased surface activity would increase their exposure to predators as well as increase their risk of desiccation.

The soil of forest habitat also contributes to its higher quality. Forest had a significantly greater proportion of locations with burrows than either field or orchard habitats at the Puddles and Ponds. MCF were never witnessed inside a burrow during this study, but burrows were observed in the area where a frog was tracked to on multiple occasions, and on one instance the transmitter's signal was noticeably louder above the burrow's opening. Upland chorus frogs are known to utilize mammal burrows (Dodd 2013), so it would not be surprising if MCF use existing burrows as refuges, too.

Similarly, the bulk density of the forest topsoil was significantly lower than orchard topsoil at both the Puddles and Ponds. There are no previous reports of MCF actively burrowing, and it was not observed here. However, southern chorus frogs were observed

digging burrows when kept in captivity (Carr 1940), so it is possible MCF dig burrows for refuge. Whether the burrows are dug by MCF themselves or other animals, it is more energetically costly for organisms to burrow in denser soils (Vleck 1979; Ducey et al. 1993).

Thus, forest habitat ultimately has more available refuges than other habitats due to its greater presence of burrows and lower bulk density soil. These properties, soil compaction and burrow presence, have also been shown to influence habitat selection by metamorph ringed salamanders (*Ambystoma annulatum*) and spotted salamanders (*A. maculatum*) leaving their natal pools (Osborn et al. 2014). Soil compaction significantly reduced the proportion of individuals of both species that settled within enclosures extending 20 m from the pools, and high burrow density significantly increased the proportion of spotted salamanders which settled within the enclosures.

Soil texture can influence bulk density. Fine-textured soils frequently have lower bulk densities because their particles form aggregates, which contain micropores (Brady and Weil 2004). Coarser-textured soils can vary. Sandy soils with uniformly sized particles usually have lower bulk densities. Sandy soils with particles of various sizes usually have higher bulk densities because the smaller particles fill in the pores between larger particles (Brady and Weil 2004). The forest soil in this study had significantly greater percentage composition of fine particles and significantly smaller percentage composition of sand than orchard (at Ponds only) and field habitats. Thus, the lower bulk density of the forest soil could be due to its finer texture. However, forest soils are not always more finely textured than grassland habitats. For instance, in the shortgrass steppe of Colorado this relationship is somewhat altered (Dodd et al. 2002). The dominance of shrubs and C<sub>3</sub> grasses is associated with sandy soils, and the dominance of C<sub>4</sub> grasses is associated with fine-textured soils (Dodd et al. 2002).

The soil moisture in the forest was significantly lower than that of the other habitats. Initially, it might seem counterintuitive that MCF would select for forest habitat if it was drier than orchard and field. However, they might be selecting for the stability of the soil moisture rather than the actual values. James et al. (2003) noted that the temporal heterogeneity of soil moisture was lower in Canadian aspen forest than in adjacent grassland. Specifically, the grassland soils were moister than forest soils when soil moisture was high, and drier than forest soils when soil moisture was low. Therefore, MCF may prefer forest because its soil is more reliably moist. During periods without rain, field soils may become drier than forest soils, thus creating a relatively higher risk of desiccation. Furthermore, I tended to measure soil moisture within the couple days following rain in order to prevent an excess of the soil moisture values being zeroes. That timing could also have exaggerated the differences in soil moisture among the habitats.

In conclusion, MCF were likely migrating to forest habitat after breeding due to its higher habitat quality (i.e., lower risk of desiccation, more refuges, etc.). Future research should still continue to investigate post-breeding habitat use of MCF throughout its range. A single year may not completely reflect a species' habitat preferences. For instance, southern chorus frogs were observed to prefer longleaf pine savanna over slash pine plantation in one year of a three year study, but had no preference during another (Palis and Aresco 2007). Furthermore, it would be beneficial to study habitat selection in landscapes less altered by human activity. A variety of human activity occurs at the seed orchard, including the use of heavy machinery, and habitats like the orchard and feed plot are not naturally occurring. Thus, in order to understand habitat use in more natural settings, similar studies should be conducted in landscapes with less human disturbance.

Until further study, I recommend conservation biologists and land managers to maintain forest habitat within 200 m of known MCF breeding sites. In addition, riparian areas near known breeding sites could also be maintained in order to provide refuge during dry years when post-breeding movement might be hindered. Finally, soil compaction should be minimized to facilitate the creation of burrows.

## CHAPTER TWO: MOVEMENTS AND HABITAT USE OF BOG TURTLES

### *(Glyptemys muhlenbergii)* IN ATYPICAL WETLAND HABITAT

#### **Introduction**

Turtles are one of the most threatened taxonomic groups across the globe (Buhlmann et al. 2009). According to the IUCN Red List of Threatened Species (2015) 32% of assessed turtle species are considered critically endangered or endangered, and 26% are considered vulnerable. Primary threats to these taxa are anthropogenic habitat loss and fragmentation, and collection for the illegal pet trade (Buhlmann et al. 2009). Unfortunately listing does not always translate into research and recovery for these species (Lovich and Ennen 2013), and at present no turtle species has ever been removed from the federal list of endangered species. Furthermore, research and conservation efforts are not even among species. Large, wide-ranging turtle species receive the most attention, whereas species that are smaller and/or have limited ranges receive less (Lovich and Ennen 2013).

The bog turtle (*Glyptemys muhlenbergii*) is the smallest freshwater turtle and one of the most endangered turtle species in North America (Shoemaker et al. 2013). It ranges in the eastern United States from Georgia to New York and is made of two disjunct populations. The northern population (Maryland to New York) is federally threatened, while the southern population (Virginia to Georgia) is federally threatened due to similarity of appearance with the northern population (USFWS 1997). The species receives some level of protection in every state in its range (Ernst and Lovich 2009), and is listed as state threatened in North Carolina (NCWRC 2015).

Bog turtles occupy spring-fed bogs, fens, swamps, and wet meadows, but the specific habitat varies throughout its range. In its southern distribution, these wetlands tend to have fairly open canopy, contain deep, soft mud, are dominated by emergent vegetation (e.g. rushes, sedges, etc.) and sphagnum moss, and contain pools and rivulets, including muskrat runways and beaver channels (Arndt 1977, Kiviat 1978, and Herman and Tryon 1997). These wetlands are also usually ephemeral or transitional (Kiviat 1978, Herman and Tryon 1997). Historically beavers were likely the primary creator and maintainer of habitat, but currently livestock grazing is the foremost habitat maintainer (Kiviat 1978, Herman and Tryon 1997). In fact, moderate livestock grazing is currently recommended as a habitat management strategy because it prevents canopy closure and woody stem succession, and livestock hoof prints provide microhabitat bog turtles occasionally use (Tesauro and Ehrenfeld 2007). Furthermore, the majority of southern bog turtle sites and those with the strongest populations are located within livestock pastures (Tryon and Herman 1990). These “meadow bogs” are likely the remnants of natural wetlands, such as southern Appalachian bogs and swamp forest-bog complexes, that human activity altered, like draining for agriculture (Weakley and Schafale 1994).

The present study focused on the bog turtle population in Whiteoak Bottoms (WOB), a 2.5 ha wetland along the Nantahala River in Macon County, North Carolina. WOB has been in existence for 15,000 years, and is currently maintained by hillslope seeps, groundwater, and beavers (McDonald and Leigh 2011). It is an example of a southern Appalachian bog, and consists of a mosaic of herbaceous- and shrub/scrub-dominated areas with sphagnum moss throughout (Weakley and Schafale 1994). Approximately 45% of WOB’s habitat is shrub/scrub, 45% is emergent, and the remainder consists of a small, dense patch of rhododendron and a

beaver pond (Figure 19). There is an extensive network of rivulets, which are beaver channels, that runs throughout the wetland.

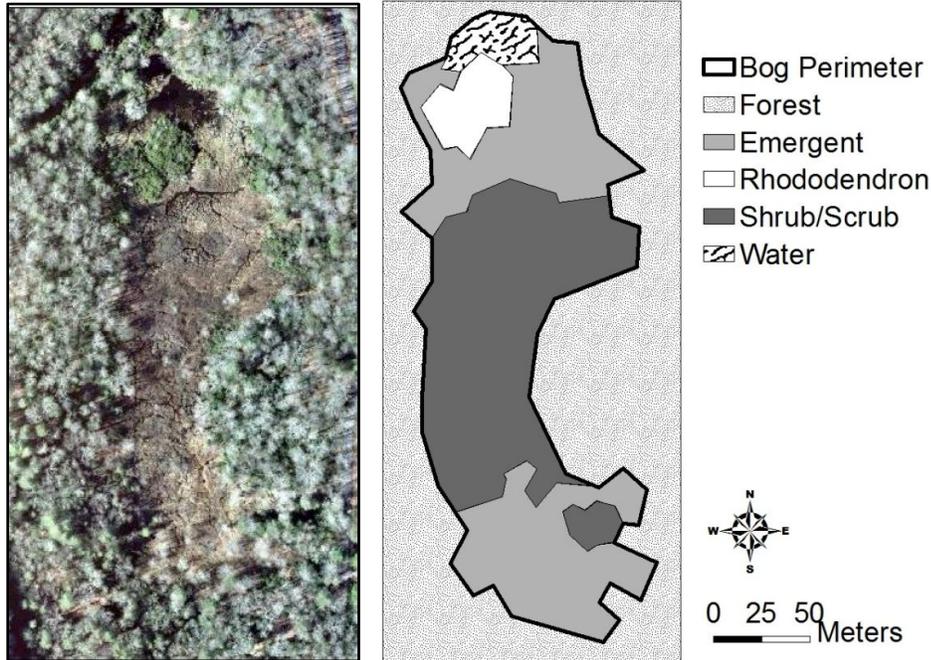


Figure 19: Aerial photograph of Whiteoak Bottoms (left), and map depicting habitats within Whiteoak Bottoms (right).

Southern Appalachian bogs are a rare habitat, with only an estimated 23% of their original extent in North Carolina remaining (Weakley and Schafale 1994). Much of this habitat has been lost to succession, possibly due to lack of historical disturbance (e.g. beavers, fire, etc.) or anthropogenic modifications (e.g. overgrazing, eutrophication, etc.) allowing invasive vegetation to outcompete native species (Schafale and Weakley 1990). WOB is a part of Nantahala National Forest. The U.S. Forest Service purchased the land containing the wetland in 1918 from the W.M. Ritter Lumber Company with the agreement that timber could be harvested for another 20 years (M. Southard, pers. comm.). While no records exist if the wetland itself was logged or what impact the logging camp may have had on the wetland, WOB has had approximately 80 years of recovery time from any disturbance.

Thus, compared to the meadow bogs typical of the bog turtle's southern distribution, WOB is unique. A large proportion of the available habitat is shrub/scrub, and it has relatively little human disturbance. The purpose of this study was to investigate the movements and habitat use of bog turtles at WOB, and compare that to other populations in more "typical" wetlands. In their review on the southern distribution of this species, Tryon and Herman (1990) observed that bog turtles are not found everywhere that it appears they should be based on habitat. This observation suggests that the conservation biology and wildlife management community does not fully understand the habitat requirements of this species, and could therefore benefit from studying a population occupying a wetland that may better reflect its habitat before anthropogenic change became systemic.

## **Methods**

### **Population Structure and Sex Ratio**

I trapped bog turtles May 15–July 29, 2015 at WOB. Sixty trap-door traps were placed within rivulets and pools, and were partially submerged in the water and mud. The tops of the traps were covered with mud and vegetation to prevent captured animals from overheating. Traps were left open for intervals of three to eight days, and checked daily. I measured straight carapace length (SCL), maximum carapace length ( $CL_{max}$ ), straight plastron length (SPL), maximum plastron length ( $PL_{max}$ ), nuchal length (NL), shell height (SH), and mass on every turtle captured. I estimated minimum age by counting the annuli on scutes, and determined the sex of sexually mature individuals by visual examination. I gave each individual captured a unique mark by scute notching (Appendix C), and adults also received a passive integrated transponder (PIT) tag. Observations on weather, microhabitat, and location of capture were recorded.



## **Movement and Activity Patterns**

I attached radio transmitters to adults captured according to the methods of Eckler et al. (1990). Transmitters with a mass of 3.8 g and expected six-month battery life (Holohil Systems Ltd., PD-2, Ontario, Canada,  $n = 4$ ) and transmitters with a mass of 3.6 g and expected 14 month battery life (Advanced Telemetry Systems, Inc., R1680, Insanti, Minnesota,  $n = 2$ ) were coated with waterproof epoxy, and attached to the third or fourth costal scute. Upon being fitted with transmitters, individuals were returned near their site of capture. I located individuals two or three times per week from May–July when activity is greatest (Ernst and Lovich 2009), and one or two times per week during August–November because certain populations are speculated to aestivate during this time due to the lack of moisture (Ernst and Lovich 2009). I recorded a GPS point for every turtle location using a Garmin eTrex Vista HCx (Garmin International Inc., Olathe, Kansas). I removed transmitters during October–November when an individual was repeatedly tracked to the same location, indicating preparation for hibernation. Step distances (straight-line distance between consecutive turtle locations) and home ranges calculated as minimum convex polygons were determined using Geospatial Modelling Environment software (Spatial Ecology LLC, Brisbane, Australia) for each individual.

Minimum convex polygons (MCP) were used due to its historical precedence in home range estimation. However, MCP has the potential to overestimate the home range area due to its sensitivity to outliers, thus a second calculation was used as well (Cuevas et al. 2007). For the second home range calculation, I used Animal Space Use 1.3 (Horne and Garton 2009) to select the best home range model given my data. This program compares six different home range estimation models using Akaike's information criterion (AIC) and likelihood cross-validation (CVC). I chose to use fixed kernel density as the second home range estimation method because

it was the best model or a close second for all turtles. Fixed kernel density estimation considers each animal location a probability density function, and the smoothing parameter has a constant value for all locations (Worton 1989). I used likelihood cross-validation to select the value of the smoothing parameter due to my small sample sizes of locations (i.e., < 50 locations/individual) and its ability to identify high use areas (Horne and Garton 2006). I estimated home range as 95% kernel density and core activity areas as 50% kernel density using Geospatial Modelling Environment software (Spatial Ecology LLC, Brisbane, Australia).

### **Habitat**

At each radio tracked turtle location, I measured the height of the dominant (i.e., most prolific) vegetation and tallest vegetation within 1 m, air temperature, and soft substrate depth. For aquatic locations I also measured water temperature and water depth. I measured water and soft substrate depth with a ¼-inch dowel rod according to Carter et al. (1999). Water depth was measured from the surface to the substrate, and for soft substrate depth, I pushed the dowel into the mud until it could be pressed no farther. I also noted macrohabitat type and microhabitat soil, water, and vegetation. Microhabitat was defined as what was touching the turtle.

These same habitat parameters were measured at randomly selected sites within the wetland complex. For each unique tracked turtle location, I sampled paired random locations (Carter et al. 1999; Dubois et al. 2009; O'Bryan 2014). Random locations were determined by choosing a random direction (0-359°; Carter et al. 1999) and a random distance (1-25 m) to travel from the tracked turtle location. I chose 25 m as the maximum distance because that encompasses the average distances travelled between consecutive tracked turtle locations in other bog turtle telemetry studies (Lovich et al. 1992; Carter et al. 2000). GPS points were recorded for all random locations.

I measured pH using a pH tester (Hanna Instruments HI98127, Woonsocket, Rhode Island) at randomly selected locations within the wetland monthly from June–August to describe the pH ranges present in the wetland during the turtle’s most active season. I randomly picked at least one trap from each geographic area in the wetland where I placed turtle traps, and took pH readings of the water the trap was in or near.

Bog turtles typically mate during April and May, nest in June, and hatchlings emerge in late August and September (Ernst and Lovich 2009). I monitored the gravid/non-gravid status of all tracked females, and attempted to locate any nests. I made observations on the number of eggs laid and the nesting habitat for all discovered nests. I placed nest protectors around nests to guard them against predators. I constructed these protectors from chicken wire, and they were cut into the soil approximately two inches using a sod knife (Appendix D). I checked nests weekly during June and July to ensure the protective boxes were intact. Beginning in August, I checked nests during every visit in order to detect hatching date.

## **Analysis**

All statistical analyses were conducted in R software version 3.1.2 (R Core Team 2014). Seasonal differences in daily movement rate were investigated using a mixed-model ANOVA, in which individual was considered a random effect and all individuals were nested within season. I classified my seasons as ‘nesting,’ ‘summer,’ and ‘autumn.’ Nesting season for bog turtles has been defined as May to July (Ernst and Lovich 2009), June and July (Ernst and Barbour 1989), and 7 June to 6 July specifically in Maryland (Wilson et al. 2004). Thus, I considered nesting season beginning on the first date of my field study (15 May 2015) through the date when all gravid females no longer felt gravid (6 July 2015) (Litzgus and Mousseau 2004). I defined

summer as continuing from the end of nesting season through 31 August, and defined autumn as 1 September through the final date on which I conducted telemetry (5 November 2015).

I conducted a chi-squared analysis to evaluate macrohabitat preference. I calculated the proportion of the wetland's area composed by each habitat type (open water, rhododendron thicket, emergent, and shrub/scrub) using ArcGIS software version 10.3 (Esri, Redlands, California) in order to determine the expected number of turtle locations within each macrohabitat type. I compared the habitat composition of each turtle's home range (95% fixed kernel density) to all other individuals' home ranges using the Bray-Curtis similarity index. Seasonal differences in habitat were investigated using a G-test with Holm's sequential Bonferroni. I used rank-preference indices to determine preference for water and soil components of microhabitat. Categories for water were 'none,' 'standing,' and 'rivulet,' and categories for soil were 'silt,' 'clay,' 'sand,' and 'muck,' which was a very liquid mud. Data from turtle locations were used to calculate usage ranks, and data from random locations were used to calculate availability ranks.

I converted quantitative soft substrate depths into categories of shallow ( $x < 30$  cm), intermediate ( $30 \text{ cm} \leq x < 60$  cm), and deep ( $60 \text{ cm} \leq x$ ) because there were many observations where the soft substrate depth exceeded the length of the dowel rod (91.5 cm). These soft substrate depth profiles were then compared between location types (i.e., tracked turtle locations and random locations) and among macrohabitats using G-tests. I used ANOVA to compare turtle depth in mud among macrohabitats. To investigate the relationship among location type, macrohabitat type, and soft substrate depth I used the Cochran-Mantel-Haenszel (CMH) test, which is a form of three-way contingency table analysis. I conducted Woolf's test for homogeneity across strata to ensure the data met the assumptions for CMH.

## Results

### Population Structure and Sex Ratio

Nine bog turtles were captured in the traps (2 adult males, 4 adult females, and 3 juveniles). All individuals were new captures except for one adult female who was originally captured as an adult in 2008. Thus, of the turtles captured in this study the age structure is 67% adults and 33% juveniles, and the sex ratio is 33% male and 67% female.

### Movement and Activity Patterns

Radio transmitters were fitted to all adults ( $n = 6$ ). The mass of the adults ranged from 99-158 g and the mass of the transmitter and epoxy used per individual ranged from 4-8 g. Thus, the mass of the transmitter package used on each individual was less than 7% of that individual's body mass (Eckler et al. 1990). One individual (turtle 0.4) was found deceased in the mud of a dry rivulet on 5 October. All other individuals were tracked until they were repeatedly located at the same location during late autumn (25 Oct–5 Nov) indicating preparation for hibernation. Individuals were located 35-39 times during the course of this study for a grand total of 218 turtle locations.

Mean step distance ranged from 13.4-35.2 m per individual and mean daily movement rate, including movements of 0 m, ranged from 4.3-11.0 m/day per individual. Mean step distance was 30.6 m (SE = 2.9 m, min = 0.0 m, max = 184.1 m) for females and 17.0 m (SE = 2.5 m, min = 0.0 m, max = 128.2 m) for males, and mean daily movement rate was 8.3 m/day (SE = 0.9 m/day, min = 0.0 m/day, max = 49.6 m/day) for females and 5.0 m/day (SE = 0.8 m/day, min = 0.0 m/day, max = 42.7 m/day) for males. Overall, the grand mean step distance was 26.0 m (SE = 2.2 m), and the grand mean daily movement rate was 7.2 m/day (SE = 0.6 m/day). The proportion of short step distances (i.e. < 20 m) was 59%.

Daily movement rate did not significantly differ amongst seasons ( $F = 1.12$ ,  $df = 2$ ,  $p = 0.36$ ) (Figure 20). The mean daily movement rate was 9.1 m/day (SE = 1.2 m/day) during nesting season, 5.9 m/day (SE = 0.7 m/day) during summer, and 4.8 m/day (SE = 1.0 m/day) during autumn. However, the majority of large daily movement rates (i.e.,  $\geq 30$  m/day) occurred during nesting season (Figure 21). Thus, I investigated seasonal differences in the maximum daily movement rate per individual. Maximum daily movement rate was significantly greater during nesting season than during summer or autumn ( $F = 45.46$ ,  $df = 2$ ,  $p < 0.001$ ) (Figure 22). The mean maximum daily movement rates for nesting season, summer, and autumn respectively were 39.8 m/day (SE = 4.8 m/day), 17.1 m/day (SE = 3.7 m/day), and 16.3 m/day (SE = 3.8 m/day).

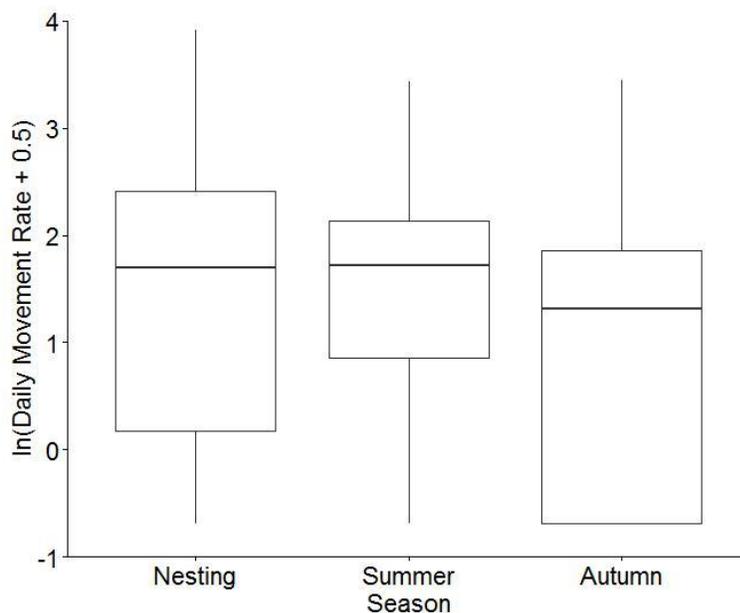


Figure 20: Daily movement rates by season.

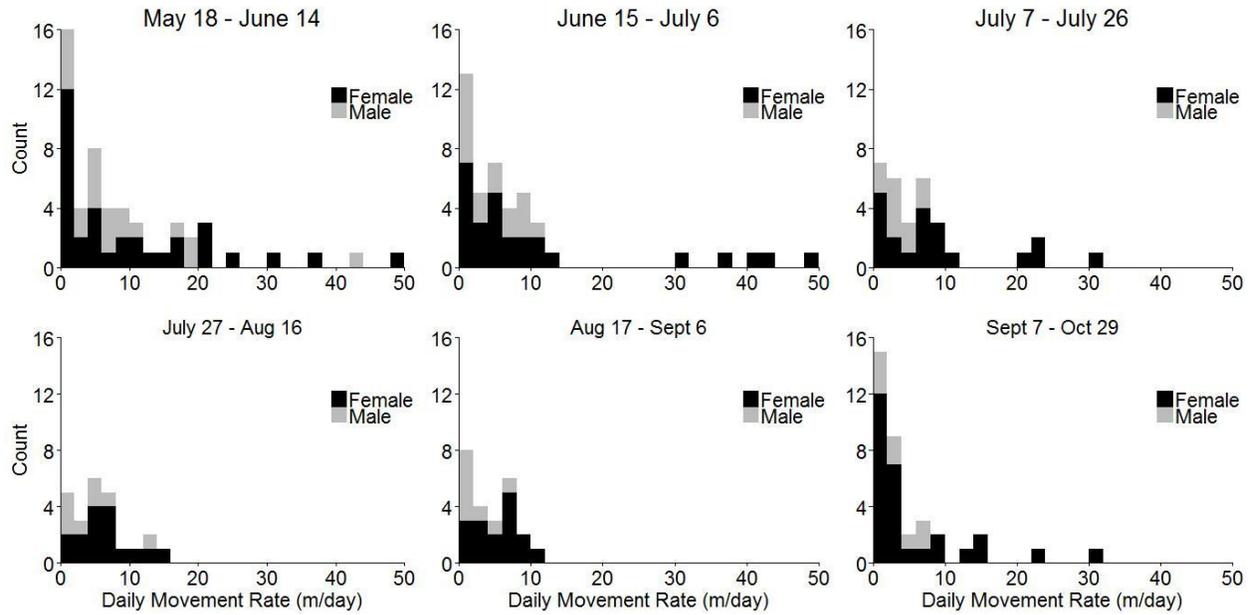


Figure 21: Histograms of daily movement rates in 3-7 week intervals.

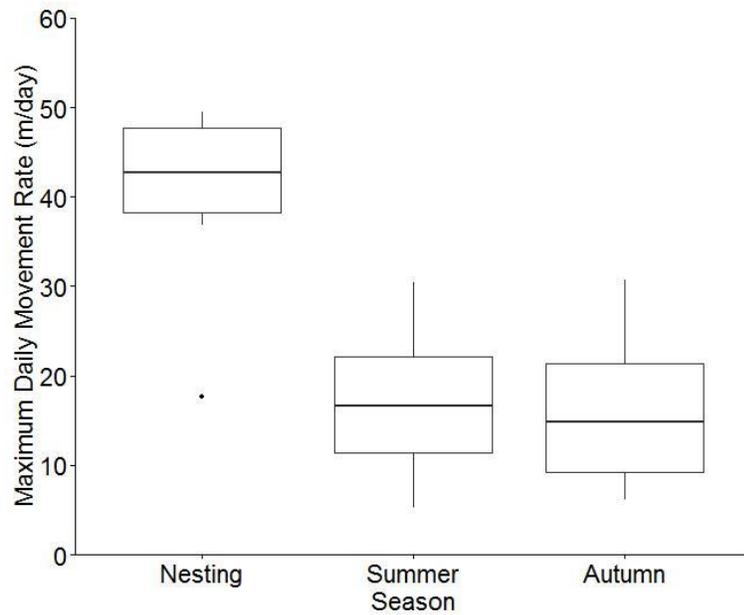


Figure 22: Maximum daily movement rates by season.

Home ranges calculated as minimum convex polygons (MCP) ranged from 0.1081-1.0187 ha per individual, and ranged from 0.2387-0.9027 ha when calculated as 95% kernels (Appendices E and F). Core area ranged from 0.0369-0.2002 ha per individual (Appendices E and F). Mean female home range was 0.6970 ha (SE = 0.4118 ha) and 0.6064 ha

(SE = 0.1202 ha) when calculated as MCP and 95% kernels respectively, and mean female core area was 0.1255 ha (SE = 0.0336 ha). Mean male home range was 0.3706 ha (SE = 0.2625 ha) and 0.4458 ha (SE = 0.2071 ha) when calculated as MCP and 95% kernels respectively, and mean male core area was 0.0940 ha (SE = 0.0442 ha). Overall, the grand mean home range was 0.5882 ha (SE = 0.1266 ha) as MCP and 0.5528 ha (SE = 0.0989 ha) as 95% kernel, and the grand mean core area was 0.1150 ha (SE = 0.0250 ha).

### **Habitat**

Habitat measurements were not always taken at random locations on the same day that turtle location habitat was measured due to weather, daylight, etc. constraints. For the purposes of comparison, I only report here on the random location habitat that was measured on the same day as its respective turtle location habitat. The mean dominant vegetation height was 212.0 cm (SE = 8.5 cm) at turtle locations and 224.3 cm at (SE = 6.8 cm) random locations. The mean maximum vegetation height was 238.5 cm (SE = 7.0 cm) and 254.7 cm (SE = 6.2 cm) at turtle and random locations respectively. At turtle locations the mean water depth was 15.8 cm (SE = 1.4 cm), the mean soft substrate depth was 61.4 cm (SE = 2.9 cm), and turtles were found at a mean depth of 11.9 cm (SE = 0.9 cm) below the surface (Table 2). At random locations the mean water depth was 2.5 cm (SE = 0.4 cm) and the mean soft substrate depth was 60.6 cm (SE = 2.4 cm) (Table 2).

Nine pH measurements were taken throughout the wetland each month from June through August. Throughout the summer individual pH measurements ranged from 3.86 to 8.53. The mean monthly pH was 4.55 (SE = 0.21), 6.98 (SE = 0.14), and 6.10 (SE = 0.61) for June, July, and August respectively. Overall the mean pH of the Whiteoak Bottoms wetland was 5.88 (SE = 0.29).

Table 2: Water depths, substrate depths, and turtle depths by location type.

<b>Location Type</b>	<b>Water Depth (cm)</b>			<b>Soft Substrate Depth (cm)</b>			<b>Turtle Depth (cm)</b>		
	<b>Mean</b>	<b>Max.</b>	<b>Min.</b>	<b>Mean</b>	<b>Max.</b>	<b>Min.</b>	<b>Mean</b>	<b>Max.</b>	<b>Min.</b>
Turtle	15.8	50.1	0.0	61.4	91.5	5.6	11.9	38.9	0.0
Random	2.5	40.3	0.0	60.6	91.5	0.0			

For all habitat selection analyses, rhododendron thicket habitat was pooled with shrub/scrub habitat due to its small proportion of the wetland (6%) and its similarity to shrub/scrub habitat. Overall, chi-squared analysis indicates that these turtles preferentially selected shrub/scrub habitat ( $X^2 = 25.28$ ,  $df = 2$ ,  $p = 3.23 \times 10^{-6}$ ; Figure 23). Only 52% of the wetland’s habitat was shrub/scrub or rhododendron thicket, but 68% of the turtle locations were within it. In contrast, 43% of the wetland was emergent habitat, and only 32% of turtle locations were within it. The mean Bray-Curtis similarity of habitat within each turtle’s home range compared to all other individuals ranged from 0.60-0.82 per individual (Figure 24). Habitat use did not significantly vary seasonally (pairwise *p-values* for each comparison: nesting and summer = 0.095, nesting and autumn = 0.493, and summer and autumn = 0.038). However, habitat use between summer and autumn was nearly significant different with autumn having a greater proportion of locations in emergent habitat. In summer 23% of locations were in emergent habitat and 77% were in shrub/scrub, whereas in autumn 40% of locations were in emergent habitat and 60% were in shrub/scrub.

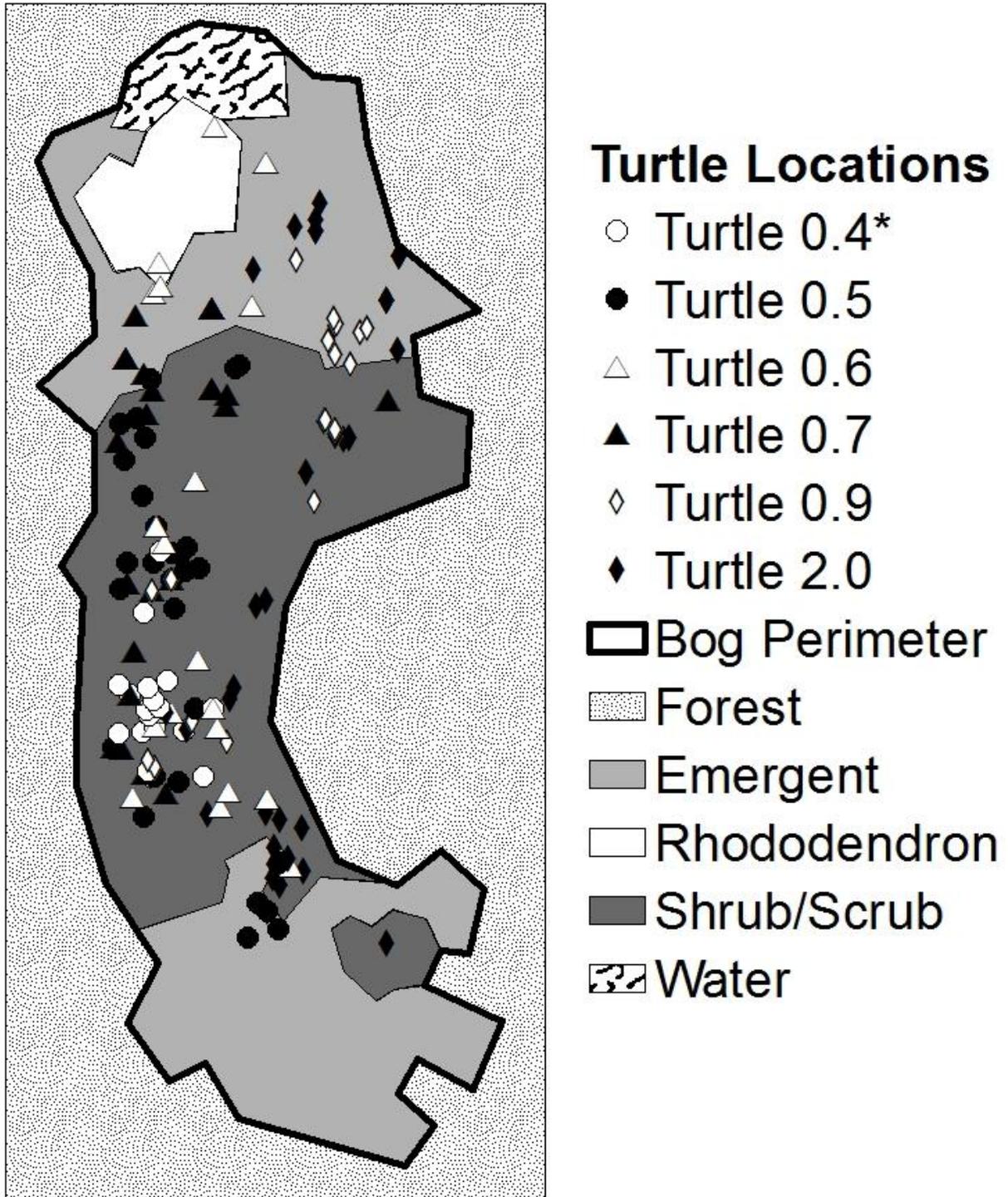


Figure 23: Turtle locations by individual throughout the wetland.

\* Turtle 0.4 was found dead on 5 Oct 2015.

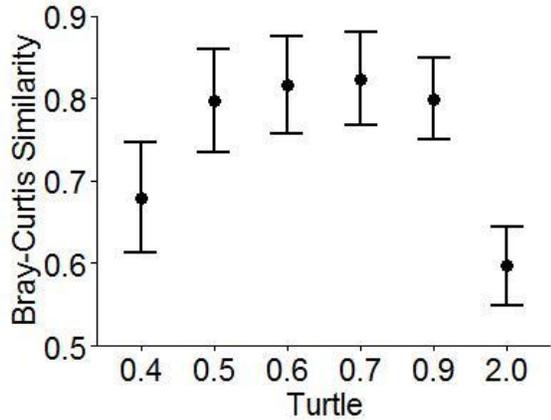


Figure 24: Mean  $\pm$  standard error of Bray-Curtis similarity of habitat within the home range (95% kernel) of each turtle compared to all other individuals.

The microhabitat water rank preference index indicates that these turtles most preferred the rivulets, but also showed preference for standing water (Table 3). They avoided being out of water. The microhabitat soil rank preference index shows that turtles preferred sand substrates, avoided silt, and had neutral preference towards muck substrates (Table 4).

Mud depth profile did not differ between random and turtle locations ( $G = 1.10$ ,  $df = 2$ ,  $p = 0.58$ ; Table 5), but did differ significantly between habitats ( $G = 25.20$ ,  $df = 2$ ,  $p = 3.4 \times 10^{-6}$ ). In general, shrub/scrub habitat contained more deep mud. Sixty-seven percent of locations in shrub/scrub habitat contained deep mud, whereas only 42% of locations in emergent habitat did (Table 6). Conversely, emergent habitat had a higher proportion of its locations in shallow and intermediate mud than did shrub/scrub habitat (Table 6). However, there was no significant difference in turtle depth between habitats ( $F = 0.71$ ,  $df = 1$ ,  $p = 0.40$ ; Figure 25). Results of the Cochran-Mantel-Haenszel test (CMH) indicate that location type and mud depth are not independent when controlling for habitat ( $M^2 = 7.50$ ,  $df = 2$ ,  $p = 0.02$ ),

Table 3: Rank-Preference Index of the water component of microhabitat.

Turtle →	Usage Rank ( $r_i$ )						Availability Rank ( $s_i$ )						Rank Differences ( $t_i$ )						Mean $t_i$	Rank	Result
	0.4	0.5	0.6	0.7	0.9	2.0	0.4	0.5	0.6	0.7	0.9	2.0	0.4	0.5	0.6	0.7	0.9	2.0			
None	3	3	3	3	3	3	1	1	1	1	1	1	2	2	2	2	2	2	2	3	Avoid
Standing	2	2	2	2	2	2	2.5	2	3	3	3	3	-0.5	0	-1	-1	-1	-1	-0.75	2	Prefer
Rivulet	1	1	1	1	1	1	2.5	3	2	2	2	2	-1.5	-2	-1	-1	-1	-1	-1.25	1	Prefer

Table 4: Rank-Preference Index of the soil component of microhabitat.

Turtle →	Usage Rank ( $r_i$ )						Availability Rank ( $s_i$ )						Rank Differences ( $t_i$ )						Mean $t_i$	Rank	Result
	0.4	0.5	0.6	0.7	0.9	2.0	0.4	0.5	0.6	0.7	0.9	2.0	0.4	0.5	0.6	0.7	0.9	2.0			
Muck	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	2	Neutral
Silt	2.5	2.5	2	2.5	3	3	2	2	2	2	2	2	0.5	0.5	0	0.5	1	1	0.58	3	Avoid
Sand	2.5	2.5	3	2.5	2	2	3	3	3	3	3	3	-0.5	-0.5	0	-0.5	-1	-1	-0.58	1	Prefer

55

Table 5: Proportion of locations by location type within each mud depth category.

	Random	Turtle
Deep	0.56	0.58
Intermediate	0.20	0.23
Shallow	0.24	0.19

Table 6: Proportion of locations by habitat type within each mud depth category.

	<b>Emergent</b>	<b>Shrub/Scrub</b>
<b>Deep</b>	0.42	0.67
<b>Intermediate</b>	0.32	0.20
<b>Shallow</b>	0.26	0.13

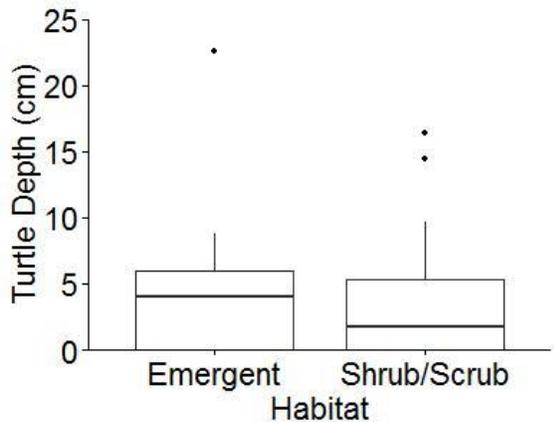


Figure 25: Turtle depth in mud by habitat.

and Woolf's test for homogeneity across strata confirmed that this test was appropriate for this scenario ( $X^2 = 3 \times 10^{-4}$ ,  $df = 1$ ,  $p = 0.99$ ). Within each habitat, the proportion of turtle locations with deep mud was greater than the proportion of random locations with deep mud (Table 7). Furthermore, the proportion of random locations in deep mud (i.e., availability of deep mud) was greater in shrub/scrub habitat than in emergent habitat (Table 7).

Table 7: Proportion of locations by location type in each mud depth category. Each habitat shown separately.

<b>Emergent</b>			<b>Shrub/Scrub</b>		
	<b>Random</b>	<b>Turtle</b>		<b>Random</b>	<b>Turtle</b>
<b>Deep</b>	0.36	0.48	<b>Deep</b>	0.63	0.73
<b>Intermediate</b>	0.32	0.31	<b>Intermediate</b>	0.21	0.18
<b>Shallow</b>	0.32	0.21	<b>Shallow</b>	0.16	0.09

Three of the females being radio tracked were gravid. I determined the general vicinity of one nest, which was in the emergent macrohabitat, in an elevated mound of sphagnum moss

surrounded by rushes. I found the exact location of a second female's nest. It too was in the emergent macrohabitat, in a sphagnum mound surrounded by rushes. The nest contained four eggs buried 5.5 cm deep in a 23 cm-tall mound of sphagnum moss. I only constructed a nest protector box around the nest whose exact location was known.

No evidence of hatching was observed for either nest during the regular hatching season (Aug–Sept). On 19 October I presumed the nest whose exact location was known had failed, and excavated it. All four eggs were still present and unhatched. The eggs at the bottom of the nest were resting in a small amount of water and had ice on them. I collected the eggs and gave them to the North Carolina Wildlife Resources Commission (NCWRC) as voucher specimens.

## **Discussion**

### **Movement and Activity Patterns**

The home range sizes reported here are within the ranges of those observed in other bog turtle populations. In Whiteoak Bottoms, females had a mean home range of 0.6970 ha (MCP) and 0.6064 ha (95% fixed kernel), and males had a mean home range of 0.3706 ha (MCP) and 0.4458 ha (95% fixed kernel). Ernst (1977) observed mean home ranges (MCP) of 1.26 ha and 1.33 ha for females and males respectively in southeastern Pennsylvania. The smallest home ranges were reported by Pittmann and Dorcas (2009) from the Piedmont of North Carolina. They observed mean home ranges (MCP) of 0.0796 ha and 0.16123 ha for females and males respectively. In the study population, turtle 0.4 (♂) had the smallest home range by both methods, and was found deceased, buried in the mud, on 5 Oct 2015. Based on necropsy, he died from emaciation (SCWDS 2016). It is possible that poor health reduced his movements and home range size during the course of this study.

Due to small sample size, significant differences in home range size due to sex were not investigated. However, it is interesting to note that in this population mean home range size was larger for females than males. Most previous studies report either significantly larger home ranges for males or no significant difference between sexes. In Maryland, Chase et al. (1989) observed significantly larger home ranges for males. Morrow et al. (2001b) found no significant difference in home range size between sexes in their study which shared a site with Chase et al. (1989), but did note that the male home ranges were insignificantly larger. In southwestern Virginia, Carter et al. (1999) also found no significant difference in home range size between sexes.

Females in WOB could have had larger home ranges than males due to the zoned nature of the habitats within the wetland. The middle of the wetland is shrub/scrub habitat, whereas the north and south ends are primarily emergent (Figure 20). Overall this population preferred the shrub/scrub habitat, but females nested in emergent habitat. Bog turtle nests are typically made in elevated mounds of sphagnum moss or hummocks of sedges and rushes, and are usually exposed to sunlight (Ernst and Lovich 2009). Thus, females may have had to make an intra-wetland nesting migration in order to find suitable nesting habitat. In other freshwater turtles, females that conduct nesting migrations also have larger home ranges than males. Gravid female spotted turtles (*Clemmys guttata*) in South Carolina migrate to nesting habitat and consequently have significantly larger home ranges than males (Litzgus and Mousseau 2004). Similarly, in Ontario gravid female Blanding's turtles (*Emydoidea blandingii*) nest approximately 1 km away from the wetland, and home range size is significantly larger for gravid females than males or nongravid females (Millar and Blouin-Demers 2011). On the contrary, eastern long-necked turtles (*Chelodina longicollis*) in Australia nest within their

wetland and do not make nesting migrations, and home range size does not vary between sexes (Roe and Georges 2008).

Movement rate follows a similar pattern to home range size. The observed movement rates for bog turtles in WOB were 8.3 m/day and 5.0 m/day for females and males respectively. These values are slightly greater than those reported elsewhere. Morrow et al. (2001b) observed a mean movement rate of 3.3 m/day for bog turtles in Maryland, and Lovich et al. (1992) observed mean movement rates of 1.1 m/day and 2.1 m/day for females and males respectively in North Carolina.

Statistical differences in movement rate between sexes were not investigated due to limited sample size, but females in this population did have a greater mean movement rate than males. This finding differs from other studies. Morrow et al. (2001b) found no significant difference in movement rate between sexes, and Lovich et al. (1992) found males had a significantly greater movement rate. A nesting migration would explain the larger movement rate of females in this population. Gravid female spotted turtles conduct nesting migrations and have greater movement rates than males during the spring (Litzgus and Mousseau 2004). Likewise, Millar and Blouin-Demers (2011) found a significant month  $\times$  reproductive class interaction on mean monthly movement rates for Blanding's turtles. Gravid female Blanding's turtles had significantly greater movement rates than males and nongravid females during June when they nest. However, there was no significant effect of sex on monthly mean movement rate for eastern long-necked turtles (Roe and Georges 2008).

A potential nesting migration is also supported by seasonal differences in movement. There was no significant seasonal effect on mean movement rate for this population, which is consistent with the findings of Morrow et al. (2001b) for Maryland bog turtles. However, the

maximum movement rate for this population was significantly greater during nesting season. Seasonal differences existed for maximum movement rate but not mean movement rate, likely because nesting migrations were conducted as a single, consistent move.

Other freshwater turtles are known to make nesting migrations in one, cohesive movement. Painted turtles (*Chrysemys picta*) in Michigan nested a mean distance of 60 m (range: 1-164 m) away from aquatic habitat, and 75% of migrations, including the act of nesting and the return trip, were completed within a single day (Congdon and Gatten 1989). Some female snapping turtles (*Chelydra serpentina*) in Michigan were found to make nesting migrations instead of nesting adjacent to their marsh of residence (Congdon et al. 1987). Females that migrated moved 500-1625 m from their marsh of residence within 1-8 days, and two females made their migrations (750 m and 1625 m respectively) within one day. Rovero and Chelazzi (1996) observed nesting migrations of the European pond turtle (*Emys orbicularis*) in Italy. Gravid females from the same pond traveled to two different nesting areas, 150 m and 600 m away respectively. Individuals took one day to reach the nearer of the nesting areas and two days for the farther area.

## **Habitat**

This population of bog turtles showed definite preference for shrub/scrub habitat. Only 52% of the wetland was shrub/scrub and rhododendron habitat, but 68% of the turtle locations were within it. Both the predominance of and selection for shrub/scrub habitat make WOB and its bog turtle population unique. Most southern bog turtle sites contain little to no shrub/scrub habitat. Trees, shrubs, and taller herbaceous vegetation typically occur in patches or isolated individuals in southern bog turtle sites (Kiviat 1978), and the majority of these sites as well as those containing the strongest populations are livestock pastures (Tryon and Herman 1990). In

addition, the previously studied sites that do contain shrub/scrub habitat do not proportionally contain as much, and the turtle populations did not exhibit selection for it. Tesauro and Ehrenfeld (2007) compared bog turtle abundance and density between actively and formerly grazed habitat in New Jersey. Both habitat types contained less shrub habitat than does WOB (10.4% and 14.5% in actively and formerly grazed respectively), and bog turtle captures and density were also significantly greater in actively grazed habitat. Likewise, in southwestern Virginia bog turtles most preferred wet meadow habitat, and utilized shrub (i.e., alder) habitat in proportion to its availability (Carter et al. 1999). However, use of shrub/scrub habitat is not entirely unheard of for bog turtles. In the Lake Ontario coastal plain of New York, they frequently use red maple and tamarack swamps that are adjacent to open-canopy, emergent vegetation-dominated areas (Rosenbaum and Nelson 2010).

Other habitat factors aside from vegetation likely also influence bog turtle habitat selection. Carter et al. (1999) observed that bog turtles prefer locations with deeper mud and water, and concluded that mud and water are more important than vegetation to habitat selection. Similarly, in their study of the soil properties of bog turtle wetlands, Feaga et al. (2013) noted that the soil at turtle locations has significantly lower resistance compared to the soil at randomly selected locations throughout the wetland.

In Whiteoak Bottoms, it appears that mud depth and vegetation are correlated. Mud depth profile significantly varied between habitats, and a greater proportion of shrub/scrub locations contained deep mud than did emergent locations. Furthermore, habitat explained the relationship between mud depth and location type. Turtles showed preference for deep mud, even in shrub/scrub habitat where deep mud was twice as abundant as in emergent habitat. One possible explanation is soil strength. Globally, shrubs have a deeper mean root depth than do

herbaceous vegetation (Canadell et al. 1996), and deeper root penetration can be an indication of lower soil strength (Brady and Weil 2004). If it is easier for roots to penetrate through the soil in the shrub/scrub habitat, then it might also be easier for the turtles to burrow in this habitat. De Baets et al. (2008) determined that in Mediterranean environments fibrous root systems are better at increasing soil shear strength than taproot systems. Specifically, grasses and *Juncus* spp. provide the greatest increase in shear strength of topsoil (0-10 cm). Thus, it might be more difficult for turtles to burrow through the surface soil layer in emergent habitat than in shrub/scrub habitat.

Factors other than soil could also explain this population's preference for shrub/scrub habitat. In the present study as well as others bog turtles have been observed occupying the root cavities of vegetation during the active season and hibernation (Tryon and Herman 1990, Ernst and Lovich 2009, and Feaga and Haas 2015). The shrub/scrub vegetation could have greater root structures and cavities than the emergent vegetation. Alternatively, simply the wetness of the shrub/scrub habitat and the presence of rivulets within it could have allowed turtles to utilize it. Morrow et al. (2001a) studied bog turtles in Maryland in a site 2 ha in size that contained emergent and shrub/scrub habitats, and the turtles preferred emergent vegetation. In fact, most individuals stayed within one emergent area; only 18% of individuals crossed the shrub/scrub habitat to utilize multiple emergent areas (Morrow et al. 2001b). Unlike Whiteoak Bottoms, the shrub/scrub habitat at their site was dry and composed primarily of invasive multiflora rose (*Rosa multiflora*; Morrow et al. 2001a). Likewise, bog turtles are known to use rivulets including muskrat and beaver channels (Arndt 1977, Kiviat 1978, and Ernst and Lovich 2009), and the network of beaver channels in WOB extended throughout the shrub/scrub habitat.

Whiteoak Bottoms offers a unique glimpse at a relatively undisturbed bog turtle habitat. Not only does this wetland contain a habitat type not typically found in southeastern bog turtle sites, shrub/scrub habitat, but the turtle population favored it throughout the study. Wildlife managers who only consider “suitable” bog turtle habitat to be wetlands dominated by emergent vegetation, like those frequently found in livestock pastures in the southeast, might be oversimplifying this species’ available habitat. This oversimplification could lead to shrub/scrub wetlands being overlooked for bog turtle surveys and conservation prioritization even though this habitat was more abundant historically in the landscape than it currently is. Thus, wildlife management and conservation biologists must factor in wetlands with large proportions of shrub/scrub habitat when considering suitable habitat for this species. In addition, land managers could manage known bog turtle wetlands for a mosaic of vegetation cover instead of solely an open canopy. As long as some open canopy habitat is available for nesting, then the remainder could include some shrub/scrub habitat.

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APPENDIX A

Table A1: Mass and snout-vent length (SVL) of all MCF captured during study.

Site	Sex	Mass (g)	SVL (mm)	Site	Sex	Mass (g)	SVL (mm)	Site	Sex	Mass (g)	SVL (mm)
Ponds	Female	3.47	35	Ponds	Female	3.36	32	Ponds	Male	2.92	28
Ponds	Female	3.28	35	Ponds	Female	3.20	29	Ponds	Male	2.39	27
Ponds	Female	5.02	37	Ponds	Female	2.87	33	Ponds	Male	2.68	31
Ponds	Female	3.22	32	Ponds	Female	3.20	33	Ponds	Male	2.86	29
Ponds	Female	3.87	35	Ponds	Female	3.00	32	Ponds	Male	3.06	32
Ponds	Female	3.22	33	Ponds	Male	3.15	30	Ponds	Male	2.81	28
Ponds	Female	3.44	33	Ponds	Male	2.70	28	Ponds	Male	2.69	26
Ponds	Female	3.07	32	Ponds	Male	2.79	28	Ponds	Male	2.14	27
Ponds	Female	3.24	34	Ponds	Male	2.61	30	Puddles	Female	2.99	31
Ponds	Female	3.36	34	Ponds	Male	2.64	31	Puddles	Female	3.77	36
Ponds	Female	2.88	30	Ponds	Male	2.88	29	Puddles	Female	4.27	36
Ponds	Female	3.08	32	Ponds	Male	3.22	30	Puddles	Female	3.95	33
Ponds	Female	3.43	32	Ponds	Male	2.41	29	Puddles	Female	2.45	29
Ponds	Female	3.23	34	Ponds	Male	3.02	28	Puddles	Female	3.57	36
Ponds	Female	3.52	33	Ponds	Male	2.27	28	Puddles	Female	3.46	34
Ponds	Female	3.20	34	Ponds	Male	2.85	29	Puddles	Female	2.73	32
Ponds	Female	4.24	36	Ponds	Male	2.70	29	Puddles	Male	2.77	29
Ponds	Female	3.44	34	Ponds	Male	2.02	26	Puddles	Male	2.25	27
Ponds	Female	3.62	33	Ponds	Male	2.74	27	Puddles	Male	1.97	26
Ponds	Female	3.56	34	Ponds	Male	2.31	27	Puddles	Male	2.99	28
Ponds	Female	3.44	33	Ponds	Male	2.67	31	Puddles	Male	2.88	28
Ponds	Female	3.42	30	Ponds	Male	2.86	31	Puddles	Male	2.71	30
Ponds	Female	3.02	32	Ponds	Male	2.10	28	Puddles	Male	3.10	31
Ponds	Female	2.99	30	Ponds	Male	2.68	28	Puddles	Male	2.88	32

APPENDIX B

Table B1: Results of pairwise G-tests for differences in burrow presence among habitats (top) and location types (bottom) at the Puddles. Significant results are indicated with asterisks (\*). Specific  $\alpha$  values for each pairwise comparison from Holm's sequential Bonferroni are reported.

<b>Comparison</b>	<b><i>p</i></b>	<b><math>\alpha</math></b>
Field/Forest*	$5.1 \times 10^{-8}$	0.0167
Orchard/Forest*	$5.1 \times 10^{-3}$	0.025
Field/Orchard*	0.024	0.05
Pass-Through/Random	0.045	0.0083
Tracked/Random	0.066	0.01
Pass-Through/Extrapolated	0.422	0.0125
Tracked/Extrapolated	0.476	0.0167
Extrapolated/Random	0.527	0.025
Tracked/Pass-Through	0.916	0.05

Table B2: Results of pairwise G-tests for differences in burrow presence among habitats (top) and location types (bottom) at the Ponds. Significant results are indicated with asterisks (\*). Specific  $\alpha$  values for each pairwise comparison from Holm's sequential Bonferroni are reported.

<b>Comparison</b>	<b><i>p</i></b>	<b><math>\alpha</math></b>
Field/Forest*	$3.2 \times 10^{-14}$	0.0167
Orchard/Forest*	$3.9 \times 10^{-6}$	0.025
Field/Orchard*	0.013	0.05
Tracked/Random	0.023	0.0083
Tracked/Extrapolated	0.087	0.01
Tracked/Pass-Through	0.137	0.0125
Pass-Through/Random	0.283	0.0167
Extrapolated/Random	0.369	0.025
Pass-Through/Extrapolated	0.816	0.05

APPENDIX C

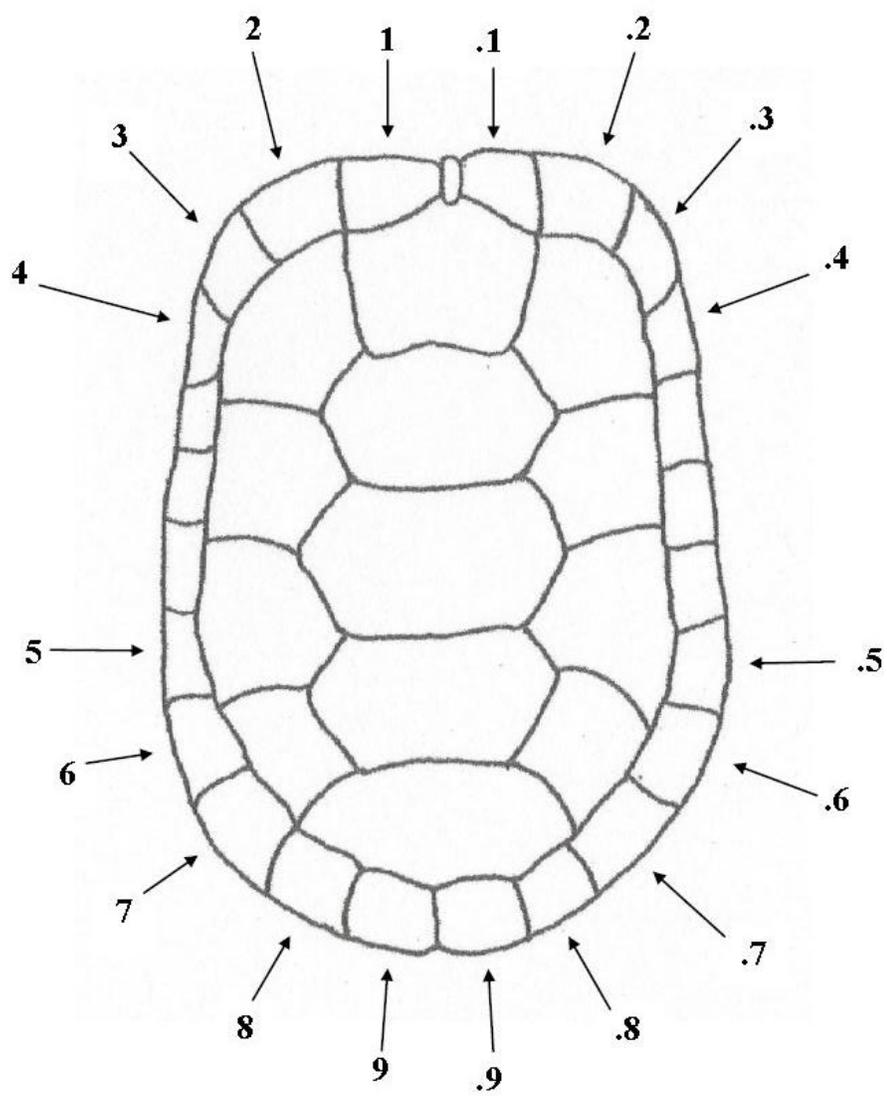


Figure C1: Scute notching system used to individually mark turtles.

APPENDIX D



Figure D1: Photograph of nest protector box placed around bog turtle nest.

APPENDIX E

Table E1: Home ranges and core areas per individual.

<b>Turtle</b>	<b>Sex</b>	<b>Core Area (ha)</b>	<b>95% Kernel Area (ha)</b>	<b>MCP Area (ha)</b>
0.4	Male	0.0498	0.2387	0.1081
0.7	Male	0.1382	0.6529	0.6331
0.5	Female	0.1365	0.6152	0.5795
0.6	Female	0.1285	0.5934	0.7720
0.9	Female	0.0369	0.3142	0.4178
2.0	Female	0.2002	0.9027	1.0187

APPENDIX F

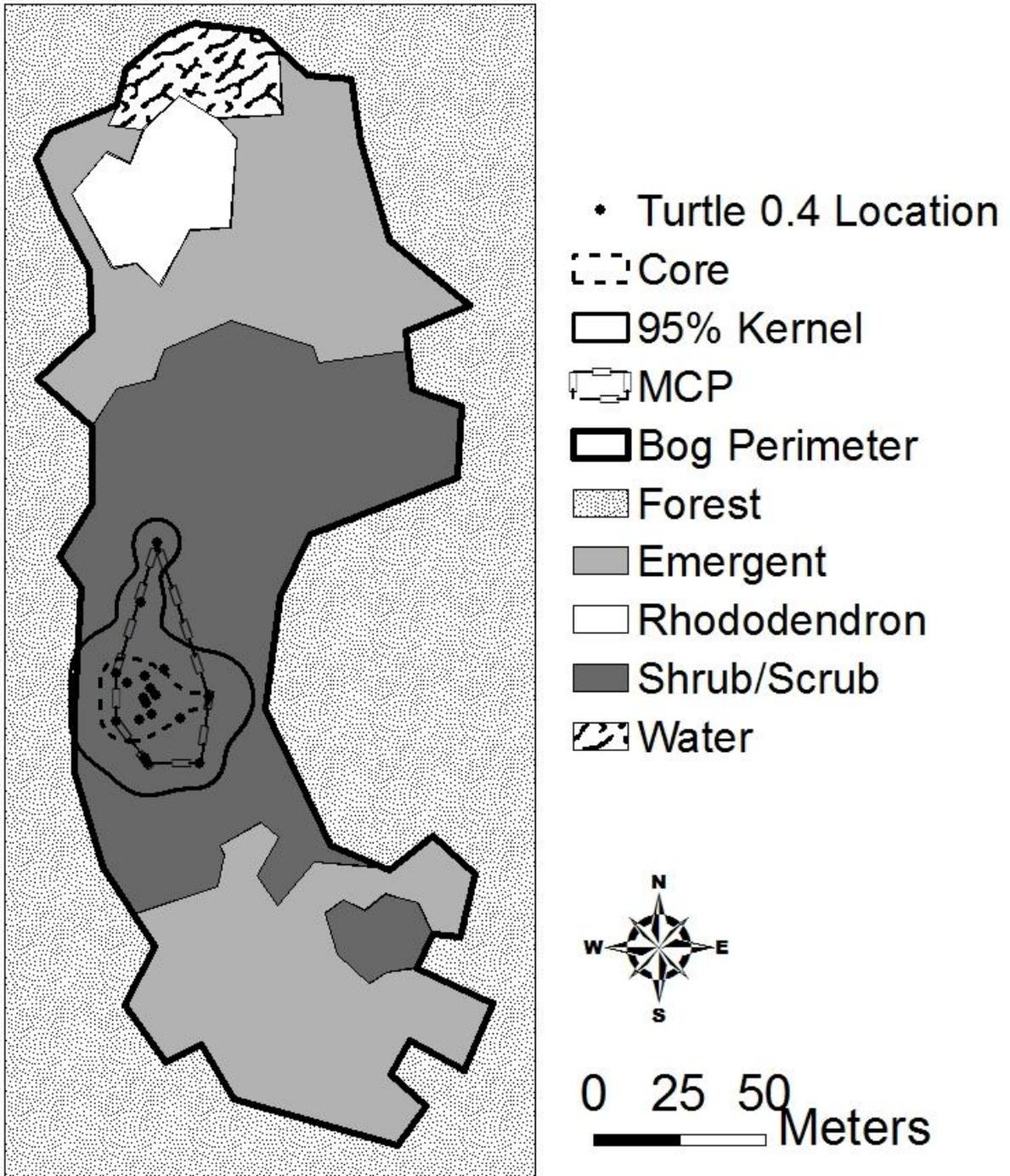


Figure F1: Home ranges and core area for turtle 0.4 (male).

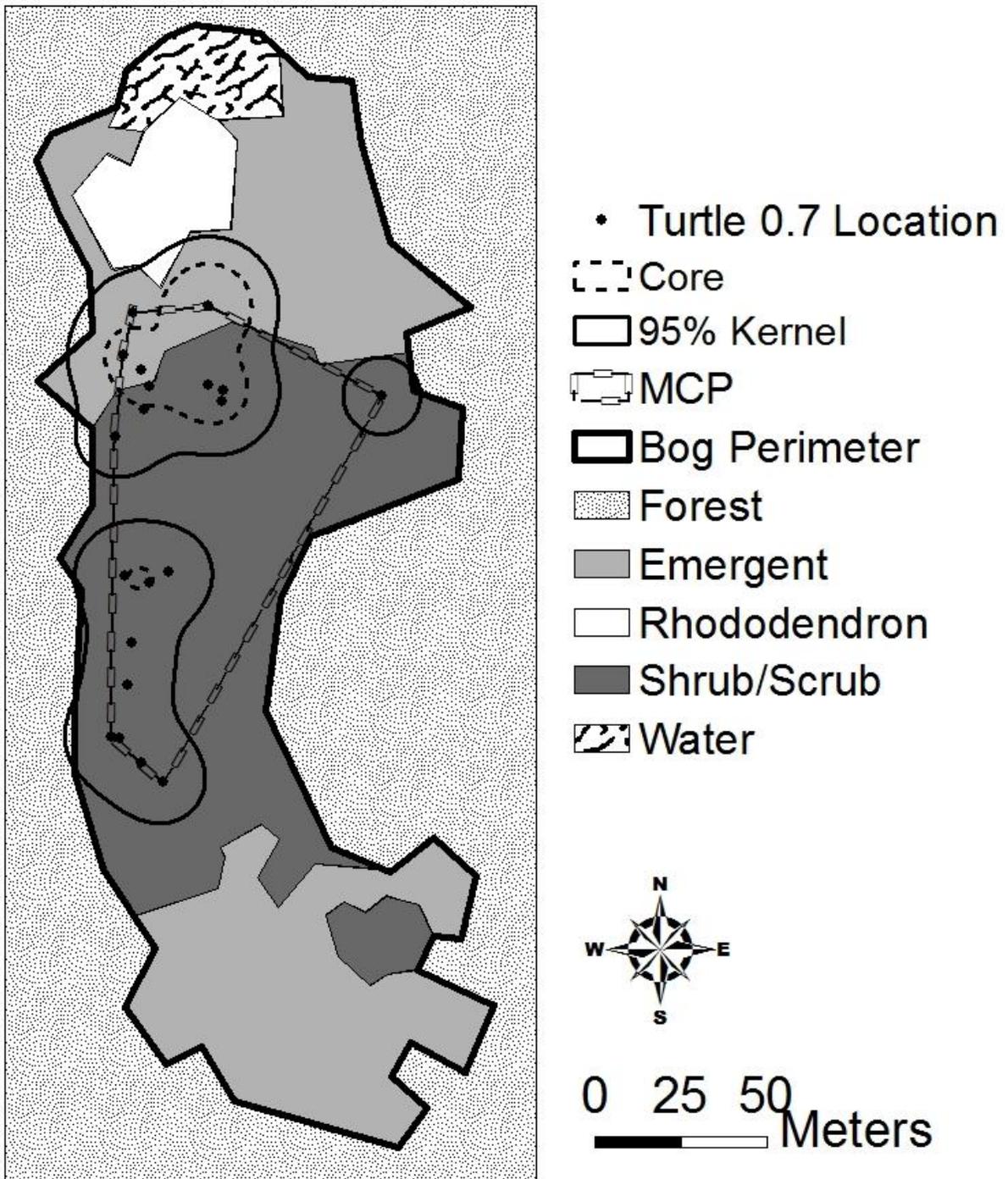


Figure F2: Home ranges and core area for turtle 0.7 (male).

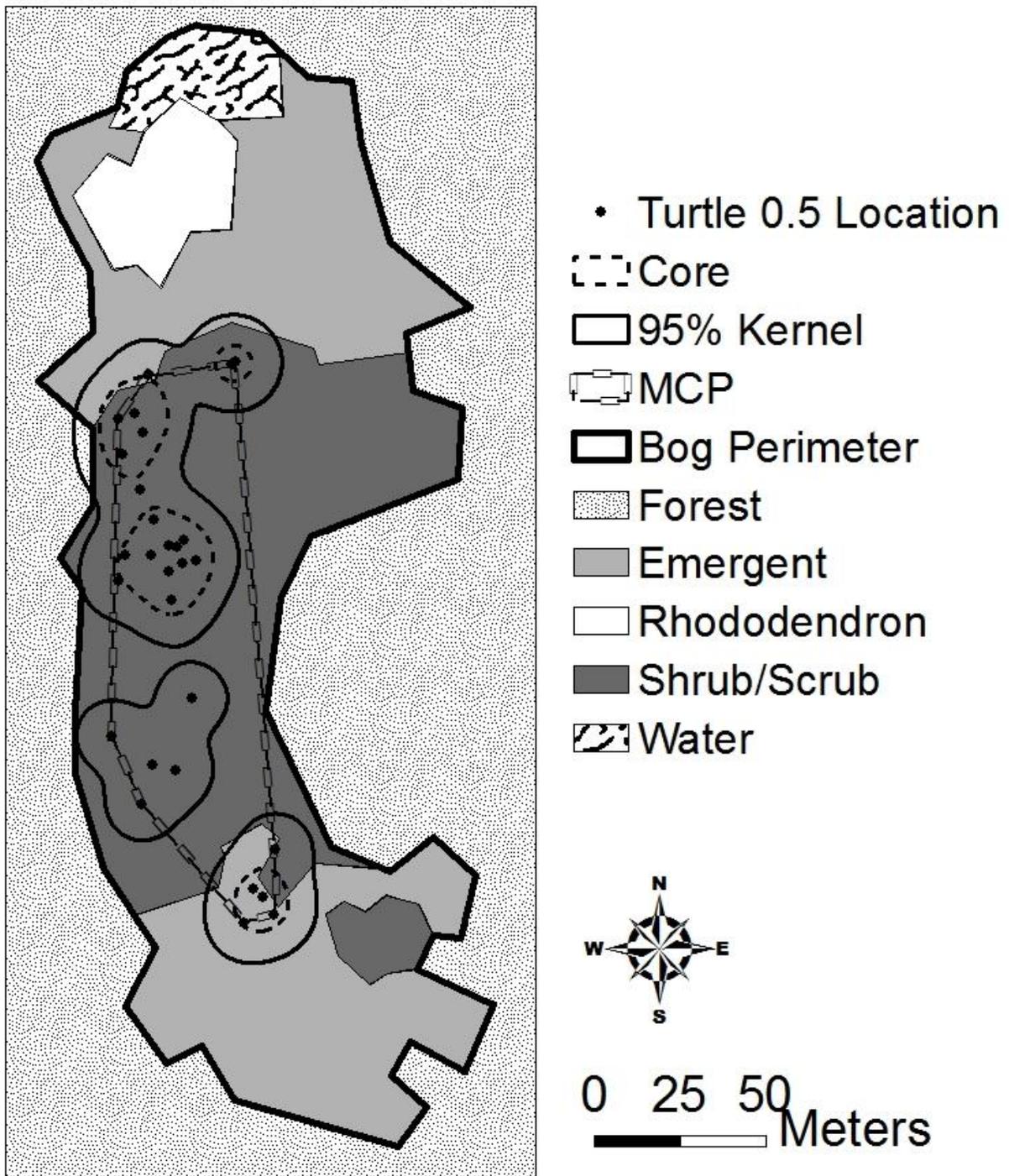


Figure F3: Home ranges and core area for turtle 0.5 (female).

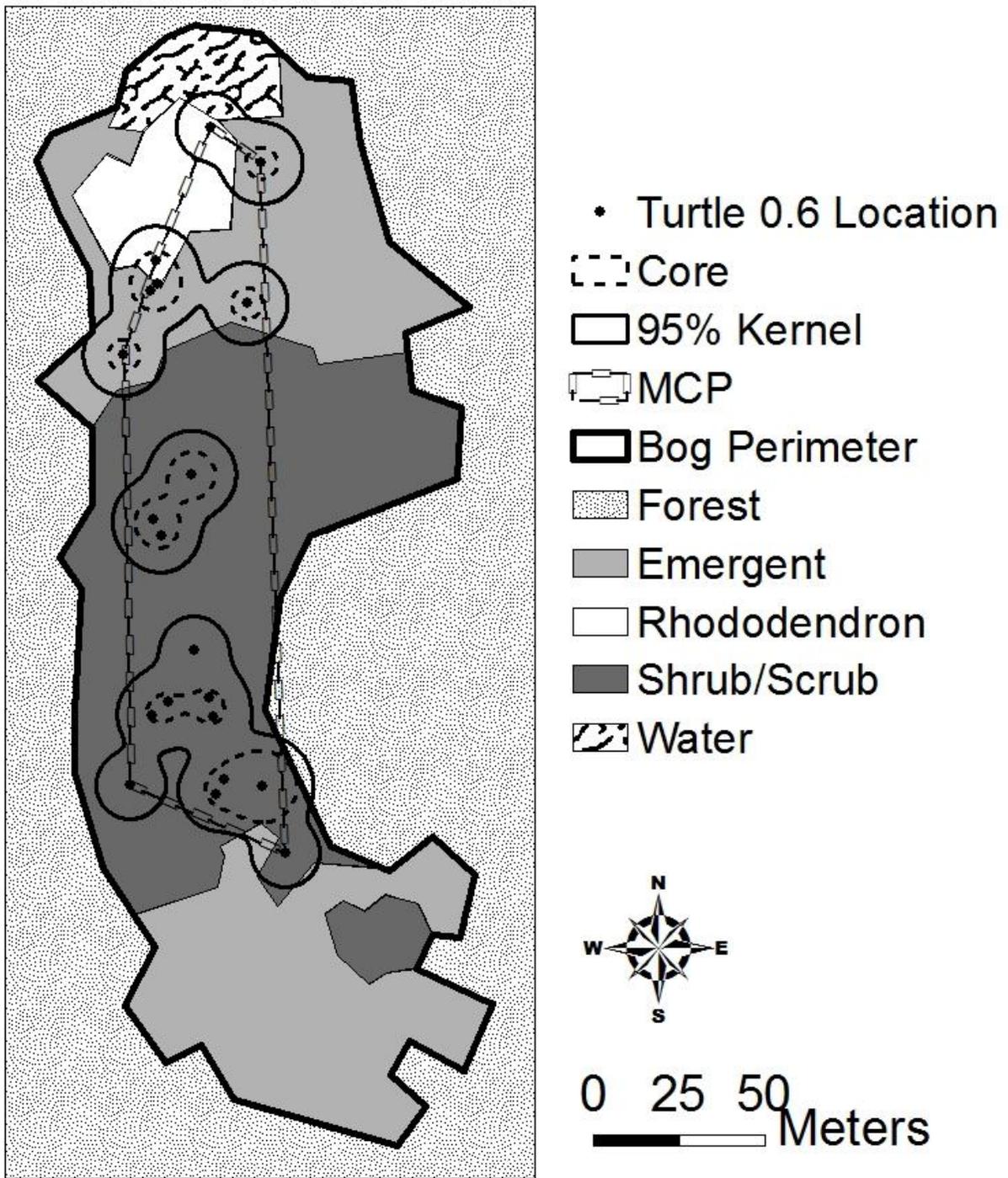


Figure F4: Home ranges and core area for turtle 0.6 (female).

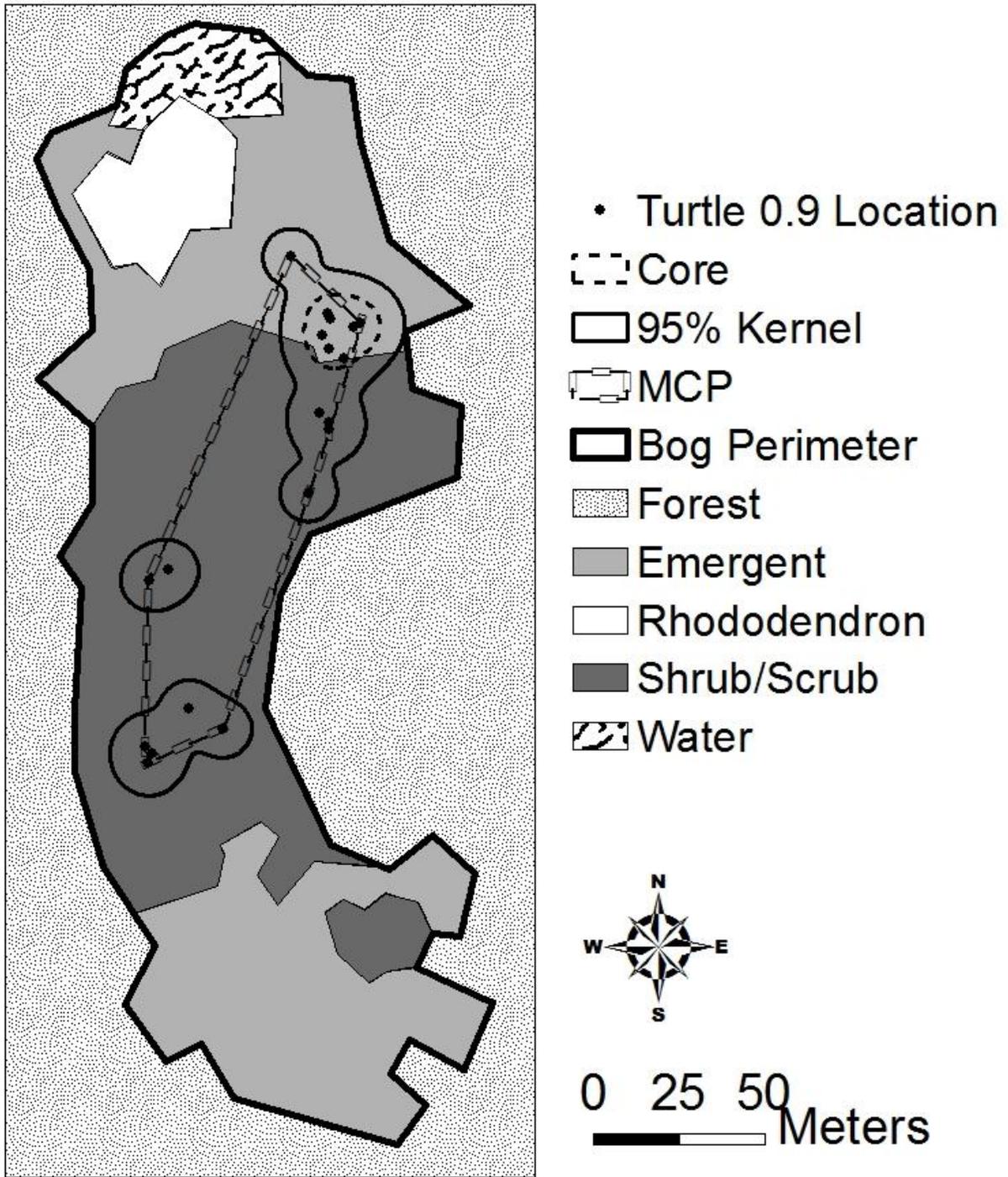


Figure F5: Home ranges and core area for turtle 0.9 (female).

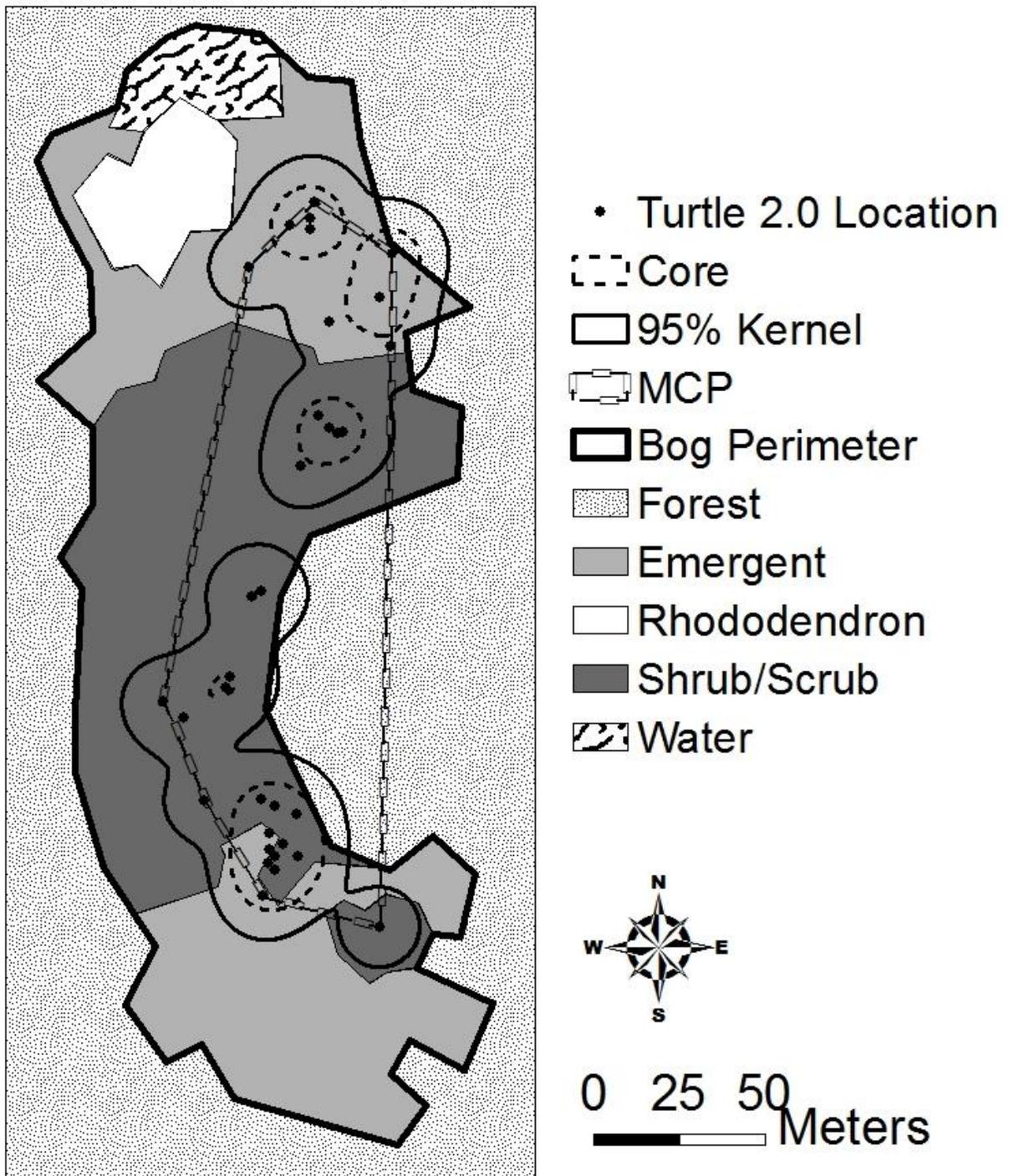


Figure F6: Home ranges and core area for turtle 2.0 (female).