# SPATIAL ANALYSIS OF AMPHIBIANS AND REPTILES IN THE OAK OPENINGS PRESERVE

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# ABSTRACT

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The Oak Openings Preserve is the largest park within the Oak Openings Region in northwestern Ohio, a biodiversity hotspot where there have been no previous studies examining its herpetofauna biodiversity. Surveying herpetofauna can be impacted by the sampling methods; integrating information on the effectiveness of surveys can improve models on species distributions. Once an understanding of general herpetofauna distributions is described, we can begin to examine how individual species interact with their environment by measuring influential environmental factors. Using tracking methods can help us to further understand how organisms disperse and use habitats. We extensively surveyed herpetofauna biodiversity within the Oak Openings Preserve from 26 April to 27 September 2014 to understand spatial patterns and ecology within this landscape and to provide essential foundational research for future surveys.

We created herpetofauna distribution maps and found that some species, especially amphibians, were more detectable. We used quadrat and opportunistic surveys, and found that fewer species and individuals, especially reptiles, were detected by quadrat sampling, except for *Plethodon cinereus*. We examined environment variables influencing species presence-absence and found that leaf litter, coarse woody debris, conifer needles, moist soil and plants were important factors. Detected herpetofauna tended to occupy forested areas; however this may have been partially a result of limitations within our sampling design.

We further examined the details of spatial patterns by tracking box turtles using three methods to examine fine-scale movements. Previous studies have used thread trailing and radio

telemetry; however this is the first study to compare and contrast these methods with fluorescent powder. Movement patterns by turtles were underestimated for thread trailing and radio telemetry when compared to fluorescent powder. On average per day, box turtles traveled for: thread trailing, 28.4 m, fluorescent powder, 46.0 m, and radio telemetry, 17.68 m. We found that thread trailing paths were more linear and fluorescent powder trails showed curves in the turtles' pathways, while radio telemetry was the best method for relocating turtles. Our research results have helped us understand species occurrence across time and space, provided new insight for sampling herpetofauna and provided critical data on important taxa for conservation.

This research is dedicated to Slither (1999-2009), my first snake, who is the reason that I'm pursuing my dream to study amphibians and reptiles and to Callie (1994-2014) and Blackie (1994-2015) for being there for me.

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# INTRODUCTION

The Oak Openings Region in northwestern Ohio is a biodiversity hotspot in which many taxa have made their home. This variety of species occurs as a result of having highly heterogeneous habitats, even though it lies within a human-dominated landscape. Many taxa have been studied within this area; however there have been no studies that have examined the distributions and spatial patterns of amphibians and reptiles, known as herpetofauna. Herpetofauna are important species for their ecosystem because they have dual roles acting as both predators and prey, while acting as indicator species of ecosystem health. Without these important organisms, pest species such as mosquitos or rodents would increase in abundance, and mammalian and avian predators would lose an important food source. As the world continues to change, herpetofauna are especially vulnerable because they use the environment to regulate their body temperature. When habitats begin to degrade, whether it is a result of natural or anthropogenic causes, herpetofauna are the first vertebrate organisms to disappear (Cabrera-Guzman & Reynoso 2012). We can determine if ecosystems are degrading by monitoring herpetofauna populations and identifying when populations are declining.

In this thesis, I have provided critical ecological knowledge on the distributions and abundances of herpetofauna within the Oak Openings Preserve and have utilized a new method that has not previously been used to track eastern box turtles (*Terrapene carolina carolina*). The goals of this research were to: (1) examine spatial patterns at different scales, (2) compare methodologies, (3) understand underlying ecological processes influencing distributions, and (4) examine in depth fine-scale movements for one species, eastern box turtles. The research is presented as four stand-alone chapters and final conclusions.

The purpose of Chapter 1 was to create a small paper for publication by highlighting the general herpetological biodiversity within the Oak Openings Preserve. This paper identifies each detected species and their abundances, while providing information about general habitat occupation. It will be formatted for submission to *Herpetological Conservation and Biology* under general herpetology. The purpose of Chapter 2 was to examine how our sampling methods impacted our results. I expected that the quadrat method would yield greater species detections than opportunistic method and was surprised when the results did not support my prediction. Few studies examine how the methods impact their results and this chapter served to identify how to continue forward surveying herpetofauna within this region to examine spatial and temporal factors. The purpose of Chapter 3 was to identify how the environment influences spatial patterns. Knowing where species occur is only part of the larger picture; managers require further knowledge such as why are they there. By examining the important factors influencing species presence-absence managers can use this information to create suitable habitat to increase herpetofauna abundances. The purpose of Chapter 4 was to provide preliminary data on the use of a new tracking technique, fluorescent powder, to study the movements of eastern box turtles. Radio telemetry provides details on tracking box turtles; however critical information is missing between detection points. This chapter has shown movement patterns of box turtles and compared the three tracking methods.

# CHAPTER 1: SPATIAL PATTERNS OF HERPETOFAUNA BIODIVERSITY IN OHIO

*Abstract*

The Oak Openings Region has many protected areas, but the Oak Openings Preserve is the largest in this biodiversity hotspot. To date there have not been any studies that have examined herpetofauna biodiversity within this highly diverse region that lies within a human-dominated landscape. We examined the spatial distributions of herpetofauna using quadrat sampling and opportunistic finds by mapping GPS coordinates of each detected individual within the Oak Openings Preserve. Our results suggest that amphibians were widespread across the preserve and easier to detect, while reptiles were rarer and more dispersed throughout the preserve. Some species were much more detectable than others, especially for amphibians. We found that Urodela were distributed across the landscape and there was significant spatial clustering for all herpetofauna except for Squamata. These data provide an understanding of species diversity, richness and abundance of herpetofauna while providing a representative foundational dataset for spatial patterns for future monitoring. These data are particularly valuable because the Oak Openings Preserve should be a good model for other parks with similar land cover features within the Oak Openings Region and herpetofauna diversity and distributions should follow similar patterns.

*Introduction*

It is well known that many ecological systems are suffering from habitat loss, destruction, pollution and fragmentation and that these effects can interact with each other exacerbating biodiversity loss. It is important to understand the current natural state of each ecosystem in order to assess how it changes over time. Studying these changes will help in conserving global biodiversity, which will benefit humans and other organisms. Preserving global biodiversity is

essential for humans because there are many different ecological benefits such as medical applications, economic gain, resources and ecosystem services (Diaz et al. 2006). These benefits demonstrate that biodiversity is critical and that conservation efforts should focus on understanding the value of each different ecosystem. Our current understanding of global biodiversity is still incomplete. There are many species that have not been discovered yet and our knowledge of the true distributions for even the best-studied taxa is quite limited (Ficetola et al. 2013). This occurs because quantifying biodiversity patterns can be challenging, but it is an important step in prioritizing which areas should be the focus of conservation efforts. Amphibians and reptiles, herpetofauna, in particular, are priority species because they are climatically restricted and have limited dispersal abilities in a changing world (Carvalho et al. 2010). As the environment changes, it is predicted that thermal stress and disease susceptibility will increase, while suitable breeding habitats may dry up (Ryan et al. 2014). This elevates the risk of extinction for herpetofauna as their habitat degrades over time.

Herpetofauna are important for their ecosystem because they have a dual role, acting as both predators and prey species, especially amphibians which act as consumers and prey in both aquatic and terrestrial systems (Blaustein et al. 2011). For example, amphibian larvae have voracious appetites and they consume invertebrates while also acting as prey for other wildlife like small mammals, birds and other herpetofauna (DuRant & Hopkins 2008). Their metamorphosis from aquatic larvae to terrestrial adults allows them to occupy a diversity of ecological niches fostering a multitude of species interactions (Hopkins 2007). Both amphibians and reptiles are ectothermic; they behaviorally regulate their body temperature by moving to and from areas with more or less heat, which may restrict their distribution based on temperature (Vega-Trejo et al. 2013). As habitat degrades, herpetofauna tend to be the first vertebrate

organisms to disappear with disturbance making them useful indicator taxa for habitat health (Cabrera-Guzman & Reynoso 2012). Although reptiles are more susceptible to disturbances than mammals or birds, amphibians will be more likely to show immediate signs of detrimental effects within a system because their permeable skin readily absorbs substances such as toxins from the environment (Hopkins 2007).

To better understand ecosystems and their dynamics, we examined the distribution of the component species. Very little work has been done in the Oak Openings Region to study the overall distributions and abundances of the herpetofauna species as a whole. We examined species range maps and expected to find 32 herpetofauna species within the Oak Openings Region. Since larger protected areas should hold greater biodiversity, our study focuses on the distribution and abundance of herpetofauna within the Oak Openings Preserve (Abella et al. 2007). The Oak Openings Preserve is heterogeneous and we focused on understanding how environmental heterogeneity affects the spatial and temporal patterns in diversity at a landscape scale (Sutherland et al. 2013). By examining these spatial patterns, we have created a foundational data set which describes the current herpetofauna spatial patterns and how they relate to environmental features. We examined herpetofauna species diversity within the Oak Openings Preserve for (1) species richness; (2) species abundance; and (3) spatial distributions. We would expect the patterns to be similar for other heterogeneous protected areas surrounded by human land uses, such as urban development and agriculture, as a result of having similar land cover and surrounding matrix.

## *Methods*

### ***Study Area***

The Oak Openings Region is a biodiversity hotspot, which contains an abundance of diverse species. The heterogeneous area hosts five globally significant communities: Great Lakes Twig-rush Wet Meadow (Wet Prairie), Great Lakes White Oak-Pin Oak Flatwoods, Mesic Sand Prairie, Midwest Sand Barrens, and Black Oak/Lupine Barrens (Oak Savanna) (EPA 2012). The region supports a variety of species, like the endangered Karner blue butterfly, *Lycaeides melissa samuelis*, and has 177 rare species, along with other organisms from different taxa (EPA 2012). It encompasses approximately 40,000-ha and it extends from northwestern Ohio to parts of southern Michigan. It was shaped by glaciation and subsequent anthropogenic influences (e.g., water drainage and fire suppression) and alterations (e.g., urban expansion and agricultural intensification). There are several protected areas within this region, but our study focused on the largest preserve.

We sampled the Oak Openings Preserve, Figure 1.1, in Swanton, Ohio, from 26 April 2014 to 27 September 2014, to investigate the spatial distribution and abundance of herpetofauna. The 1618-ha preserve is the largest contiguous protected area that contains a high amount of biodiversity for all taxa (The Ohio Ornithological Society 2014). The preserve contains many different land cover types; we used a land cover map (Schetter & Root 2011) to combined the similar land covers into four main groups: forests (swamp forest, conifers, upland forest, floodplain forest, and shrub), prairies (Eurasian meadows, prairie, barrens, savanna, and wet prairie), agricultural (cropland, residential, turf, and asphalt) and water (pond). The oak savanna has steadily transitioned into oak woodland over time with fire suppression, and park managers have implemented prescribed burning plans to reduce woody understory biomass. For our study, we excluded areas with ground nesting birds to avoid disturbing them.

***Quadrat Sampling***

We sampled 189 quadrats for herpetofauna, which were created in ArcGIS 10.1 (ESRI Inc., Redlands, CA, USA) using random sampling within the Oak Openings Preserve. Points were randomly placed on a land cover map (Schetter and Root 2011) within forested areas comprised of savanna, swamp forest, upland forest, floodplain forest and conifers and each point had to be at least 50 m apart to reduce possible sampling of the same individuals. Quadrats were not surveyed if they were placed within restricted ground nesting bird areas. Each day, we sampled one to five points that were located in similar areas. The sample point was used as the corner of the quadrat. We set up the quadrat by walking 20 meters north or south from the initial point. The quadrat encompassed a 400 $m^2$ area, which we surveyed. We recorded the date, the number of observers, start and end time of the survey, weather, cardinal direction, and the coordinates for the quadrat corner points. After the quadrat was set up, we started a timer for 15 minutes in order to reduce searching bias when sampling the area.

We searched the area within the quadrat visually and checked underneath logs. The timer was stopped when an individual was located in order to prevent time loss when recording data; this suggests that our diversity measures were likely to be minimum estimates rather than maximum estimates. For each individual located, we recorded species, time, approximate body size, behavior, coordinates, air temperature and humidity, surface temperature and humidity and presence of vegetation. We photographed each individual when located, if feasible, and identified them to species. After the 15 minute search, we noted general environmental characteristics for the center point and every two meters north, east, south and west of the center point. If we detected any individuals outside the specified search time, we recorded the data as the quadrat detection, but uniquely marked it to identify that it was not located during our search time. We only recorded individuals that we could identify by sight; we excluded any

vocalizations that were not confirmed visually because we would be unable to specifically pinpoint their coordinates.

***Opportunistic Observations***

When traveling to our sample points, we had the potential to encounter herpetofauna along the way. We recorded individuals that we found opportunistically as a separate data set. We recorded the same information as we did for the quadrat searches and mapped each of the individuals found with those found in quadrats, Figure 1.1. Individuals that we were unable to identify to the species level were recorded by order: Anura (frogs and toads), Urodela (salamanders), Squamata (snakes), and Testudines (turtles). This occurred when Anura jumped too quickly into the water or the individuals were too far away to properly identify them by species.

***Data Analysis***

Total species richness was recorded as the total number of each species detected. We measured species diversity by calculating Simpson's index (D). As another estimate of species diversity we calculated the Shannon-Wiener diversity index (H) and calculated Shannon's evenness (J) by dividing the Shannon-Wiener diversity index by the natural logarithm of the number of species found. Using ArcGIS we identified the number of each taxa found in four general land cover types: forested, prairie, agricultural and water. We mapped the GPS coordinates of each individual detected from both quadrat surveys and opportunistic finds using ArcGIS to create a distribution map. We calculated group clustering using the average nearest neighbor analysis in ArcGIS. All calculations and percentages are based from the inclusion of both quadrat and opportunistic data. We used ArcGIS to randomly place 1345 points within the Oak Openings Preserve to identify how many individuals would be expected to find within each

land cover and used a Wilcoxon rank sum test in JMP® 11.0 (SAS Institute, Inc., Cary, NC, 1989-2007) to compare each order to random habitat occupancy.

*Results*

***Species Richness***

We found a total of 1345 individuals encompassing 21 species within the Oak Openings Preserve, Table 1.1. Amphibian abundance totaled to 1286 individuals for 11 species, accounting for 96% of total species found; of these, we detected 905 Anura, and 381 Urodela. Reptile abundance totaled to 59 individuals for 10 species accounting for 4% of the total of individuals found; of these we detected 11 Squamata, and 48 Testudines.

We examined species richness, diversity and evenness for herpetofauna, amphibians, reptiles, and for each order. A high species richness index indicates a higher chance of encountering two individuals from different species. We found that Urodela had the lowest species richness, while all others had high species richness. A high species diversity index depicts a greater number of species within the area. We found that herpetofauna had the greatest number of species detected followed by amphibians and then reptiles. The species evenness index shows the relative abundance of each species and a high index value indicates that the species are more equally abundant, while a low index value indicates that some of the species are rarer. We found that Squamata had the highest evenness, which indicates that most of each species was equally detected. Overall, Urodela had the lowest diversity index estimates and species richness, diversity and evenness indexes can be seen in Table 1.2.

Species Diversity

In our study, 70% of amphibians detected were Anura and 30% were Urodela. For reptiles, 19% detected were Squamata and 81% were Testudines. The most abundant amphibian

species detected was *Plethodon cinereus,* which comprised of 27.6% of the amphibian samples, Table 1.1. We found two Urodela species, *Ambystoma laterale* complex and *Plethodon cinereus*, the *Plethodon cinereus* was the most abundant Urodela species found with the striped morph more abundant (55%) than the lead back morph (45%). Of the Anura species, *Lithobates sylvaticus* comprised the most of the detected individuals (14.4%) and *Lithobates clamitans* comprised 9.3% of individuals, as the second most abundant species, Table 1.1. We were unable to verify the species of 20.3% of the Anura individuals we detected. The most abundant Squamata species was *Heterodon platirhinos* (0.3%) followed by *Coluber constrictor foxii* (0.2%) for the second most abundant species detected, Table 1.1. The most abundant Testudines species detected was *Chrysemys picta* (1.4%), followed by *Terrapene c. carolina* (0.8%), Table 1.1. We were unable to verify the species of 17.4% of the Testudines encountered.

***Spatial Distribution***

Urodela were widely distributed across the preserve, Figure 1.1. They were found throughout different areas of the park, with a majority of the detections within forests (95%), and agricultural (5%), Table 1.3. Almost every Urodela was detected under a log with varying degrees of decomposition and only two individuals were observed moving on the ground. When we located Urodela, 13% of the time there were two or more salamanders underneath the log and 3% of the Urodela were found within a 50 m buffer from streams. For Anura, when we located a frog or toad, 16% of the time we found two or more individuals. Anura were mostly found within forests (93%), followed by agricultural (5%), and prairies (2%), Table 1.3, and 11% of the Anura were found within a 50 m buffer around the streams. Using the average nearest neighbor calculation, we found statistical significant clustering ($p < 0.01$) for all herpetofauna, for amphibians, for Anura and for Urodela. Reptiles comprised a small percentage of the detected

individuals (4%). As seen in Figure 1.1, Squamata were found in different areas of the park, except with one case, where three *Coluber constrictor foxii* were found close together. Squamata were mostly found within forests (73%), prairies (9%), agricultural (9%), and water (9%), Table 1.3, and 27% of the Squamata were found within a 50 m buffer around the streams. Testudines were also found throughout the park within forests (79%), prairies (8%), and agricultural (13%), Table 1.3, and 21% of the Testudines were found within a 50 m buffer around the streams. All aquatic Testudines were found in rivers, temporary ponds or temporary streams, while terrestrial *Terrapene c. carolina* were found in a variety of forest and prairie habitats. Using the average nearest neighbor calculation, we found statistical significant clustering ($p < 0.01$) for Testudines and for reptiles as a whole and we did not find statistical significant clustering ($p > 0.01$) for Squamata. Using the Wilcoxon rank sum test, we found no difference from a random distribution for habitat occupancy for forests, agriculture and water. However, there was a significant difference in occupancy of prairies by herpetofauna; the data suggests that the different orders were occupying prairie more (e.g., Squamata) or less (e.g., Anura), than expected based on the amount of prairie on the landscape.

*Discussion*

Understanding where organisms occur is important for understanding natural ecosystems and evaluating potential impacts. Species distribution patterns vary over time and space as they experience different environmental factors (Gerick et al. 2014). As these changes occur, whether as a result of natural or anthropogenic causes, we must continually monitor populations and examine their spatial patterns. We identified species richness and diversity for the Oak Openings Preserve and created a spatial representation of the herpetofauna biodiversity in order to understand current abundance and distribution. The spatial distribution data will help

researchers assess what type of changes are occurring, whether populations are decreasing, increasing in size or if they are stable over time, and identify critical areas of high herpetofauna diversity. This study is unique because we examined spatial patterns instead of relying on simple census data. Our study not only identified current patterns, but can assists future studies that are interested in assessing spatial patterns over time.

We identified 21 species which encompassed 1345 individual detections using quadrat and opportunistic sampling when we surveyed forest, prairie and agricultural land covers. We found no significant differences between habitat occupancy by herpetofauna and random for forests, agriculture and water land covers. This provides evidence that herpetofauna are probably occupying these habitats in proportion to the land cover occurrence on the landscape. The exception to this was for prairie, for which we found occupancy patterns significantly different from our predictions based on the proportions on the landscape. We found that 2% of Anura were found within prairies, 0% of Urodela, 9% of Squamata and 8% of Testudines compared to 11% of random points, Table 1.3. Overall, the Oak Openings Preserve land cover is dominated by forests, which supports habitat occupancy for forested area. Other studies have shown that amphibians, which are primarily forest dwellers, and reptiles, select for forested areas more than open areas (Bury 2004). Within northern California, frogs, salamanders, and snakes had higher relative abundance within forested areas while lizards were found within open grasslands (Bury 2004). Perry, Rudoph & Thill's (2009) herpetofauna survey led to the capturing 2,592 reptiles and 2,493 amphibians within forested areas, which supports our results that herpetofauna are potentially more abundant in forested areas than open areas like prairies. Herpetofauna abundances may be influenced by canopy cover, which provides cover from avian predators within forests and not prairies and forested areas have deep layers of leaf litter for

shelter and protection than prairies (Hu et al 2013). These past studies support our results that these herpetofauna are occupying forested habitat, although we did not find the difference statistically significant from the proportion of forest on the landscape. Our study shows that forested areas are probably more important for amphibians and less important for reptiles when compared to a random pattern. This suggests that managers should maintain forested habitats to increase and help stabilize herpetofauna populations. Long-term removal of forested habitats (e.g., to create oak savanna) could detrimentally effect amphibian populations and could disrupt the ecological system. Amphibians are essential organisms because they impact both terrestrial and aquatic systems. Declining amphibian populations will result in the loss of prey items, especially tadpoles, for mammals, birds and other herpetofauna and disrupt the food chain with the loss of important predators. Open areas with less canopy cover are also important and should also be maintained, however forested should not be largely converted into prairie habitats. We found very few individuals within our water land cover; this may have occurred because the land cover map (Schetter & Root 2011) did not include temporary vernal ponds, which could increase the number of individuals using water sources.

Our quadrat sampling points were always located within the same land cover type, which eliminated any mixing of habitats. For an example, the quadrat point was located within a conifer forest and not within a mixed gradient of conifer forest and deciduous forest. However, ecotones or edges between two habitats, such as Oak savanna, should be examined in order to potentially increase species detections. It has been shown that edge habitats have increased species richness (e.g. Ries et al. 2004, Urbina-Cardona, Olivares-Perez & Reynoso 2006). Our study landscape is heterogeneous; which may influence the herpetofauna spatial patterns. Examining ecotones or edges may increase our estimates of species richness and diversity

because areas with edges between two different habitats typically have more species diversity than within homogeneous habitats such as prairies or forests alone (Urbina-Cardona, Olivares-Perez & Reynoso 2006). Because of the sampling focus on primarily forest habitats, our results are probably minimum estimates and it is highly likely that our survey underestimated the current herpetofauna population within the Oak Openings Preserve. Although these are minimum estimates, we have sufficiently sampled specific habitats (e.g., forest) and provided starting data in the exploration of the herpetofauna biodiversity.

Our study showed that the spatial distributions are different for each taxon and that reptiles may be more difficult to detect or are rarer than amphibians as a result of our low abundance numbers. From our observations, we show that *Plethodon cinereus* were found daily throughout the preserve where 4.11 individuals were found per day and spatially we found 1.44 individuals per quadrat. We found two Urodela species, *Plethodon cinereus* and *Ambystoma laterale* complex with numerical counts highly skewed towards *Plethodon cinereus*. Since *Plethodon cinereus* is terrestrial, it should be less confined by aquatic habitats and able to disperse evenly across the preserve. This pattern has been widely supported in many studies where one species is numerically dominant and it is often *Plethodon cinereus* (McGhee & Killian 2010, MacArthur 1972, and Preston 1948). Its dominance is probably a result of being a habitat generalist and having a smaller body size; this may allow it to exploit soil systems when prey is limited, more so than larger sized salamanders like *Ambystoma laterale* complex. We found significant clustering for Anura, and that they were more likely found within 50 m of streams than Urodela. This may have occurred because amphibians need water sources such as ponds, creeks or lakes for reproduction, oxygen exchange and prevent desiccation. Reptiles were difficult to find and we cannot make any definitive conclusions about their spatial patterns except

that they can be found within the preserve. This suggests that our methods were possibly not sufficient enough to fully detect reptile populations. We may need to use other methods such as rock flipping, pitfall traps, drift fences, artificial cover boards, and leaf litter removal to increase reptile detections (McDiarmid 2012). However, they may be rarer than amphibians and more research should be conducted to solidify our results.

We examined the Oak Openings Preserve biodiversity throughout the park and identified which species are most likely to be encountered at the preserve. We created a useful spatial data set to examine current species distributions, which highlighted the importance of forest land covers. Our survey provided a map of the park's herpetofauna biodiversity with emphasis on forested habitats. As with many surveys, it is likely that we have underestimated the herpetofauna population. It is highly likely that the park is more diverse and has greater population abundances than our study is able to reveal. This suggests that the park has a healthy herpetofauna population and this may be a result of having high heterogeneity, large amount of area and/or successful management strategies. We expect that other parks within this region have smaller herpetofauna diversity and abundances because they have less area. It would be helpful for future studies to examine herpetofauna distributions within these other parks to identify if they too have healthy populations and compare the amount of heterogeneity, area and management strategies to the Oak Openings Preserve. We have successfully provided current abundance data and identified species richness while providing a useful tool for herpetofauna conservation. We found 21 detectable species within this heterogeneous landscape and our sampling efforts revealed that the park has an abundance of amphibians and may have relatively few reptiles. The Oak Openings Preserve is the largest of the Metroparks and contains similar land cover types as other parks within the region. This suggests that our results are likely to be

similar to these other protected areas that are also highly heterogeneous and diverse that are found in a human-dominated landscape.

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*Tables*

Table 1.1: Herpetofauna abundance for each species detected within the Oak Openings Preserve and percentage of abundance for all individuals and within the order. All calculations include individuals detected by quadrat and opportunistic sampling and exclude individuals not identified to species.

| Scientific Name | # Found | Abundance (%) | Abundance in Order (%) |
|---|---|---|---|
| *Anaxyrus americanus* | 126 | 8.20 | 16.8 |
| *Anaxyrus fowleri* | 24.0 | 1.60 | 3.20 |
| *Hyla versicolor* | 10.0 | 0.60 | 1.30 |
| *Pseudacris crucifer* | 136 | 8.80 | 18.1 |
| *Pseudacris triseriata* | 40.0 | 2.60 | 5.30 |
| *Lithobates catesbeianus* | 47.0 | 3.00 | 6.30 |
| *Lithobates clamitans* | 144 | 9.30 | 19.1 |
| *Lithobates pipiens* | 2.00 | 0.10 | 0.30 |
| *Lithobates sylvaticus* | 223 | 14.4 | 29.7 |
| *Ambystoma laterale* complex | 12.0 | 0.80 | 3.30 |
| *Plethodon cinereus* | 369 | 23.9 | 100 |
| *Coluber constrictor foxii* | 3.00 | 0.20 | 27.3 |
| *Diadophis punctatus* | 2.00 | 0.10 | 18.2 |
| *Heterodon platirhinos* | 4.00 | 0.30 | 36.4 |
| *Nerodia sipedon* | 1.00 | 0.10 | 9.10 |
| *Apalone spinifera* | 2.00 | 0.10 | 4.30 |
| *Chelydra serpentina* | 2.00 | 0.10 | 4.30 |
| *Chrysemys picta* | 19.0 | 1.20 | 41.3 |
| *Emydoidea blandingii* | 3.00 | 0.20 | 6.50 |
| *Graptemys geographica* | 3.00 | 0.20 | 6.50 |
| *Terrapene c. carolina* | 11.0 | 0.70 | 23.9 |
| Anura | 153 | 9.90 | 20.3 |
| Squamata | 1.00 | 0.10 | 9.10 |
| Testudines | 8.00 | 0.50 | 17.4 |

Table 1.2: Species richness represented by Simpson's diversity (D), species diversity represented by Shannon Wiener index (H) and Shannon's evenness (J) for herpetofauna found in both quadrats and opportunistic detections. Calculations exclude individuals identified by order: Anura, Urodela, Squamata or Testudines.

| Taxa | Simpson's Diversity | Shannon Wiener Diversity | Evenness |
|---|---|---|---|
| Amphibians and Reptiles | D = 0.82 | H = 2.05 ± 0.11 SD | J = 0.67 |
| Amphibians | D = 0.81 | H = 1.88 ± 0.12 SD | J = 0.78 |
| Reptiles | D = 0.80 | H = 1.87 ± 0.09 SD | J = 0.81 |
| Anura | D = 0.81 | H = 1.80 ± 0.13 SD | J = 0.81 |
| Urodela | D = 0.06 | H = 0.14 ± 0.06 SD | J = 0.20 |
| Squamata | D = 0.78 | H = 1.28 ± 0.06 SD | J = 0.92 |
| Testudines | D = 0.65 | H = 1.40 ± 0.10 SD | J = 0.78 |

Table 1.3: Herpetofauna from both quadrat and opportunistic detections found within each land cover: random, forests, prairies, agricultural, and water by percentage.

| Order | Forests | Prairies | Agricultural | Water |
|---|---|---|---|---|
| Random | 79% | 11% | 10% | 0% |
| Anura | 93% | 2% | 5% | 0% |
| Urodela | 95% | 0% | 5% | 0% |
| Squamata | 73% | 9% | 9% | 9% |
| Testudines | 79% | 8% | 13% | 0% |

*Figures*

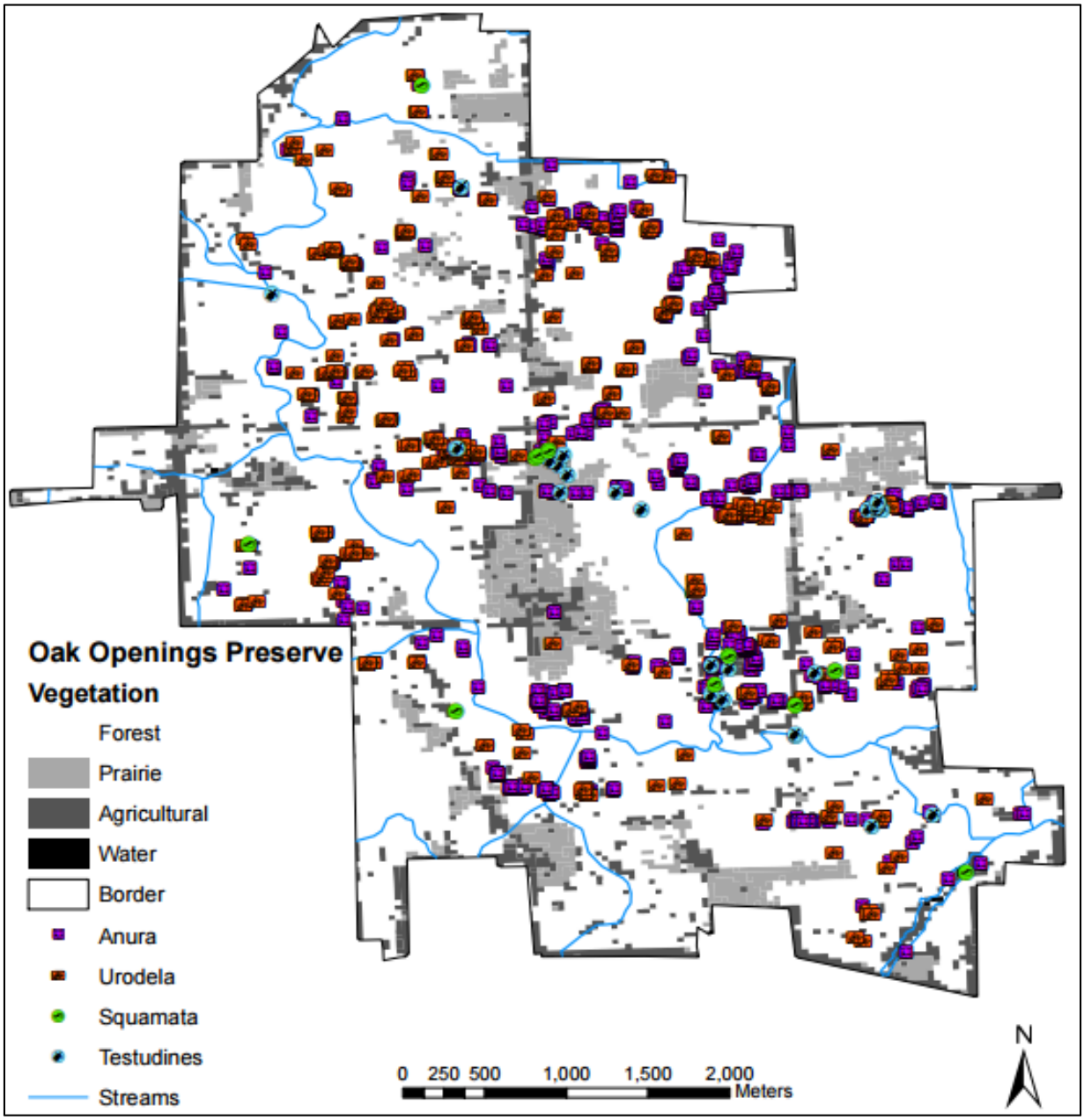


Figure 1.1: Spatial locations of each amphibian and reptile individual sampled within the Oak Openings Preserve with streams.

# CHAPTER 2: SURVEYING HERPETOFAUNA USING COMPLEMENTARY NON-INVASIVE METHODS

*Abstract*

We examined herpetofauna biodiversity within the Oak Openings Preserve using visual encounter surveys with two method variations: quadrat and opportunistic sampling. We have compared and contrasted the results from each method in order to determine if visual encounter surveys are adequate for sampling herpetofauna. We found that fewer species and individuals were detected in quadrat sampling and that opportunistic sampling detected 12 unique species. Quadrat sampling detected a greater number of individuals for one species, *Plethodon cinereus*, but only detected one Squamata species. These results suggest that searching in fixed locations may not yield as many species and individuals as searching a larger extent of suitable habitats. We found significant differences in the number and species detected by the two methods, which suggests that relying on one method would provide different species abundance and richness estimates. Visual encounter surveys appear to be adequate for detecting herpetofauna and can be used for providing foundational research in heterogeneous study areas. Our methods are valuable because they can be applied to other studies with similar land covers to survey herpetofauna and would be effective for long-term monitoring programs.

*Introduction*

Examining species diversity and abundance of ecological systems is important for conservation efforts. Conservation plans are hindered by the lack of data on the distribution, habitat associations and community dynamics of local ecosystems (Swan et al 2014). As the world changes, each taxon is affected differently and one of the most vulnerable groups are herpetofauna, which consists of reptiles and amphibians. This group is vulnerable to drastic

population changes because they are ectothermic; they change their body temperature by moving in and out of areas with heat (Cabrera-Guzmán & Reynoso 2012). Herpetofauna as a whole have been poorly studied in the Oak Openings Region, a highly biodiverse region in northwestern Ohio. There have been amphibian call surveys which rely on auditory data. These studies focus on the specific order Anura and are not applicable for studying Urodela and reptiles. Amphibian call surveys occur during the breeding season to examine distribution and abundance and the surveys can be impacted by different factors such as date, time, survey methods, environmental factors, and even observer experience (Pellet & Schmidt 2005). Observer experience can substantially change the results of call surveys, especially if a novice observer takes over an experienced observer's route, who may underestimate abundance, thus implying there are declines when that is not necessarily true (Shirose et al. 1997). We chose to use visual surveys instead of call surveys because we wanted to visually identify each individual detected to avoid any sampling biases for one specific order. We attempted to photograph every individual encountered in order to support our visual identification. Our goal was to create a spatial map of herpetofauna as a whole and auditory calls would have prevented us from identifying the exact Global Position System coordinates (GPS) of each individual detected. Using call surveys would have also potentially biased which individuals were detected such as not recording females, juveniles or silent males because they would not be calling during auditory surveys. We are not dismissing auditory surveys, which can be useful for monitoring Anura abundances; however, they are less useful for a comprehensive herpetofauna study.

Herpetofauna population data have been collected in a variety of ways using different trapping methods (funnel, pitfall and drift fences), cover boards, and call surveys and visual encounters (Dodd 2010). Many of these methods require extensive time and effort and can be

quite costly. Heterogeneous habitats can influence the consistency of different techniques through factors such as species detectability, abundances and any changes within environmental gradients (Swan et al 2014). Many scientists have found that different methods work more efficiently for certain species and a single approach is not sufficient to detect all of the species within the area (Garden et al 2007). There are many trade-offs when choosing a specific technique and, as a result, many herpetofauna surveys choose to use several techniques which complement each other (Doan 2003; Linares & Eterovick 2013; Ryan et al. 2002). A complementary approach is especially valuable for studies, like ours, that examined biodiversity of multiple species. Here, we have chosen to sample our study site using cost-effective visual encounter surveys with two variations: quadrat sampling and opportunistic detections.

Our study is the first comprehensive herpetofauna study to examine spatial distribution, abundance and species diversity of the Oak Openings Preserve, the largest protected area of the Oak Openings Region. Our overall goal was to provide critical foundational work for future studies by examining the current abundance and biodiversity of herpetofauna. We expected that quadrat sampling would detect a greater number of smaller amphibians as a result of the intensive searching and opportunistic detections would detect a greater number of reptiles as a result of extensive searching. We examined general herpetofauna biodiversity described in detail in Chapter 1. This study provides a comparison between our sampling methods looking at the two variations (quadrat and opportunistic) in order to examine the similarities and differences.

*Methods*

***Study Area***

The Oak Openings Region is a biodiversity hotspot, which contains an abundance of diverse species. The heterogeneous area hosts five globally significant communities: Great

Lakes Twig-rush Wet Meadow (Wet Prairie), Great Lakes White Oak-Pin Oak Flatwoods, Mesic Sand Prairie, Midwest Sand Barrens, and Black Oak/Lupine Barrens (Oak Savanna) (EPA 2012). The region supports a variety of species, like the endangered Karner blue butterfly, *Lycaeides melissa samuelis*, and has 177 rare species, along with other organisms from many different taxa (EPA 2012). It encompasses approximately 40,000-ha and it extends from northwestern Ohio to parts of southern Michigan. It was shaped by glaciation and subsequent anthropogenic influences (e.g., water drainage and fire suppression) and alterations (e.g., urban expansion and agricultural intensification). There are several protected areas within this region, but our study focused on the largest preserve, see Figure 2.1.

We sampled the Oak Openings Preserve in Swanton, Ohio, from 26 April 2014 to 27 September 2014, to investigate herpetofauna species diversity and abundances. The 1618-ha preserve is the largest contiguous protected area that contains a high amount of biodiversity for all taxa (The Ohio Ornithological Society 2014). The preserve contains many different land cover types; we used a land cover map (Schetter & Root 2011) to combine similar land covers into four main groups: forests (swamp forest, conifers, upland forest, floodplain forest, and shrub), prairies (Eurasian meadows, prairie, barrens, savanna, and wet prairie), agricultural (cropland, residential, turf, and asphalt) and water (pond). For our study, we excluded areas with ground nesting birds to avoid disturbing them.

***Quadrat Sampling***

Using ArcGIS 10.1 (ESRI Inc., Redlands, CA, USA), we created random points in the Oak Openings Preserve within forested areas such as savanna, swamp forest, upland forest, floodplain forest and conifers and each point was at least 50 m apart to reduce the possibility of sampling the same individuals. Any point that fell within the restricted ground nesting bird areas

was removed. We uploaded the points into our handheld GPS unit (Garmin eTrex), which had and accuracy of 3-10 m, and used it to locate each point. The random points were used as the corner of the quadrat. We sampled a total of 189 quadrats, a 75600 $m^2$ area, Figure 2.2, which encompassed 15% of the entire area. Each day, we sampled one to five quadrats that were within similar areas of the park and on average, we traveled an average of 212 m between each quadrat. Quadrats were not randomly selected to prevent sampling quadrats on opposite sides of the park.

We set up the quadrat by walking 20 meters north or south from the initial corner point; each quadrat encompassed a 400 $m^2$ survey area. Flag markers were placed at each of the four corner points and we wrapped rope around the quadrat to prevent sampling outside of the desired area. We recorded the date, the number of observers, start and end time of the survey, weather, cardinal direction, and the coordinates for the quadrat corner points. After the quadrat was set up, we started a timer for 15 minutes in order to reduce searching bias when sampling the area. One observer searched the quadrat by slowly walking up and down in zigzag transect lines until the entire quadrat had been searched. If time was still left, then the observer would carefully recheck the quadrat until the timer ran out. When there were two observers, one observer led the walk through and the other observer would follow and check for any individuals that may have been missed by the first observer. We searched the area within the quadrat visually and checked underneath logs. The timer was stopped when an individual was located in order to prevent time loss when recording data. For each individual located, we recorded species, time, approximate body size, behavior, coordinates, air temperature and humidity, surface temperature and humidity and presence of vegetation. We photographed each individual when located, if feasible, and identified them by species.

After the 15 minute search, we noted general environmental characteristics for the center point and every two meters north, east, south and west of the center point. We photographed each point and categorized the ground cover vegetation as proportions, such as coarse woody debris, mud, grass, etc. If we detected any individuals outside the specified search time, we recorded the data as the quadrat detection, but uniquely marked it to identify that it was not located during our search time. We only recorded individuals that we could identify by sight; we excluded any vocalizations that were not confirmed visually.

***Opportunistic Observations***

When traveling to our sample points, we had the potential to encounter herpetofauna. We recorded individuals that we found opportunistically as a separate data set for 111 days and we searched an approximately 82503 $m^2$ area. We recorded the same individual information as we did for the quadrat searches and mapped each individual found. Individuals that we were unable to identify to the species level were recorded by order: Anura (frogs and toads), Urodela (salamanders), Squamata (snakes), and Testudines (turtles). This occurred when individuals jumped too quickly into the water or they were too far away for proper identification.

***Data Analysis***

We identified species richness, species abundance, species diversity, and species evenness for both methods. Total species richness was recorded as the total number of each species detected. Species diversity was measured by calculating Simpson's index (D) for each method. Species diversity was calculated with the Shannon-Weiner diversity index (H) and Shannon's evenness (J) was calculated by dividing the Shannon-Weiner diversity index by estimating the natural logarithm of the number of species detected. For each method, we mapped the individuals using ArcGIS to create distribution maps. We conducted an independent-sample

t-test ($\alpha = 0.05$) to compare the two methods for statistical differences using species richness and number of individuals detected.

*Results*

***General Success***

Using the quadrat and opportunistic methods we detected nine species in common: *Anaxyrus americanus*, *Pseudacris crucifer*, *Pseudacris triseriata*, *Anaxyrus fowleri*, *Hyla versicolor*, *Lithobates sylvaticus*, *Ambystoma laterale* complex, *Plethodon cinereus*, and *Diadophis punctatus*. The quadrat method resulted in detection of only those nine species and opportunistic method resulted in detection of a total of 21 species (including the nine species in common), see Table 2.1. A large number of both amphibians and reptiles were detected using the opportunistic method, 11 amphibian species and 10 reptilian species, while we detected six amphibian species and one Squamata species using quadrat surveys. We found that the number of individuals detected between the two methods was significantly different ($t = 4.46$, d.f. = 92, $P < 0.001$). Also, we found that the number of species detected were significantly different ($t = 8.38$, d.f. = 108, $P < 0.001$) between the two methods.

We examined species richness, diversity and evenness with each method. A high species richness index indicates a higher chance of encountering two individuals from different species. We found that the opportunistic method had the greatest species richness, for both amphibians and reptiles, see Table 2.2. A high species diversity index depicts a greater number of species within the area. We found that the opportunistic method resulted in more species detected than the quadrat method and there were more amphibian species detected than reptile species, see Table 2.2. The species evenness index shows the relative abundance of each species and a high index value indicates that the species are more equally abundant, while a low index value

indicates that one species may be rare and another common. The highest evenness index values were for amphibians detected opportunistically, then reptiles, all individuals detected opportunistically, amphibians detected by quadrats and quadrat detections, see Table 2.2. Overall, utilizing the opportunistic method resulted in the highest index values and amphibians had higher values than reptiles.

***Quadrat Surveys***

We sampled 189 quadrats: 130 quadrats (69%) had at least one detected individual and 59 quadrats (31%) had zero detected individuals, Figure 2.3. Quadrat sampling efforts yielded a grand total of 457 individuals for nine species. Quadrat detections accounted for 34% of all detections within the Oak Openings Preserve. During the time-constrained search, we found 409 individuals (89%) and during setup, collection of vegetation data and take-down, we found 48 individuals (11%). Individuals detected outside of our search time occurred in 32 quadrats and ranged from one to five individuals detected. On average, we found 2.4 individuals per quadrat and when individuals detected outside of the specified search time were excluded, we found on average 2.2 individuals per quadrat. We competed on average, 2.2 quadrats per day and found on average, 5 individuals per day. We detected in quadrats a range of zero to 18 individuals and each contained on average one species, with a range of zero to four species. We detected four Anura that were not identified to the species level.

For amphibians, we found 178 Anura from 6 species (*Anaxyrus americanus*, *Pseudacris crucifer*, *Pseudacris triseriata*, *Anaxyrus fowleri*, *Hyla versicolor* and *Lithobates sylvaticus*) and 278 Urodela from two species (*Ambystoma laterale* complex and *Plethodon cinereus*), see Table 2.1. For reptiles, we found 1 Squamata (*Diadophis punctatus*) and zero Testudines, see Table 2.1. For amphibians, *Plethodon cinereus* and *Lithobates sylvaticus* were the most detected

species, while *Diadophis punctatus* was the only reptile detected. Figure 2.4 shows the spatial distribution of each individual detected by quadrat sampling.

***Visual Encounters***

We sampled herpetofauna for 111 days and detected at least one individual on 90 days (81%) and had no detections on 21 days (19%). Using opportunistic detection yielded a grand total of 888 individuals for 21 species. Opportunistic detections accounted for 66% of all detections within the Oak Openings Preserve. When we detected individuals opportunistically, on average, we found 16 individuals per day with a range of zero to 106 detected individuals. On average we found 2.2 species per day, with a range of zero to ten species. The most detected species of all the herpetofauna were *Lithobates sylvaticus* and *Lithobates clamitans*. There were 149 Anura, eight Testudines and one Squamata that were not identified to species level.

For amphibians, we found 727 Anura from nine species (*Lithobates catesbeiana*, *Lithobates clamitans* , *Anaxyrus americanus*, *Anaxyrus fowleri*, *Hyla versicolor*, *Pseudacris triseriata*, *Lithobates sylvaticus*, *Pseudacris crucifer*, and *Lithobates pipiens*) and 103 Urodela from two species (*Ambystoma laterale* complex and *Plethodon cinereus*), see Table 2.1. *Lithobates sylvaticus* and *Lithobates clamitans* were the most detected amphibian species. For reptiles, we found ten Squamata from four species (*Heterodon platirhinos*, *Diadophis punctatus*, *Nerodia sipedon* and *Coluber constrictor foxii*) and 48 Testudines from six species (*Graptemys geographica*, *Chrysemys picta*, *Apalone spinifera*, *Chelydra serpentina*, *Terrapene c. carolina* and *Emydoidea blandingii*), see Table 2.1. *Chrysemys picta* and *Terrapene c. carolina* were the most detected reptile species. Figure 2.5 shows the spatial distribution of each individual discovered through opportunistic detection.

*Discussion*

Currently our knowledge of biodiversity is still limited, even for the best studied organisms, we know little about their distributions (Ficetola et al. 2013). We have provided critical foundational work by examining herpetofauna distributions and abundances within the Oak Openings Preserve using minimally invasive visual encounter surveys. In general, surveying herpetofauna requires different methods in order to detect different species. Some species will be more likely detected by actively searching, such as using quadrats, which can readily detect small frogs and leaf litter lizards, while other species like faster moving lizards, many snakes and arboreal frogs, will be more likely detected through visual opportunistic detections (Doan 2003). We found that all of the species detected within quadrats were also detected within opportunistic surveys. This suggests that we did not lose any data by using multiple methods. Many studies have shown that using different methods yields detection of different species and that detection of at least one species does not overlap between methods (Doan 2003; Garden et al. 2007; Lowe & Parmley 2008). Each method has the potential to detect different species providing at least a minimum estimate of the current biodiversity. Therefore it would be useful to use additional methods: netting, pitfall trapping, torching, searching refugia, such as using artificial cover objects, and funnel traps (McDiarmid et al. 2012) to maximize the detection of the diversity present. Using additional methods may reveal species that we did not detect using our visual encounter surveys.

We had higher detections for both amphibians and reptiles with our opportunistic method than our quadrat sampling. We did survey more of the area opportunistically than with our quadrats, but it only slightly varied and the data is comparable. These results suggests that opportunistic surveys or moving from area to area may allow an observer to detect more individuals than searching a fixed area for herpetofauna, at least for some species. One

exception, though, was the *Plethodon cinereus*, of which we detected more individuals in quadrat sampling than opportunistic surveys. *Plethodon cinereus* is a terrestrial species with fossorial tendencies and spends relatively little time actively moving on the ground surface (Hocking & Babbitt 2014; Olson & Kluber 2014). Using our quadrat survey, we intensively sampled a fixed area which included lifting all logs, large sticks and bark from the ground, which increased our ability to find Urodela. When searching opportunistically, we did not intensively search under logs and because *Plethodon cinereus* is not actively moving on the ground surface, we were less likely to find them with opportunistic walks. In addition, opportunistic searches could also have been impeded by habitat factors such as ground cover vegetation (Olson & Kluber 2014), which may explain why we found relatively fewer *Plethodon cinereus* individuals when searching visually than when we searched fixed areas.

Even though we had greater detection data for opportunistic surveys, quadrat sampling can be a useful tool for targeting certain amphibian species such as *Anaxyrus americanus*, *Pseudacris crucifer*, *Lithobates sylvaticus*, *Plethodon cinereus* and *Ambystoma laterale* complex. However, there were three Anura species that the quadrat method did not detect that were present within the Oak Openings Preserve: *Lithobates catesbeianus*, *Lithobates clamitans* and *Lithobates pipiens*. This may have occurred because these three species tend to be semi-aquatic, especially *Lithobates catesbeianus*, which are more likely to be found near lakes, ponds and slow-flowing streams, but they do inhabit terrestrial environments (Clarkson & DeVos Jr. 1986). Our quadrats were set up in areas with ground cover vegetation and were not located within large bodies of water in which these species are typically found. We were able to detect *Lithobates sylvaticus* in some of our quadrats that had small ephemeral flooded areas within the boundaries.

For our surveys, we found that quadrats were insufficient for detecting reptiles. We found one Squamata, *Diadophis punctatus*, within one quadrat, hidden underneath a log. It is possible that when we approached and set up our quadrats, reptiles within that area may have fled after detecting our presence by sensing vibrations. As shown in other studies (Doan 2006; Garden et al 2007), reptiles were more likely to be detected through direct observation, like our opportunistic observations, than standardized methods. Our quadrat method was our focal surveying tool, but we found that its species richness and species diversity indexes for both herpetofauna and just amphibians were lower than in our opportunistic detections, Table 2.2. Therefore, using quadrat surveys we detected fewer species and had a smaller chance of encountering two individuals from different species. Quadrat sampling also resulted in a smaller evenness index indicating that the relative abundance of each species was less uniform, see Table 2.2. We had a relatively small number of reptile detections within the preserve; it is likely that reptiles are rarer and/or more difficult to detect than amphibians. It is possible that our reptile detections may have increased if we had sampled the ground nesting bird areas which had less canopy cover than most of our survey locations. We found two of our *Coluber constrictor foxii* and four *Terrapene c. carolina* within prairie areas, which suggests that reptiles are occupying areas with less canopy cover. We suggest that future studies perform intensive surveys focused on reptiles in order to confirm these findings. If reptiles are indeed rarer, then it suggests that the habitat may not be as suitable for reptiles as it is for amphibians and highlights a need for management of the habitat to increase suitability for reptiles.

When completing our quadrat sampling, several of the individuals detected, 11%, were detected outside of our time constrained search. This occurred either when we were setting up or taking down the quadrat and during our vegetation survey. Although this was a small portion of

our detections, this suggests that our designated time of 15 minutes may not have been enough to properly detect all of the individuals within a quadrat. We wanted to have an efficient, systematic, replicable search method for each quadrat to prevent over searching areas that appeared more suitable and under searching areas that appeared less suitable. Some of the individuals detected within our vegetation surveys may have migrated within our search area during the survey. Detectability of herpetofauna was also most likely impacted by vegetation density; some of our quadrats were more uniform and had less vegetation, see Figure 2.6 A, than others which had dense vegetation, see Figure 2.6 B. For some quadrats, it would have been better to have a longer sampling time, e.g., in denser vegetation, while others, e.g., in sparse vegetation, did not need as much search time. For future studies we would suggest using species accumulation curves to set a minimum search time for all quadrats. This would require preliminary work before surveying by completing a series of timed searches while gradually increasing the time for each survey to identify when adding more time does not yield more species. This would help other studies in different areas find the appropriate sampling time to account for differences in vegetation cover between survey sites. Our time constraint of 15 minutes may have led us to underestimate the number of individuals within each quadrat. The high level of biodiversity probably helped increase the number of individuals detected; we found that 31% of the quadrats had zero individuals detected. It is possible that we would have detected zero individuals in as many as half of our quadrats if the level of biodiversity was much smaller.

There are relatively few studies that have examined herpetofauna diversity and abundances, many studies examine targeted species. We compared our survey results to two studies, Killbuck Marsh Wildlife Area (similar in size) and Little Black Creek (similar in sampling time) to highlight how methodology can impact survey results. We found that the Oak

Openings Preserve is less diverse than Killbuck Marsh Wildlife Area in Ohio, but has greater species abundances. Wicknick, Anthony, & Reblin's (2005) four year amphibian survey used visual encounter surveys, call surveys and dip netting to survey amphibians and found 16 species with a total of 439 individuals, Table 2.3. Surprisingly we found 1286 amphibian individuals within our 1618 ha, while they only detected 439 individuals within their 2222 ha site over four years. Although they detected more Urodela species than we did, seven versus two, we both found nine Anura species with one different species, *Lithobates palustris* versus *Anaxyrus fowleri*. We did not use dip netting which is likely the reason why we did not find as many Urodela species. We were only able to detect the terrestrial *Plethodon cinereus* and terrestrial adult *Ambystoma laterale* complex and missed many of the aquatic Urodela species. It is interesting that we were able to detect the same number of Anura species as the Killbuck Marsh study even though they used call surveys. This suggests that call surveys are not detecting more Anura species than visual encounter surveys. The substantially large difference in number of species is most likely a result because of habitat richness. Both studies had similar intensive sampling hours: they sampled 20 sites with subsampling (53.55 person hours), while we sampled 189 quadrats (47.25 person hours) along with extensive opportunistic sampling time (~30 person hours).

We also compared our study to Lowe & Parmley's (2008) study in Little Black Creek, Georgia. This study was much smaller, <25 ha, however sampling time was more equivalent to our study, 8 months vs. our 6 months sampling. They used terrestrial drift fences and pitfall traps to examine vertebrate biodiversity and found 803 herpetofauna from 33 different species, Table 2.3. Although we found more individuals, our species richness was smaller, 21 species. It is interesting that our study detected fewer species than their study, but this may be a result of using

trapping techniques which can detect cryptic species that are not easily visible, location near water source and having different climates. Comparing these two studies supports the idea that using drift fences or pitfall traps can help to increase the number of species we could detect, especially for increasing Urodela detections.

From this study, we found that quadrat sampling can used to sample amphibian biodiversity; however, it should be used together with other methods to get a more complete understanding of population estimates. For this initial study of herpetofauna diversity in the Oak Openings Preserve, we wanted to maximize the number of individuals detected by covering a larger portion of area rather than to resample and look at population estimates, which can be done in future studies. The visual encounter surveys have most likely underestimated herpetofauna populations within the preserve and many secretive species like snakes could have been missed or underrepresented. Future studies may want to consider other more invasive methods to try to detect cryptic species. We found that visual encounter surveys can be used to detect herpetofauna and provide important information on biodiversity in heterogeneous landscapes, forming the basis for monitoring biodiversity changes across time and space.

***Management Implications***

We found that visual encounter surveys are a suitable method for surveying herpetofauna within heterogeneous landscapes. We recommend that surveyors use multiple complementary methods in order to detect a larger set of species. We found that the quadrat method detected fewer species; however we are not recommending one method over another, simply that it may yield fewer numbers of individuals. Either of these sampling methods provides a simple, replicable and cost-effective technique that could be applied to a citizen science program to examine both spatial and temporal trends in biodiversity with long-term monitoring. Observers

can be quickly trained on how to set up and survey specified areas, which allows for multiple surveys within a season and over time. Surveyors can be trained to identify each species and/or the use of photographs can confirm species identification at a later date by experts. Quadrat sampling requires only a few supplies: meter tape, rope, flag markers, camera, data sheets; which allows for an inexpensive assessment of biodiversity that is relatively quick; one quadrat takes approximately one hour and thirty minutes to complete with two people. We highly recommend that quadrats are set up within uniform habitats with thin vegetation; otherwise the search time should be increased. For denser vegetation, opportunistic surveys will most likely yield higher detections as shown in Doan's (2003) study within rainforests. Park size may also influence which methods are chosen. Large parks such as the Oak Openings Preserve may be more suitable for opportunistic surveys that may cover more ground in less time versus smaller parks that can be surveyed more intensively with quadrat surveys. Our study was limited by having one two-person team survey each day; citizen science provides a pool of volunteers who can help collect large amounts of data that is cost-efficient. Having multiple teams per day is beneficial because true random sampling can occur which eliminates some biases in our survey. Volunteers can be trained to identify species, but our method allows for confirmation with experts, which allows the researcher to assess observer bias. This is a workable program that can get the public excited about relatively underappreciated taxa while providing reliable data for long-term temporal and spatial dynamics of herpetofauna diversity. It is crucial that we continue to monitor herpetofauna populations in a cost-effective manner in order to monitor spatial and temporal changes.

*Acknowledgements*

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*Tables*

Table 2.1: Abundance of each herpetofauna found within the Oak Openings Preserve and the percent of individuals found per method (Quadrat or Opportunistic).

| Scientific Name | Quadrat | % Quadrat | Opportunistic | % Opportunistic |
|---|---|---|---|---|
| Anura | 4.00 | 0.90 | 149.0 | 16.8 |
| *Anaxyrus americanus* | 48.0 | 10.5 | 78.0 | 8.80 |
| *Anaxyrus fowleri* | 4.00 | 0.90 | 20.0 | 2.30 |
| *Hyla versicolor* | 2.00 | 0.40 | 8.00 | 0.90 |
| *Pseudacris crucifer* | 45.0 | 9.80 | 91.0 | 10.3 |
| *Pseudacris triseriata* | 3.00 | 0.70 | 37.0 | 4.20 |
| *Lithobates catesbeianus* | 0.00 | 0.00 | 47.0 | 5.30 |
| *Lithobates clamitans* | 0.00 | 0.00 | 144 | 16.2 |
| *Lithobates pipiens* | 0.00 | 0.00 | 2.00 | 0.20 |
| *Lithobates sylvaticus* | 72.0 | 15.8 | 151 | 17.0 |
| Urodela | 0.00 | 0.00 | 0.00 | 0.00 |
| *Plethodon cinereus* | 273 | 59.7 | 96.0 | 10.8 |
| *Ambystoma laterale* complex | 5.00 | 1.10 | 7.00 | 0.80 |
| Squamata | 0.00 | 0.00 | 1.00 | 0.10 |
| *Diadophis punctatus* | 1.00 | 0.20 | 1.00 | 0.10 |
| *Heterodon platirhinos* | 0.00 | 0.00 | 4.00 | 0.50 |
| *Coluber constrictor foxii* | 0.00 | 0.00 | 3.00 | 0.30 |
| *Nerodia sipedon* | 0.00 | 0.00 | 1.00 | 0.10 |
| Testudines | 0.00 | 0.00 | 8.00 | 0.90 |
| *Chelydra serpentina* | 0.00 | 0.00 | 2.00 | 0.20 |
| *Emydoidea blandingii* | 0.00 | 0.00 | 3.00 | 0.30 |
| *Terrapene c. carolina* | 0.00 | 0.00 | 11.0 | 1.20 |
| *Graptemys geographica* | 0.00 | 0.00 | 3.00 | 0.30 |
| *Chrysemys picta* | 0.00 | 0.00 | 19.0 | 2.20 |
| *Apalone spinifera* | 0.00 | 0.00 | 2.00 | 0.20 |

Table 2.2: Species richness represented by Simpson's diversity (D), species diversity represented by Shannon Wiener index (H) and Shannon's evenness (J) for herpetofauna found in quadrat, and opportunistic sampling. The individuals that were identified by their order (e.g. Anura, Urodela, Squamata and Testudines) were excluded from diversity index calculations.

| Sampling Method | Simpson's Diversity | Shannon Wiener Diversity | Evenness |
|---|---|---|---|
| Quadrat | D = 0.59 | H = 1.23 ± 0.13 SD | J = 0.40 |
| Quadrat Amphibians | D = 0.69 | H = 1.28 ± 0.16 SD | J = 0.53 |
| Opportunistic | D = 0.87 | H = 2.25 ± 0.11 SD | J = 0.74 |
| Opportunistic Amphibians | D = 0.85 | H = 2.02 ± 0.12 SD | J = 0.84 |
| Opportunistic Reptiles | D = 0.79 | H = 1.84 ± 0.10 SD | J = 0.80 |

Table 2.3: The number of species and individuals detected with the methods used for detection: visual encounter survey (VES), call survey (CS), dip netting (DN), pitfall trapping (PF) and funnel trapping (FT) for the Oak Openings Preserve (OOP), Killbuck Marsh (KM) and Little Black Creek (LBC) for amphibians (Am) and reptiles (Re). A dash (-) represents no data.

| Survey | Am Species | Am Individuals | Re Species | Re Individuals | Method |
|---|---|---|---|---|---|
| OOP | 11.00 | 1286 | 10.00 | 59.00 | VES |
| KM | 16.00 | 439.0 | - | - | VES/CS/DN |
| LBC | 17.00 | 728.0 | 16.00 | 75.00 | PF/FT |

*Figures*

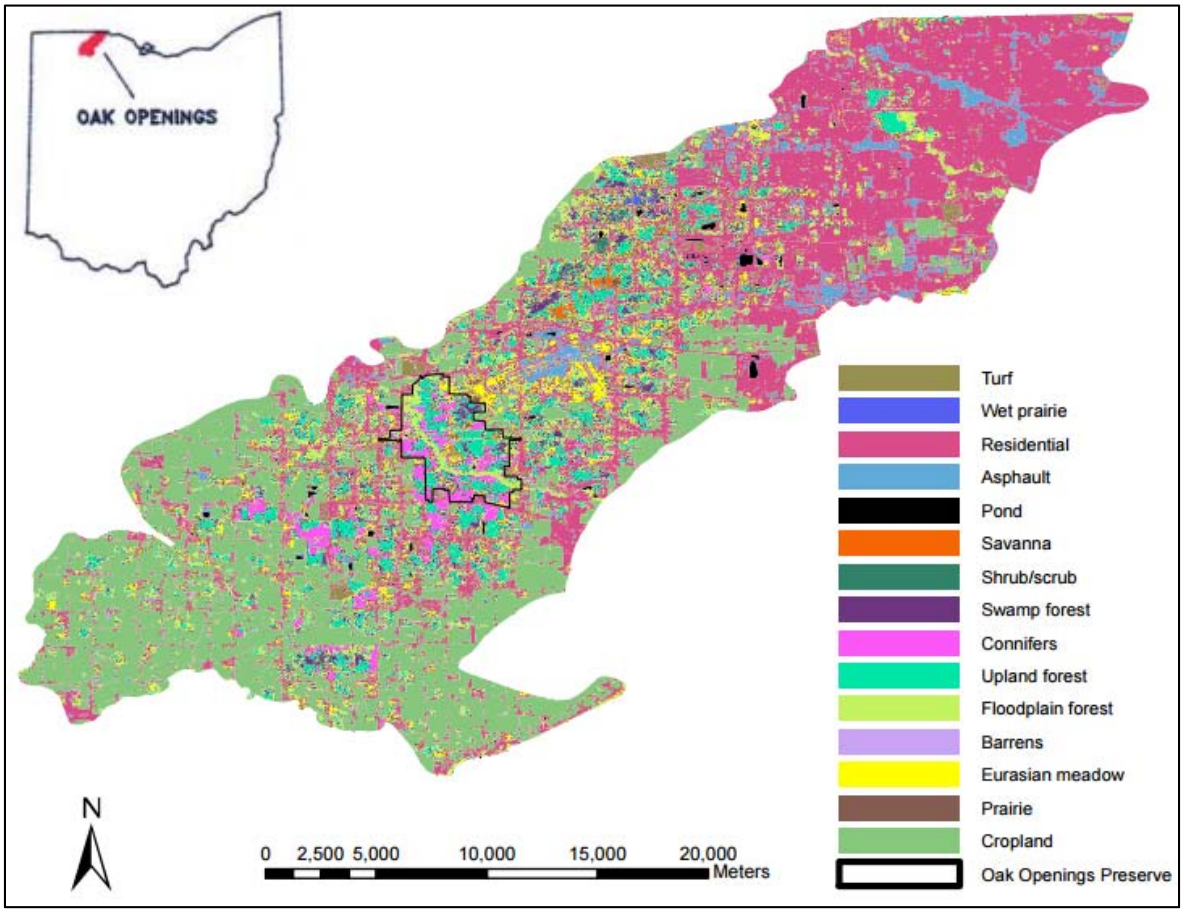


Figure 2.1: A land cover map of the Oak Openings Region based on Schetter and Root 2011.

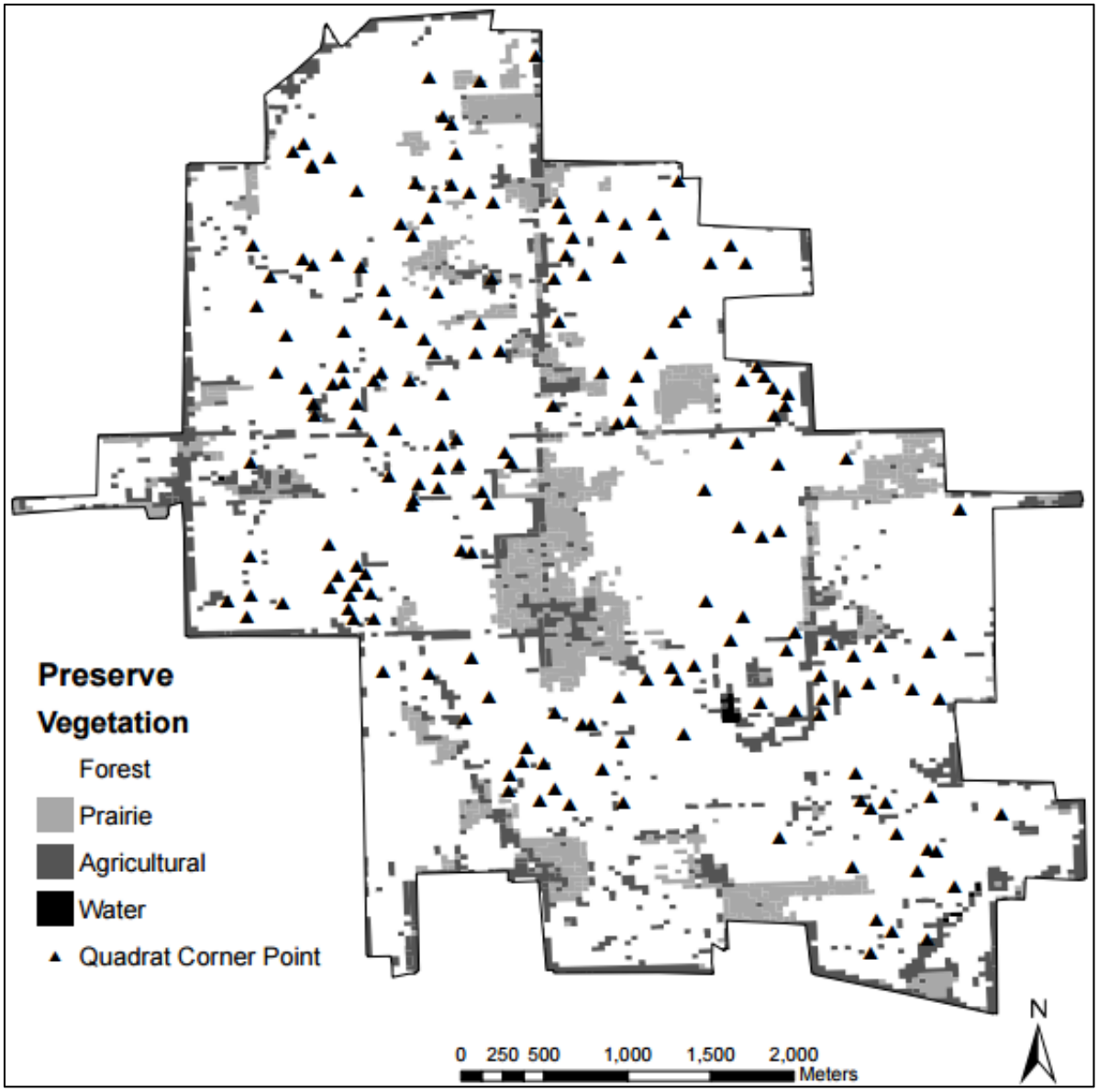


Figure 2.2: Spatial map of areas surveyed for herpetofauna using quadrats within the Oak Openings Preserve with four types of land cover: forest, prairie, agricultural and water.

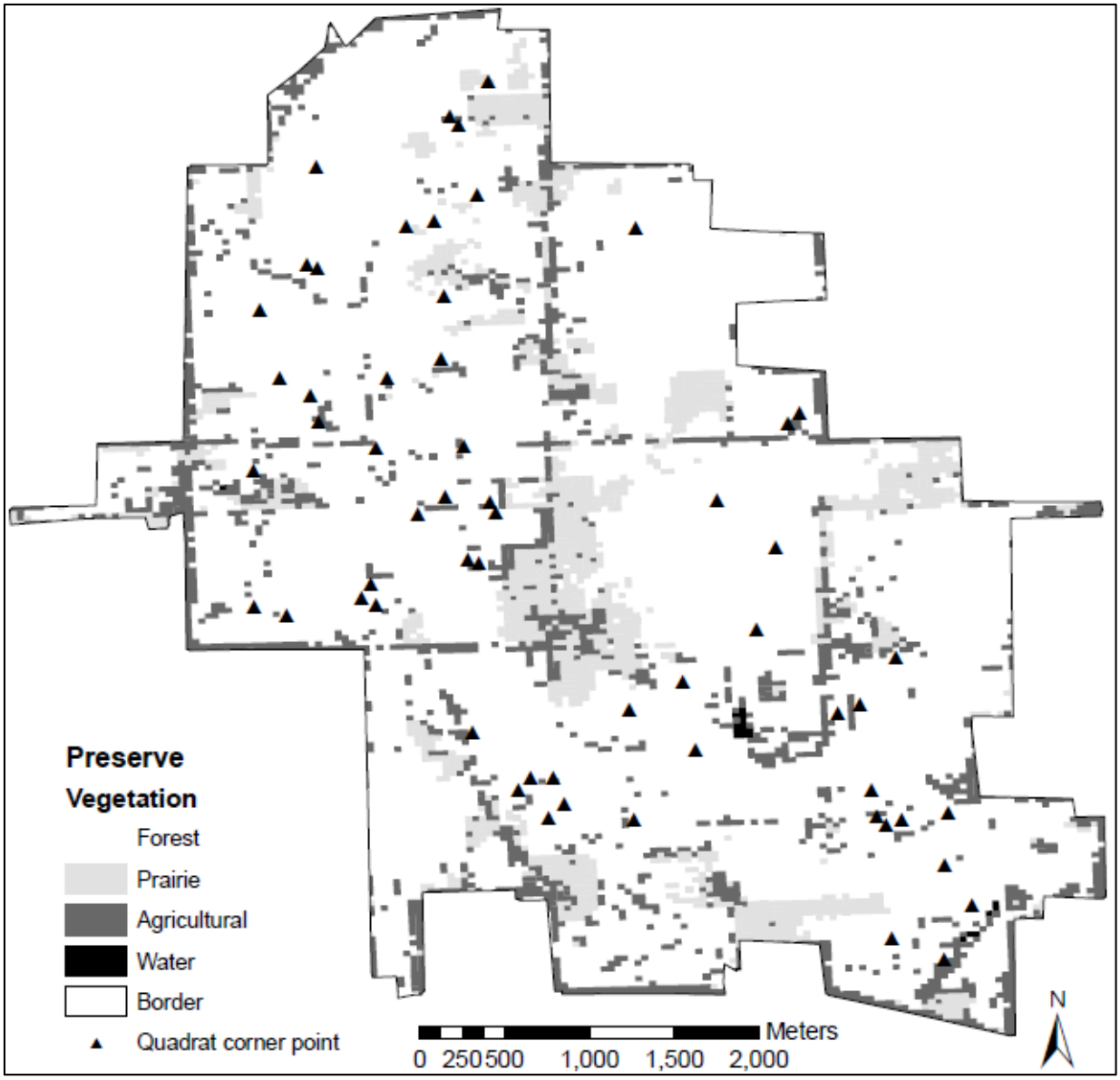


Figure 2.3: Spatial map of quadrats with zero individuals detected within the Oak Openings Preserve with four types of land cover: forest, prairie, agricultural and water.

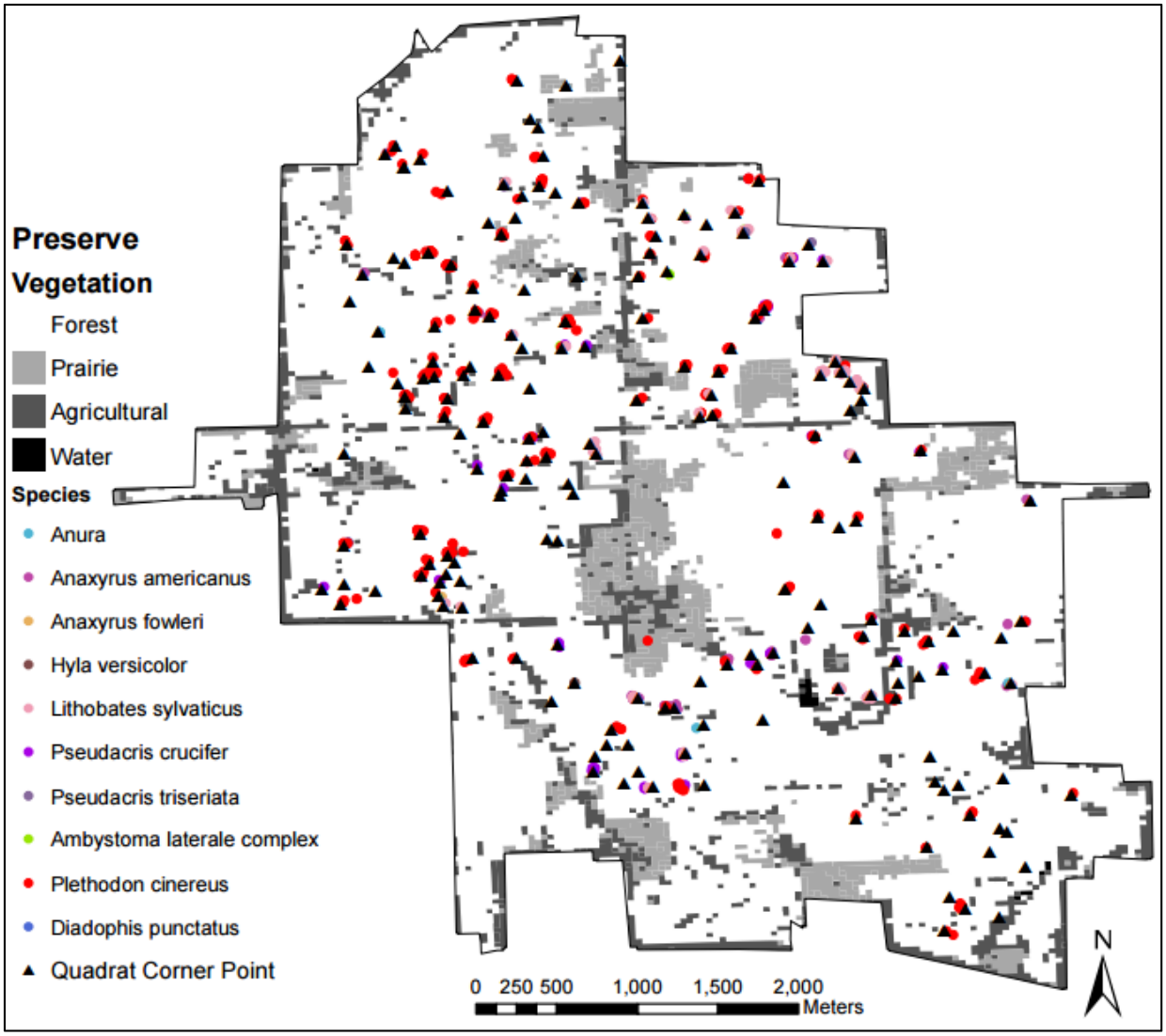


Figure 2.4: Spatial patterns of each herpetofauna species detected within quadrat sampling in Oak Openings Preserve with four types of land cover: forest, prairie, agricultural and water.

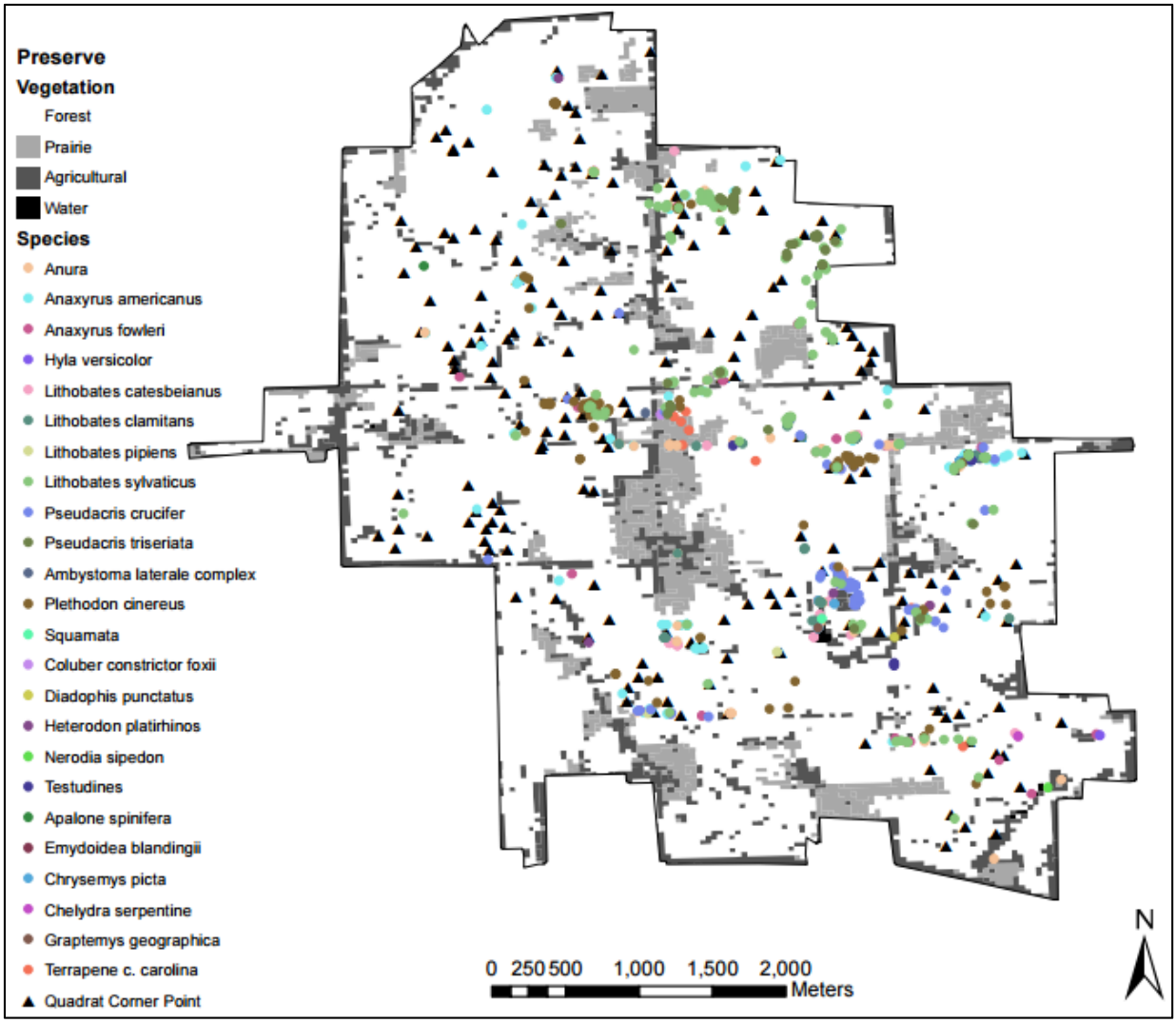


Figure 2.5: Spatial patterns of each herpetofauna species detected using opportunistic sampling within Oak Openings Preserve with four types of land cover: forest, prairie, agricultural and water.

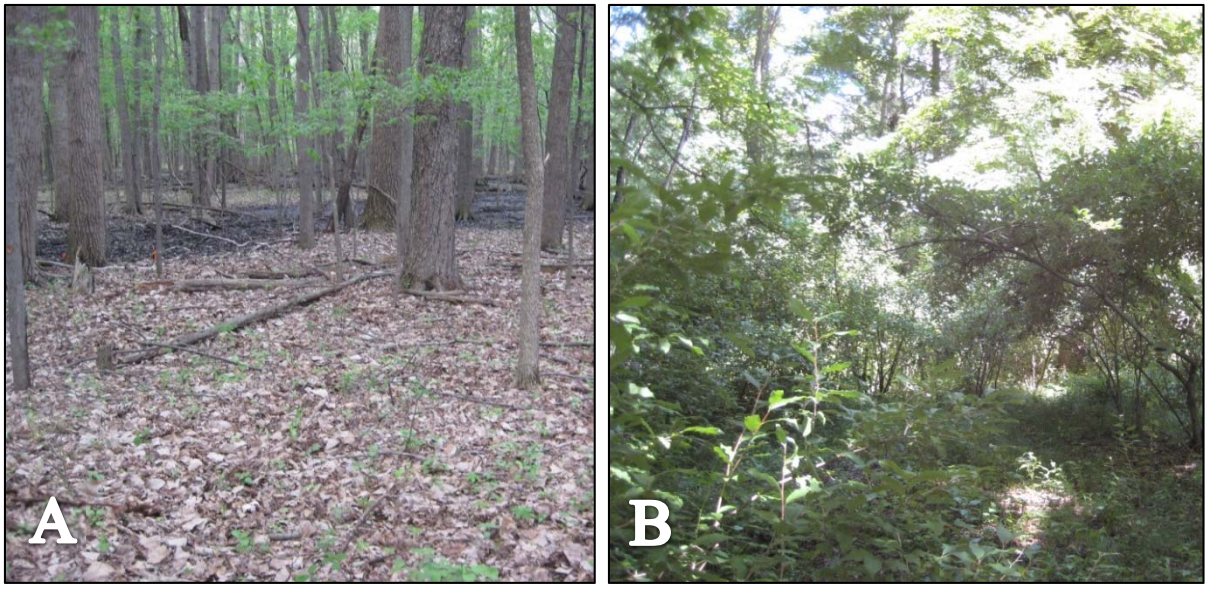


Figure 2.6: The picture on the left (A) depicts a survey quadrat with relatively sparse vegetation vs. the quadrat pictured on the right (B) which has dense vegetation in Oak Openings Preserve.

# CHAPTER 3: EXAMINING ENVIRONMENTAL INFLUENCES ON HERPETOFAUNA DISTRIBUTIONS

*Abstract*

To examine the influence of environmental factors on the distribution of herpetofauna, we surveyed herpetofauna biodiversity within the Oak Openings Preserve and documented presence. We created maps of species' spatial patterns and used the average nearest neighbor analysis to look at spatial dispersion for each species. We used principal components analysis to identify habitat variable associations. We used logistic regression to identify significant habitat variables and modeled important habitat variables using a forward stepwise logistic regression for each species. The results illustrated differences in species spatial patterns. Six species had clustered spatial patterns, while three species were randomly distributed and 11 species were dispersed throughout the park. We found a small percentage of herpetofauna were detected in areas near streams or roads. Most of the individuals detected were found in the afternoon and in April. Squamata were detected at the highest temperatures, while Anura were detected at the highest humidity. Forested areas had the greatest number of individuals present. Our principal components analysis identified that proportion of leaf litter, coarse woody debris, conifer needles, moist soil and plants were important habitat variables for herpetofauna and this was supported by our model. Our study has shown that herpetofauna, as a whole, are occupying forested areas and each species has slight variations in which habitat variables are most important. We suggest that managers use the species maps to identify which species will be impacted before implementing management plans or to strategize conservation efforts. Our results should be generalizable for other parks with similar land cover features and their herpetofauna distributions should follow similar patterns.

*Introduction*

Many herpetological surveys examine species richness and abundance; however it is important to understand how the environment influences herpetological biodiversity. Understanding how organisms interact with their environment is important for examining factors that contribute to population declines. As humans continue to convert natural habitats, protected areas become even more important to help preserve biodiversity. Managing habitat for a multitude of species can be difficult and it is critical to identify which habitat features are most important for a variety of species.

Herpetofauna, amphibians and reptiles, may not only be impacted by the physical habitat structure surrounding them, but also other environmental factors such as temperature and humidity. Herpetofauna rely on their environment not only for basic needs such as food and shelter, but they need to maintain their body temperature by moving in and out of areas with heat. Unlike mammals and birds which are endotherms, amphibians and reptiles are more sensitive to changes in the environment which can have a stronger impact. Amphibians are especially vulnerable because they have permeable skin which readily absorbs substances and a dual lifecycle, making them vulnerable to changes in either/both aquatic and terrestrial habitats (Hopkins 2007). By examining species presence and its habitat association, park managers can create management plans to better protect the desired species (Broms et al. 2014). Investigating the environmental factors influencing species presence and distributions can help park managers to provide suitable habitat for multiple species.

We examined herpetofauna biodiversity in Chapter 1 and will examine how environmental factors influenced the herpetofauna distributions in the Oak Openings Preserve. Our question focused on how spatial and temporal heterogeneity within the environment impacts

species diversity (Sutherland et al. 2013). We looked at overall species diversity, abundance and individual species spatial patterns from a landscape-level by examining density, species overlap and spatial dispersion. We analyzed species abundance based on time, month, temperature and humidity. We looked at species presence-absence and identified which habitat variables were significantly associated. Finally, we created a model for each species presence based on important habitat variables. Our overarching goal was to identify how the environment plays a role in herpetofauna species distributions.

*Methods*

***Study Area***

The Oak Openings Region is a biodiversity hotspot containing an abundance of diverse species. The heterogeneous area hosts five globally significant communities: Great Lakes Twig-rush Wet Meadow (Wet Prairie), Great Lakes White Oak-Pin Oak Flatwoods, Mesic Sand Prairie, Midwest Sand Barrens, and Black Oak/Lupine Barrens (Oak Savanna) (EPA 2012). The region supports a variety of species, like the endangered Karner blue butterfly, *Lycaeides melissa samuelis*, and has 177 rare species, along with other organisms from many different taxa (EPA 2012). It encompasses approximately 40,000-ha and it extends from northwestern Ohio to parts of southern Michigan. It was shaped by glaciation and subsequent anthropogenic influences (e.g., water drainage and fire suppression) and alterations (e.g., urban expansion and agricultural intensification). There are several protected areas within this region, but our study focused on the largest preserve.

We sampled the Oak Openings Preserve in Swanton, Ohio, from 26 April 2014 to 27 September 2014, to investigate herpetofauna species diversity and abundances. The 1618-ha preserve is the largest contiguous protected area that contains a high amount of biodiversity for

all taxa (The Ohio Ornithological Society 2014). The preserve contains many different land cover types; we used a land cover map (Schetter & Root 2011) to assess habitat associations per species with 11 land covers (out of a total of 15 for the entire Oak Openings Region): conifer, Eurasian meadow, floodplain forest, pond, prairie, residential, savanna, shrub, swamp forest, turf, and upland forest, see Figure 3.1. Areas modified for human land use were characterized as residential with mowed lawns, structures, roadways and ditches where trees are absent and for cropland as crops, refer to Schetter and Root (2011) for more detailed description. For our study, we excluded areas with ground nesting birds to avoid disturbing them.

***Quadrat Sampling***

Using ArcGIS 10.1 (ESRI Inc., Redlands, CA, USA), we created random points in the Oak Openings Preserve within forested areas such as savanna, swamp forest, upland forest, floodplain forest and conifers and each point was at least 50 m apart to reduce the possibility of sampling the same individuals. Any point that fell within the ground nesting bird areas was removed. We uploaded the points into our handheld GPS unit (Garmin eTrex), which had an accuracy of 3-10 m, and used it to locate each point. The random points were used as the corner of the quadrat. We sampled a total of 189 quadrats; which encompassed 15% of the entire area. Each day, we sampled one to five quadrats that were within similar areas of the park. Quadrats were not randomly selected to prevent sampling quadrats on opposite sides of the park.

We set up the quadrat by walking 20 meters north or south from the initial corner point; each quadrat encompassed a 400 $m^2$ survey area. Flag markers were placed at each of the four corner points and we wrapped rope around the quadrat to prevent sampling outside of the desired area. We recorded the date, the number of observers, start and end time of the survey, weather, cardinal direction, and the coordinates for the quadrat corner points. After the quadrat was set up,

we started a timer for 15 minutes in order to reduce searching bias when sampling the area. One observer searched the quadrat by slowly walking up and down in zigzag transect lines until the entire quadrat had been searched. If time was still left, then the observer would carefully recheck the quadrat until the timer ran out. When there were two observers, one observer led the walk through and the other observer would follow and check for any individuals that may have been missed by the first observer. We searched the area within the quadrat visually and checked underneath logs. The timer was stopped when an individual was located in order to prevent time loss when recording data. For each individual located, we recorded species, time, approximate body size, behavior, coordinates, air temperature and humidity, surface temperature and humidity and presence of vegetation. We photographed each individual when located, if feasible, and identified them by species.

After the 15 minute search, we noted general environmental characteristics for the center point and every two meters north, east, south and west of the center point. We photographed each point and categorized the ground cover vegetation into 12 habitat variables: proportion of leaf litter, coarse woody debris, logs, plant, tree, moist soil, grass, dry soil, conifer needles, water, sand, and wet leaf litter; for an example refer to Figure 3.2 A and B. If we detected any individuals outside the specified search time, we recorded the data as the quadrat detection, but uniquely marked it to identify that it was not located during our search time. We only recorded individuals that we could identify by sight; we excluded any vocalizations that were not confirmed visually.

***Opportunistic Observations***

When traveling to our sample points, we had the potential to encounter herpetofauna. We recorded individuals that we found opportunistically as a separate data set for 111 days. We

recorded the same individual information as we did for the quadrat searches and mapped each individual found. Individuals that we were unable to identify to the species level were recorded by order: Anura (frogs and toads), Urodela (salamanders), Squamata (snakes), and Testudines (turtles). This occurred when individuals jumped too quickly into the water or they were too far away for proper identification.

***Data Analysis***

We overlaid the Oak Openings Preserve map with a grid with 800 m by 800 m cells (each cell is equivalent to 1600 sampling quadrats) in ArcGIS to determine the number of species and individuals per grid cell, see Figure 3.3. We used this to determine which areas had the greatest abundances and richness. We mapped species spatial patterns using ArcGIS. We counted the number of species and individuals found close to streams and roads by creating a 50 m buffer in ArcGIS and clipping the data points within the buffer area. Recommended riparian buffers areas for amphibians vary, Rudolph and Dickson (1990) recommend a 30 m buffer, while DeMaynadier and Hunter (1995) recommend 10-25 m buffer and Vesely and McComb (2002) found that 47 m buffers were needed to support amphibian biodiversity. We chose to use a 50 m buffer because Crawford and Semlitsch (2007) recommended 50 m as a conservative estimate of core terrestrial habitat used by Urodela. Our 50 m buffer should include individuals using streams or roads within the area. We calculated the percentage of individuals within the stream and road buffers by dividing the number of individuals found within the buffer by the total number of individuals detected within the park. We calculated the extent of stream, road and total area using the calculate area tool in ArcGIS. We divided the extent of the stream and road areas by the total area to determine the percent area covered within the park for each feature. We compared the percent of individuals detected within 50 m of a stream or road and compared it to

the total percent of streams and roads to evaluate if the species was occupying areas with streams or roads more than expected (Martino et al. 2012).

Spatial dispersion for each species was calculated using the average nearest neighbor analysis in ArcGIS for which a z-score value less than -1.65 indicates a clustering distribution, a z-score value ranging from -1.65 to 1.65 indicates a random distribution, and a z-score value above 1.65 indicates a dispersed or uniform distribution. Survey times for herpetofauna ranged 8:00 am to 10:00 pm; we classified 8:00 am to 11:59 am as morning, 12:00 pm to 5:59 pm as afternoon and 6:00 pm to 10:00 pm as evening. We counted the number of individuals and species found per time and month to calculate species abundance and richness for morning, afternoon and evening hours and per month (April-September). We conducted an independent-sample t-test ($\alpha = 0.05$) to compare sampling time: morning and afternoon, morning and evening and afternoon and evening for the number of individuals detected per day. The dominant species was calculated by counting the total number of individuals and identifying the species with the highest abundance. We examined four environmental factors: air temperature (C°), surface temperature (C°), air humidity (%) and surface humidity (%), that were directly associated with each individual detected. We analyzed the mean, minimum, maximum and median of each factor for herpetofauna, amphibians, reptiles, Anura, Urodela, Squamata and Testudines. Using ArcGIS, we identified the number of each species found in 11 land cover types: conifer, Eurasian meadow, floodplain forest, prairie, residential, savanna, shrub, swamp forest, turf, and upland forest. We estimated habitat occupancy for each land cover by comparing the proportion of individuals of each species detected per land cover to the proportion of area sampled within each land cover. To examine habitat occupancy from a landscape-level, we quantified the proportion of available habitat sampled for each land cover and compared it to the proportion of habitat

occupied by each species for each land cover; this method is similar to Martino et al. (2012) study's examination of habitat selection at the landscape-level. If the proportion available is lower than the proportion occupied, it suggests that the species is using the habitat more than expected. For these analyses, we used individuals detected from both quadrat and opportunistic sampling.

The association between species presence detected within quadrats and habitat factors was summarized using a principle components analysis (PCA), using JMP® 11.0 (SAS Institute, Inc., Cary, NC, 1989-2007). The significance of the relationship between habitat variables was determined using the Spearman Rank Correlation and variables were eliminated if highly correlated (> 0.70). We ran a logistic regression using JMP to assess if the presence or absence of herpetofauna species was affected by any of our explanatory variables (habitat factors, month, time and date). We ran a forward stepwise logistic regression using JMP to create a parsimonious model based on the 12 habitat factors recorded by our vegetation survey for each quadrat. We examined the average, minimum, maximum and median vegetation proportions from the vegetation survey per quadrat to determine which one had the best model based on significance (p-value) and lowest Akaike's Information Criterion ($AIC_c$).

*Results*

***Grid Analysis***

We found that grid cell 33 had the greatest number of individuals (n = 285) followed by 39 (n = 132) and 11 (n = 98), see Table 3.1. We found that grid cell 27 had the greatest number of species (n = 12) followed by 25 and 40 (n = 11) and 33 (n = 10). Zero individuals and species were detected in grid cells 2, 4, 22, 23, 28, 42, 45, 46, 52, 53 and 54. Grid cells 33, 39 and 40 had

both the highest number of individuals and species detected and are located next to one another which suggest that this cluster may be a core area for herpetofauna, see Figure 3.3.

***Spatial Patterns***

We found that each species had different spatial patterns; however some species did have some overlap with one another, like *Pseudacris crucifer* and *Lithobates sylvaticus*, see Figure 3.4. While other species, like *Lithobates sylvaticus* and *Pseudacris triseriata*, many individuals overlapped with one another, see Figure 3.5. In this case, it appeared that detecting *Pseudacris triseriata* may assist in detecting *Lithobates sylvaticu*s. Some species of the same genus had very few overlapping individuals with each other, for an example *Anaxyrus americanus* and *Anaxyrus fowleri*, see Figure 3.6.

***Streams And Roads***

We examined the number of individuals and species that were found within a 50 m buffer for streams and roads, see Table 3.2. We found that 10.6% of Anura were found within 50 m of a stream and 9.2% were found within 50 m of a road. We found that 3.4% of Urodela were found within 50 m of a stream and 3.7% were found within 50 m of a road. We found that 27% of Squamata were found within 50 m of a stream and 36% were found within 50 m of a road. We found that 25% of Testudines were found within 50 m of a stream and 8% were found within 50 m of a road. The 50 m buffer around streams accounted for 20.1% of the area within the Oak Openings Preserve.

We found that 20.8% of *Anaxyrus fowleri*, 48.9% of *Lithobates catesbeianus*, 25% of *Heterodon platirhinos*, 100% of *Nerodia sipedon*, 50% of *Chelydra serpentina*, 33.3% of *Graptemys geographica*, 26.3% of *Chrysemys picta* and 50% of *Apalone spinifera* individuals were found within the stream buffer area. This suggests that these species are possibly occupying

areas with streams at a higher rate than expected. The area encompassed by 50 m buffers around roads accounted for 20.7% of the preserve. We found that 50% of *Diadophis punctatus* and 66.7% of *Coluber constrictor foxii* individuals were found within the road buffer area. This suggests that these species are possibly occupying areas with roads at a higher rate than expected.

***Dispersion Analysis***

We found with our dispersion analysis that 17 species had significant results. Six species, five from Anura and one from Testudines (*Anaxyrus americanus*, *Lithobates catesbeianus*, *Lithobates clamitans*, *Lithobates sylvaticus, Pseudacris triseriata* and *Chrysemys picta*,) had a clustered distribution ($p < 0.01$) and 11 species, three from Anura, one from Urodela, three from Squamata, and four from Testudines (*Hyla versicolor*, *Lithobates pipiens*, *Pseudacris crucifer, Plethodon cinereus*, *Coluber constrictor foxii*, *Diadophis punctatus, Heterodon platirhinos*, *Apalone spinifera*, *Chelydra serpentina*, *Emydoidea blandingii* and *Graptemys geographica*) had a dispersed distribution ($p < 0.01$). Three species, one from Anura, one from Urodela, and one from Testudines (*Anaxyrus fowleri* ($p = 0.627115$)*, Ambystoma laterale* complex ($p = 0.923048$) and *Terrapene c. carolina* ($p = 0.818028$) did not have significant results and appear to have a random distribution and one species from Squamata (*Nerodia sipedon*) was not tested because only one individual was detected.

***Time And Monthly Data***

Species abundances differed between sampling time periods, Table 3.3. We normalized the data to account for variance in sampling effort and found that the afternoon had the greatest number of individuals detected per day followed by evening and morning. There was a significant difference in the number of individuals detected per day for the morning and

afternoon (t = -2.55, d.f. = 66, P = 0.01). There were no significant differences between the number of individuals detected per day for morning and afternoon (t = -0.75, d.f. = 35, P = 0.63) and afternoon and evening (t = 0.49, d.f. = 35, P = 0.63). We found that the evening had the greatest number of species detected per day, followed by morning and then afternoon. For morning, we found that the most dominant species per order, Anura, Urodela and Testudines, respectively, were *Lithobates sylvaticus*, *Plethodon cinereus* and *Terrapene c. carolina*. For afternoon, we found that the most abundant species per order, Anura, Urodela, Squamata and Testudines, respectively, were *Lithobates sylvaticus*, *Plethodon cinereus*, *Heterodon platirhinos*, and *Chrysemys picta*. For evenings, we found that the most abundant species per order, Anura, Urodela, and Squamata, respectively, were *Lithobates clamitans*, *Plethodon cinereus*, *Diadophis punctatus* and *Nerodia sipedon* were equal.

We sampled late-April for two days, May was sampled for 24 days, June was sampled 26 days, July was sampled 29 days, August was sampled 23 days, and September was sampled 7 days. We normalized the data to account for variance in sampling effort, we found that April had the greatest number of individuals per day (n = 30), followed by August (n =15), June and July (n =13), September (n =10), and May (n = 7), see Table 3.4. We found that each month had different species abundances, see Figure 3.7 for amphibian species and Figure 3.8 for reptile species. We found that June had peak abundances for reptiles and Testudines and June and August had peak abundances for Squamata. We found that July had peak abundances for amphibians and August had peak abundances for Anura and Urodela. When we normalized the data, we found that April and September had the greatest number of species per day sampled (n = 2), followed by May, June, July, and August (n = 1).

When we examined both time and month, we found that the afternoon had the greatest number of individuals detected for all months except June, for which the evening had the greatest number of individuals detected, Figure 3.9. Anura had peak abundances in the afternoon for April, May, July, August and September and peak abundance in the evening for June. Urodela had peak abundances in the afternoon for May, June, July, August and September. Squamata had peak abundances in the afternoon for April, May, June, July (also for the evening), and August. Testudines had peak abundances in the afternoon for May, June, July, and September and had peak abundances in the morning for August.

***Temperature And Humidity***

We found that average air temperature (C°) associated with each individual detected ranged from 25.6 C° to 27.6 C° and Squamata had the highest value and Testudines had the lowest value. We found that average air humidity (%) associated with each individual detected ranged from 14.1% to 17.4% and Anura had the highest value and Squamata had the lowest value. We found that average surface temperature (C°) associated with each individual detected ranged from 25.7 C° to 27.6 C° and Squamata had the highest value while Testudines had the lowest value. We found that average surface humidity (%) associated with each individual detected ranged from 15.9% to 18.2% and Anura had the highest value and Urodela had the lowest value, refer to Table 3.5 for all temperature and humidity analyses for average, minimum, maximum and median values for herpetofauna, amphibians, Anura, Urodela, reptiles, Squamata and Testudines.

***Habitat Occupancy***

Using Schetter and Root's (2011) land cover map, we were able to estimate the number of individuals found within each land cover type: conifer, Eurasian meadow, floodplain forest,

pond, prairie, residential, savanna, shrub, swamp forest, turf, and upland forest. We found that the floodplain forests (n = 309) had the greatest number of individuals detected followed by upland forests (n = 286) and conifers (n = 276). While pond (n = 1), turf (n = 3) and savanna (n = 4) had the least number of individuals detected. Floodplain forests (n =17) also had the greatest number of species present followed by swamp forest (n =16) and upland forest (n = 13). Pond (n = 1) and turf (n = 2) had the least number of species present. There were two species (*Nerodia sipedon* and *Lithobates pipiens*) that were found only in one land cover, respectively, (floodplain forests and swamp forest). This was probably a result of low sample size of the species (n = 1 and n = 2). The percent abundance for each species detected within each land cover can be seen in Table 3.6.

Swamp forests had the greatest number of individuals detected within it than any of the other land covers for six species (*Hyla versicolor*, *Pseudacris triseriata*, *Lithobates pipiens*, *Chelydra serpentina*, *Graptemys geographica* and *Chrysemys picta*). Conifers had the greatest number of individuals detected within it than any of the other land covers for four species (*Pseudacris crucifer*, *Lithobates sylvaticus*, *Ambystoma laterale* complex, and *Diadophis punctatus*). Upland forests had the greatest number of individuals detected within it than any of the other land covers for six species (*Anaxyrus fowleri*, *Plethodon cinereus*, *Coluber constrictor foxii*, *Emydoidea blandingii*, *Terrapene c. carolina* and *Apalone spinifera*). Floodplain forests had the greatest number of individuals detected within it than any of the other land covers for nine species (*Anaxyrus americanus*, *Anaxyrus fowleri*, *Lithobates catesbeianus*, *Lithobates clamitans*, *Heterodon platirhinos*, *Coluber constrictor foxii*, *Nerodia sipedon*, *Chelydra serpentina* and *Apalone spinifera*). Eurasian meadows had the greatest number of individuals detected within it than any of the other land covers for one species (*Coluber constrictor foxii*).

Residential had the greatest number of individuals detected within it than any of the other land covers for one species (*Diadophis punctatus*).

However upland forests, floodplain forests and Eurasian meadows were tied for the greatest number of individuals for one species (*Coluber constrictor foxii*). Upland forest and floodplain forests were tied for the greatest number of individuals for two species (*Anaxyrus fowleri* and *Apalone spinifera*). Swamp forests and floodplain forests were tied for the greatest number of individuals for one species (*Chelydra serpentina*). Conifers and residential were tied for the greatest number of individuals for one species (*Nerodia sipedon*).

We calculated the proportion of land cover type occupied by each species and compared it to the proportion of land cover type sampled to determine a relative estimate of habitat occupancy for each species. We found that *Plethodon cinereus*, *Heterodon platirhinos*, *Diadophis punctatus*, *Anaxyrus americanus*, *Ambystoma laterale* complex, *Terrapene c. carolina*, *Anaxyrus fowleri*, *Pseudacris crucifer* and *Lithobates sylvaticus* were found in conifers in higher proportions than expected. *Heterodon platirhinos*, *Chelydra serpentina*, *Lithobates pipiens*, *Graptemys geographica*, *Anaxyrus americanus*, *Emydoidea blandingii*, *Hyla versicolor*, *Chrysemys picta* and *Pseudacris triseriata* were found in swamp forest in higher proportions than expected. We found that *Nerodia sipedon*, *Apalone spinifera*, *Chelydra serpentina*, *Anaxyrus americanus*, *Coluber constrictor foxii*, *Heterodon platirhinos*, *Lithobates clamitans* and *Lithobates catesbeianus* were found in floodplain forests in higher proportions than expected. *Plethodon cinereus*, *Apalone spinifera*, *Coluber constrictor foxii*, *Ambystoma laterale* complex, *Emydoidea blandingii*, *Terrapene c. carolina*, *Anaxyrus fowleri*, *Lithobates sylvaticus and Pseudacris triseriata* were found in upland forests in higher proportions than expected. We found that *Plethodon cinereus*, *Graptemys geographica*, *Diadophis punctatus*, *Anaxyrus*

*americanus*, *Anaxyrus fowleri*, *Chrysemys* picta, *Pseudacris crucifer*, *Lithobates sylvaticus* and *Lithobates catesbeianus* were found in residential areas in higher proportions than expected. *Coluber constrictor foxii*, *Terrapene c. carolina*, *Pseudacris crucifer* and *Lithobates catesbeianus* were found in Eurasian meadows in higher proportions than expected. We found that *Lithobates clamitans* and *Lithobates catesbeianus* were found in shrubs in higher proportions than expected. *Terrapene c. carolina*, *Pseudacris crucifer* and *Lithobates catesbeianus* were found in savannas in higher proportions than expected. We found that *Terrapene c. carolina*, *Lithobates clamitans* and *Pseudacris triseriata* were found in prairies in higher proportions than expected. *Pseudacris crucifer* and *Lithobates sylvaticus* were found in turf areas in higher proportions than expected. We found that *Lithobates clamitans* was found in ponds in a higher proportion than expected. The percentage of total area within each land cover can be seen in Table 3.7.

***Principal Components Analysis***

We computed principal components analysis for each species detected within quadrats and examined their relationship with 12 habitat variables, e.g. leaf litter, coarse woody debris etc. that were recorded during the vegetation surveys within the quadrats. For herpetofauna, principal component 1 explains 10.2% and is positively associated with moist soil, while principal component 2 explains 9.47% and is positively associated with conifer needles and negatively associated with leaf litter, Figure 3.10.

We also examined these relationships for individual species. For *Anaxyrus americanus*, principal component 1 explains 14.1% and is negatively associated with coarse woody debris and conifer needles, while principal component 2 explains 14% and is positively associated with plants and negatively associated with leaf litter, Figure 3.11. For *Anaxyrus fowleri*, principal

component 1 explains 14.4% and is positively associated with leaf litter and negatively associated with coarse woody debris and conifer needles, while principal component 2 explains 13.5% and is positively associated with plants and negatively associated with leaf litter, 3.12. For *Hyla versicolor*, principal component 1 explains 14.8% and is positively associated with moist soil, while principal component 2 explains 14.2% and is positively associated with conifer needles and negatively associated with leaf litter, Figure 3.13. For *Lithobates sylvaticus*, principal component 1 explains 14.5% and is positively associated with leaf litter and negatively associated with coarse woody debris and conifer needles, while principal component 2 explains 13.5% and is positively associated with plants and negatively associated with leaf litter, Figure 3.14. For *Pseudacris crucifer*, principal component 1 explains 14.3% and is positively associated with leaf litter and negatively associated with coarse woody debris and conifer needles, while principal component 2 explains 13.5% and is positively associated with plants and negatively associated with leaf litter, Figure 3.15 For *Pseudacris triseriata*, principal component 1 explains 14.3% and is positively associated with leaf litter and negatively associated with coarse woody debris and conifer needles, while principal component 2 explains 13.5% and is positively associated with plants and negatively associated with leaf litter, Figure 3.16. For *Ambystoma laterale* complex, principal component 1 explains 14.8% and is positively associated with leaf litter and negatively associated with coarse woody debris and conifer needles, while principal component 2 explains 13.5% and is positively associated with plants and negatively associated with leaf litter, Figure 3.17. For *Plethodon cinereus*, Principal component 1 explains 14.7% and is positively associated with plants, while principal component 2 explains 14.3% and is positively associated with leaf litter and negatively associated with coarse woody debris and conifer needles, Figure 3.18. For *Diadophis punctatus*, Principal component 1 explains 14.8%

and is positively associated with leaf litter and negatively associated with coarse woody debris and conifer needles, while principal component 2 explains 13.5% and is positively associated with plants and negatively associated with leaf litter, Figure 3.19.

***Logistic Regression Analysis***

Using logistic regression analysis, we examined which habitat variables were significantly associated with each species, an "*" indicates significance below 0.05 and "**" indicates significance below 0.0001. *Anaxyrus americanus* presence was positively associated with log average**, log minimum*, log median**, tree maximum* and moist soil minimum*. *Pseudacris crucifer* presence was positively associated with log average$^{**}$, log maximum$^{*}$, log median$^{**}$ and was negatively associated with wet leaf litter average* and wet lead litter maximum$^{*}$. *Pseudacris triseriata* presence was positively associated with coarse woody debris average$^{*}$ and was negatively associated with plant minimum$^{*}$and grass maximum$^{*}$. *Ambystoma laterale* complex presence was positively associated with coarse woody debris average$^{*}$ and was negatively associated with grass average$^{*}$. *Diadophis punctatus* presence was positively associated with conifer needles average$^{*}$ and conifer needles median$^{*}$ and was negatively associated with leaf litter average$^{*}$ and leaf litter maximum$^{*}$. *Lithobates sylvaticus* presence was positively associated with log maximum$^{*}$ and was negatively associated with dry soil average$^{**}$, dry soil maximum$^{**}$ and dry soil median$^{*}$. *Plethodon cinereus* presence was positively associated with leaf litter average$^{**}$, leaf litter minimum$^{**}$ and leaf litter median$^{**}$ and was negatively associated with plant average$^{*}$, plant maximum$^{**}$, plant median$^{*}$, moist soil average$^{**}$, moist soil maximum$^{**}$, grass minimum$^{*}$, grass maximum$^{*}$, dry soil maximum$^{**}$ and wet leaf litter median$^{*}$. *Hyla versicolor* presence was positively associated with moist soil average$^{**}$, moist soil maximum$^{**}$, moist soil median$^{*}$ and grass maximum$^{*}$ and was negatively associated with leaf

litter minimum* and coarse woody debris median*. *Anaxyrus fowleri* presence was negatively associated with dry soil average*.

We ran a logistic regression for month, date and time for each species. Only one species presence was associated with the month; P*lethodon cinereus* presence was negatively associated with month. Only one species presence was associated with the date; *Anaxyrus americanus* presence was negatively associated with date. Time was not associated with species presence.

Multivariate And Forward Stepwise Logistic Regression Model

Using the Spearman Rank Correlation, we found that the average, minimum, maximum and median values per habitat variable tended to be highly correlated (> 0.70). For an example: the average value of plants, minimum value of plants, maximum value of plants and median value of plants were highly correlated (all > 0.5188). The average values for each of the 12 habitat values were not highly correlated (< 0.70) and it was the same for minimum, maximum and median values, see Table 3.8.

We used the most significant and lowest $AIC_c$ value to identify the best model for each species, amphibians, Anura, and Urodela detected within quadrats. We found that the models included the habitat factors: proportion of logs, dry soil, water, moist soil, plants, trees, sand, coarse woody debris, wet leaf litter, conifer needles, leaf litter, and grass. Some of the best models used average (n = 4), minimum (n = 2), maximum (n = 5), and median (n = 1), see Table 3.9 for each individual species model. We did not run models using every available habitat factor (mix average, minimum, maximum, and median values) because they were highly correlated with each other.

*Discussion*

In order to understand the spatial patterns and distribution of herpetofauna within the Oak Openings Preserve, we analyzed how several environmental features influenced species presence and absence. From a landscape-level, we used a grid to identify which areas had the greatest number of individuals and species and found a core area (33, 39, and 40). These results suggest that this core area has important habitat features that allow multiple species to thrive. Within grid cell 40 is Mallard Lake, which may have increased the number of individuals and species within the area. Water is an important feature for herpetofauna, especially amphibians, which use water sources to decrease desiccation risks and it provides important breeding habitat (Crawford & Semlitsch 2007). The habitat on the edges of lakes generally increases the number of species found because of its additional structure. Increased habitat complexity provides refuges from predators, basking sites, and provides habitat for prey. We found that Squamata had the greatest percentage of individuals using areas near streams (27%); Testudines had 25%, Anura had 10.6% and Urodela had 3.4% of the individuals in areas near streams. Even though Squamata had the greatest percentage of individuals found near streams, there was a small sample size and we cannot conclude that Squamata were using areas near streams the most overall. We expected that more Testudines would be found near streams because all but one species is aquatic. It is possible that we had small number near streams because most of the aquatic Testudines we found were located in a pond that was not near any streams. We also expected that Anura would have a larger number of individuals in areas with streams because the terrestrial habitat adjacent to streams has been shown to be important for amphibians for foraging and reproduction, and decreased desiccation risk (Crawford & Semlitsch 2007). Urodela had the smallest number of individuals in areas near streams and this was expected because *Plethodon cinereus* is terrestrial for both larval and adult life stages. *Ambystoma laterale* complex has an aquatic larval life stage,

but we only detected the terrestrial adults. It is possible that we missed many individuals using terrestrial habitat adjacent to streams because we did not heavily sample those areas or our sample timing was not appropriate. Amphibians use aquatic habitats for breeding purposes in early spring and our surveys began after the breeding season began, which suggests why habitats near streams had fewer individuals than expected. Future studies could use transect lines on both sides of streams or rivers and use dip netting to detect aquatic larva during early spring to survey for herpetofauna using terrestrial habitats near streams. We found that only a small percentage of herpetofauna were found in areas near roads and this was expected. Roads are not natural habitats for herpetofauna and it is very likely that the individuals found near roads were using roadside ditches or crossing roads (Gooch et al. 2006).

We found different spatial patterns for each species; see Appendix A7 to A27; however we found varying degrees of species overlap, demonstrating variation in ecological niches. Some habitat factors were more important than others for each species. We found that *Lithobates sylvaticus* and *Pseudacris triseriata* individuals overlapped greatly with one another. Most of the *Pseudacris triseriata* individuals were always near *Lithobates sylvaticus* individuals, which suggest that *Lithobates sylvaticus* can be found when searching for *Pseudacris triseriata*. Two species within the same genus does not necessarily mean they will be found in similar areas. For example, *Anaxyrus americanus* and *Anaxyrus fowleri* had very little species overlap; this suggests that even though the two species are closely related, they are occupying different ecological niches. We would like to note that when examining our species maps, it may appear that multiple individuals overlapped as if they are on top of one another, see Figure 3.20 A. However, when the maps are examined at a smaller extent, see Figure 3.20 B, we see that the individuals are not sitting on top of one another and they are several meters away from one

another. Figure 3.20 shows three *Coluber constrictor foxii* locations; the far left point is 15.68 m away from the middle point and the middle point is 60.33 m away from the far right point. These individuals may be using the same general area; however, they may not even detect the other two individuals.

We analyzed the spatial arrangement for each species and found that six species had a clustered distribution, three species had no discernable spatial pattern, and 11 species had a dispersed distribution. Species with clusters are more likely to be using certain areas more so than the entire preserve, which suggests that managers could manage the habitat within the specified areas with high species density. For the three species with a random distribution, it may not matter as much which habitats they are occupying. Eleven species had a dispersed distribution which suggests that they are using the entire park equally. Since most of the herpetofauna species are dispersed throughout the park, management done within the park will most likely impact multiple species. In order to decrease impact intensity, managers can use our species maps to identify which species will most likely be impacted within the specified area and identify the costs and benefits of the management plan.

We examined herpetofauna distributions over several months and our survey times varied throughout the day. We found that the afternoon had the greatest number of individuals and species detected. This is possibly a result of encountering individuals seeking shelter from the afternoon heat or that herpetofauna are active at peak temperatures. We found that April had the greatest number of individuals detected, which is likely a result of the breeding season for multiple herpetofauna species, followed by August which could have resulted from an increase in observer experience, i.e., the more surveys completed, the greater detection ability by the observers. We found that Squamata were detected at the highest and Testudines at the lowest air

and surface temperature, while Anura were detected at the highest air and surface humidity and Squamata at the lowest air humidity and Urodela at the lowest surface humidity.

We found that forested areas: floodplain, upland, swamp and conifer forests had the greatest number of individuals and species. This is most likely occurred because herpetofauna can take shelter and maintain their body temperature using leaf litter, logs, and dense vegetation in forested areas for protection from avian predators (Hu et al. 2013). Open areas or prairies had the least number of individuals and species most likely as a result of being vulnerable to predators, especially birds. When we examined specific habitat variables associated with species presence, we found that across species, the same habitat variables tended to be associated with each. For example, the proportion of trees, wet leaf litter and moist soil were all positively associated, with varying lengths for every species examined. However some species differed in some habitat variable direction. For example, conifer needles were positively associated and leaf litter was negatively associated for *Hyla versicolor* and conifer needles were negatively associated and positively associated for *Plethodon cinereus*. We found that leaf litter, coarse woody debris, moist soil, plants and conifer needles were important habitat variables. Besides moist soil, which could be used to decrease desiccation risk, the other four variables can help provide shelter and protection from predators.

We created models for each species based on habitat variables and our top three models were for *Diadophis punctatus*: conifer needles (median), *Hyla versicolor*: moist soil (average), and *Pseudacris triseriata*: leaf litter, coarse woody debris, plants, dry soil and conifer needles (minimum). Our model results are supported by the principal components analysis for which the same important habitat variables were shown again with the additional variable of dry soil. Amphibians have been shown to occupy habitats with leaf litter and coarse woody debris for

shelter and foraging opportunities (Semlitsch & Bodie 2003). Especially the moisture in ground cover is important to help amphibians stay hydrated (Folt & Reider 2013).

Environmental factors play an important role for where individuals are located within their environment; however, these are not the only factors influencing species distributions. We found that the environment based on the factors we measured only explained 10% of the variation, but this is to be expected because species are influenced differently by environmental factors and behavior plays an important role in determining where individuals are found within their environment. This includes behaviors such as territory defense, foraging, predation, competition and geographic barriers. Individuals may not be found in some areas because that area is another individual's territory. Foraging for food is a driving factor for where species occur because finding food or resources is essential for survival. For an example, sea turtle conservation spends a lot of time and energy in protecting reproductive sites; however these turtles need protection for all of their life stages. Satellite tracking has allowed researchers to identify important nesting and foraging sites and understand migration routes, home ranges, and habitat use (Casale, Affronte, & Scaravelli 2012). The sea turtle's occurrence is influenced by where their food is located. Individuals will also have to avoid predators and may avoid specific areas for which their predator inhabits, which can deny them access to important resources. We were unable to detect predator avoidance within our data but future studies could examine the distributions of other taxa such as mammals and birds and overlay their distribution patterns with herpetofauna to see if any species overlap.

Not only are individuals interacting with their predators, but they are competing with other and their own species. Competition drives where individuals occur and can create ecological niches within different scales. Competition and predation spatially segregates three

*Desmognathus* salamander species in their terrestrial habitat in the southern Appalachian Mountains (Crawford & Semlitsch 2007). Amphibians are vulnerable to changes in body sizes associated with differences in foraging, this can impact inter- and intraspecific behavioral interactions such as competition, territory defense and predation which can drive indirect changes in community composition (Caruso et al. 2014).

Finally, geographical barriers may prevent individuals from accessing certain areas, even if that area is highly suitable. Physical barriers such as streams, roads, rivers, etc. can impede a species movement within the landscape. The Oak Openings Preserve has a large continuous river going throughout the park and we found that small groups of individuals were separated by the river. This is probably acting as a physical barrier for many amphibians and Squamata; however it could help aquatic Testudines dispersal. Roads are also found throughout the Oak Openings Preserve and can act as a barrier for herpetofauna dispersal. We did not see large gaps within our data when looking at roads, but road mortality is a large threat for herpetofauna. Ashley & Robinson (1996) examined road mortality for vertebrates and found that amphibians accounted for 92.1% of the total road mortality while reptiles had 2.7%, birds had 4.3% and mammals had 0.9%. Although we did not find any mortality from roads, it would be advantageous for future studies to examine herpetofauna road mortality to identify danger zones.

Future studies should also consider disturbances from management activities. Prescribed fires are used as a management tool in the Oak Openings Preserve and where these fires occur can impact distributions and spatial patterns by altering habitat structure and environmental conditions (Hu et al. 2013). Langford et al. (2007) found that herpetofauna abundances were greater in burned sites than unburned sites, although it may have been a result of fire adapted

species. It would be beneficial to work with managers and perform pre- and post-burn surveys to examine how prescribed fires affect herpetofauna distributions and abundances.

Future studies should also consider examining vernal pools with more detail. Our land cover map did not include vernal pools and we were unable to show correlations between amphibians and water use. We also probably did not sample enough habitats equally and it would be beneficial to include more areas with less canopy cover to check if herpetofauna are really occupying a greater number of areas with canopy cover than those with little to no canopy cover.

In conclusion, examining species abundance and diversity is important, but understanding why the species are distributed in certain spatial patterns is critical for managing habitats that are suitable for herpetofauna. We found that multiple species are dispersed throughout the Oak Openings Preserve and forested areas were important. We suggest that managers use the individual species maps to identify which species will be impacted before implementing structural changes. We found that leaf litter, conifer needles, coarse woody debris, moist soil and plants are important habitat variables and each species responds slightly differently to changes in these environmental features. This suggests that removing ground cover (e.g. leaf litter, logs, coarse woody debris) is unfavorable for herpetofauna and areas with these traits should be left intact. Identifying these important factors that influence species presence-absence can help managers create more suitable habitat for herpetofauna or modify habitats for specific species. Identifying and managing terrestrial habitat for herpetofauna is essential for maintaining existing populations and their current ecosystem functions (Crawford & Semlitsch 2007). We only detected a small number of reptiles in the Oak Openings Preserve (Chapter 1) and if managers

could restore habitats to make them more suitable for reptiles, they may be able to increase reptile abundances.

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*Tables*

Table 3.1: The number of individuals, species, quadrats and density (number of individuals detected per $m^2$) detected per 800 m by 800 m grid cell. Grid cells with at least one quadrat surveyed were reported.

| Grid Cell | Individuals | Species | Quadrats | Density |
|---|---|---|---|---|
| 2 | 0.00 | 0.00 | 1.00 | 0.000 |
| 3 | 14.0 | 3.00 | 4.00 | 0.009 |
| 4 | 0.00 | 0.00 | 1.00 | 0.000 |
| 9 | 5.00 | 2.00 | 4.00 | 0.003 |
| 10 | 74.0 | 5.00 | 14.0 | 0.013 |
| 11 | 98.0 | 7.00 | 14.0 | 0.018 |
| 12 | 35.0 | 6.00 | 4.00 | 0.022 |
| 16 | 5.00 | 3.00 | 4.00 | 0.003 |
| 17 | 62.0 | 6.00 | 18.0 | 0.009 |
| 18 | 25.0 | 3.00 | 7.00 | 0.009 |
| 19 | 62.0 | 7.00 | 8.00 | 0.019 |
| 23 | 0.00 | 0.00 | 1.00 | 0.000 |
| 24 | 85.0 | 7.00 | 16.0 | 0.013 |
| 25 | 71.0 | 11.0 | 4.00 | 0.044 |
| 26 | 97.0 | 8.00 | 7.00 | 0.035 |
| 27 | 93.0 | 12.0 | 2.00 | 0.116 |
| 30 | 9.00 | 4.00 | 5.00 | 0.005 |
| 31 | 37.0 | 5.00 | 15.0 | 0.006 |
| 32 | 7.00 | 3.00 | 1.00 | 0.018 |
| 33 | 285 | 10.0 | 10.0 | 0.071 |
| 34 | 17.0 | 5.00 | 4.00 | 0.011 |
| 38 | 1.00 | 1.00 | 1.00 | 0.003 |
| 39 | 132 | 9.00 | 17.0 | 0.019 |
| 40 | 57.0 | 11.0 | 5.00 | 0.029 |
| 41 | 19.0 | 7.00 | 9.00 | 0.005 |
| 47 | 11.0 | 4.00 | 1.00 | 0.028 |
| 48 | 27.0 | 7.00 | 10.0 | 0.007 |
| 49 | 3.00 | 3.00 | 1.00 | 0.008 |
| 55 | 3.00 | 1.00 | 1.00 | 0.008 |

Table 3.2: Each species percent abundance (%) that were detected within 50 m of a stream and each species abundance that were detected within 50 m of a road.

| Species | Streams (%) | Roads (%) |
|---|---|---|
| *Anaxyrus americanus* | 11.1 | 7.90 |
| *Anaxyrus fowleri* | 20.8 | 12.5 |
| *Hyla versicolor* | 20.0 | 0.00 |
| *Pseudacris crucifer* | 6.60 | 6.60 |
| *Pseudacris triseriata* | 0.00 | 2.50 |
| *Lithobates catesbeianus* | 48.9 | 8.50 |
| *Lithobates clamitans* | 8.30 | 2.80 |
| *Lithobates pipiens* | 0.00 | 0.00 |
| *Lithobates sylvaticus* | 5.90 | 13.5 |
| *Plethodon cinereus* | 3.50 | 3.80 |
| *Ambystoma laterale* complex | 0.00 | 0.00 |
| *Diadophis punctatus* | 0.00 | 50.0 |
| *Heterodon platirhinos* | 25.0 | 25.0 |
| *Coluber constrictor foxii* | 0.00 | 66.7 |
| *Nerodia sipedon* | 100 | 0.00 |
| *Chelydra serpentina* | 50.0 | 0.00 |
| *Emydoidea blandingii* | 0.00 | 0.00 |
| *Terrapene c. carolina* | 0.00 | 9.10 |
| *Graptemys geographica* | 33.3 | 0.00 |
| *Chrysemys picta* | 26.3 | 0.00 |
| *Apalone spinifera* | 50.0 | 0.00 |
| Anura | 11.8 | 14.4 |
| Squamata | 100 | 0.00 |
| Testudines | 50.0 | 37.5 |

Table 3.3: Each species abundance for individuals detected within three time classes: morning (8:00 am to 11:59 am), afternoon (12:00 pm to 5:59 pm), and evening (6:00 pm to 10:00 pm).

| Species | Morning | Afternoon | Evening |
|---|---|---|---|
| Anura | 8.00 | 133 | 13.0 |
| *Anaxyrus americanus* | 6.00 | 109 | 10.0 |
| *Anaxyrus fowleri* | 4.00 | 17.0 | 3.00 |
| *Hyla versicolor* | 0.00 | 10.0 | 0.00 |
| *Pseudacris crucifer* | 13.0 | 121 | 3.00 |
| *Pseudacris triseriata* | 8.00 | 26.0 | 6.00 |
| *Lithobates catesbeianus* | 11.0 | 24.0 | 12.0 |
| *Lithobates clamitans* | 10.0 | 31.0 | 103 |
| *Lithobates pipiens* | 0.00 | 2.00 | 0.00 |
| *Lithobates sylvaticus* | 33.0 | 159 | 31.0 |
| Urodela | 0.00 | 0.00 | 0.00 |
| *Plethodon cinereus* | 43.0 | 295 | 31.0 |
| *Ambystoma laterale* complex | 0.00 | 12.0 | 0.00 |
| Squamata | 0.00 | 1.00 | 0.00 |
| *Diadophis punctatus* | 0.00 | 1.00 | 1.00 |
| *Heterodon platirhinos* | 0.00 | 4.00 | 0.00 |
| *Coluber constrictor foxii* | 0.00 | 3.00 | 0.00 |
| *Nerodia sipedon* | 0.00 | 0.00 | 1.00 |
| Testudines | 3.00 | 4.00 | 1.00 |
| *Chelydra serpentina* | 0.00 | 2.00 | 0.00 |
| *Emydoidea blandingii* | 0.00 | 3.00 | 0.00 |
| *Terrapene c. carolina* | 7.00 | 4.00 | 0.00 |
| *Graptemys geographica* | 0.00 | 3.00 | 0.00 |
| *Chrysemys picta* | 0.00 | 19.0 | 0.00 |
| *Apalone spinifera* | 0.00 | 2.00 | 0.00 |

Table 3.4: The number of individuals detected per day sampled for each month.

| | April | May | June | July | August | September |
|---|---|---|---|---|---|---|
| Anura | 29 | 2.0 | 1.0 | 0.0 | 1.0 | 1.0 |
| *Anaxyrus americanus* | 0.0 | 0.0 | 1.0 | 2.0 | 1.0 | 1.0 |
| *Anaxyrus fowleri* | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Hyla versicolor* | 1.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Pseudacris crucifer* | 1.0 | 0.0 | 1.0 | 1.0 | 4.0 | 1.0 |
| *Pseudacris triseriata* | 0.0 | 0.0 | 0.0 | 1.0 | 1.0 | 0.0 |
| *Lithobates catesbeianus* | 0.0 | 0.0 | 0.0 | 1.0 | 0.0 | 1.0 |
| *Lithobates clamitans* | 0.0 | 0.0 | 4.0 | 0.0 | 1.0 | 0.0 |
| *Lithobates pipiens* | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Lithobates sylvaticus* | 0.0 | 0.0 | 1.0 | 4.0 | 3.0 | 1.0 |
| Urodela | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Ambystoma laterale* complex | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Plethodon cinereus* | 0.0 | 3.0 | 4.0 | 4.0 | 2.0 | 3.0 |
| Squamata | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Coluber constrictor foxii* | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Diadophis punctatus* | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Heterodon platirhinos* | 1.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Nerodia sipedon* | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Testudines | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Apalone spinifera* | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Chelydra serpentina* | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Chrysemys picta* | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 1.0 |
| *Emydoidea blandingii* | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Graptemys geographica* | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Terrapene c. carolina* | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

Table 3.5: Each detected individual was analyzed with four environmental variables: air temperature (C°), surface temperature (C°), air humidity (%) and surface humidity (%), which was associated with its detection. Abbreviations for environmental factors are: air temperature (air temp), surface temperature (surf temp), air humidity (air hum) and surface humidity (surf hum).

| Order | Variable | Mean | Minimum | Maximum | Median |
|---|---|---|---|---|---|
| Herpetofauna | Air temp (C°) | 26.7 | 8.80 | 34.9 | 26.8 |
| | Surf temp (C°) | 26.7 | 12.4 | 34.7 | 26.9 |
| | Air hum (%) | 16.7 | -5.80 | 31.8 | 16.8 |
| | Surf hum (%) | 17.6 | -5.30 | 31.8 | 18.2 |
| Amphibians | Air temp (C°) | 26.7 | 8.80 | 34.9 | 26.8 |
| | Surf temp (C°) | 26.7 | 12.4 | 34.7 | 26.9 |
| | Air hum (%) | 16.7 | -5.80 | 31.8 | 16.8 |
| | Surf hum (%) | 17.6 | -5.30 | 31.8 | 18.1 |
| Reptiles | Air temp (C°) | 26.0 | 17.4 | 33.6 | 25.6 |
| | Surf temp (C°) | 26.1 | 17.9 | 33.6 | 25.6 |
| | Air hum (%) | 15.8 | -4.70 | 27.6 | 16.0 |
| | Surf hum (%) | 18.0 | -5.30 | 28.0 | 19.2 |
| Anura | Air temp (C°) | 27.0 | 8.80 | 34.9 | 27.2 |
| | Surf temp (C°) | 27.0 | 12.4 | 34.7 | 27.2 |
| | Air hum (%) | 17.4 | -5.80 | 31.8 | 18.2 |
| | Surf hum (%) | 18.2 | -5.30 | 31.8 | 18.9 |
| Urodela | Air temp (C°) | 26.2 | 15.4 | 33.0 | 26.0 |
| | Surf temp (C°) | 26.2 | 15.5 | 33.2 | 26.1 |
| | Air hum (%) | 15.2 | -1.60 | 29.8 | 14.9 |
| | Surf hum (%) | 15.9 | -1.60 | 29.8 | 15.5 |
| Squamata | Air temp (C°) | 27.6 | 23.2 | 33.0 | 26.7 |
| | Surf temp (C°) | 27.6 | 23.1 | 33.3 | 26.5 |
| | Air hum (%) | 14.1 | -4.70 | 24.2 | 14.6 |
| | Surf hum (%) | 16.9 | -5.30 | 28.0 | 18.9 |
| Testudines | Air temp (C°) | 25.6 | 17.4 | 33.6 | 25.6 |
| | Surf temp (C°) | 25.7 | 17.9 | 33.6 | 25.6 |
| | Air hum (%) | 16.2 | 0.70 | 27.6 | 16.5 |
| | Surf hum (%) | 18.2 | 3.00 | 27.8 | 19.2 |

Table 3.6: Each species percent abundance (%) detected within each land cover: swamp forest (A), conifer (B), upland forest (C), floodplain forest (D), prairie (E), savanna (F), turf (G), shrub (H), Eurasian meadow (I), pond (J), and residential (K).

| Species | A | B | C | D | E | F | G | H | I | J | K |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Anaxyrus americanus* | 27 | 21 | 17 | 30 | 0.0 | 0.0 | 0.0 | 1.0 | 0.0 | 0.0 | 5.0 |
| *Anaxyrus fowleri* | 11 | 21 | 30 | 30 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 8.0 |
| *Hyla versicolor* | 60 | 10 | 0.0 | 30 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Pseudacris crucifer* | 18 | 34 | 13 | 22 | 1.0 | 1.0 | 2.0 | 2.0 | 2.0 | 0.0 | 7.0 |
| *Pseudacris triseriata* | 58 | 5.0 | 28 | 8.0 | 3.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Lithobates catesbeianus* | 11 | 0.0 | 0.0 | 62 | 0.0 | 2.0 | 0.0 | 15 | 2.0 | 0.0 | 9.0 |
| *Lithobates clamitans* | 8.0 | 1.0 | 8.0 | 79 | 1.0 | 0.0 | 0.0 | 3.0 | 0.0 | 1.0 | 0.0 |
| *Lithobates pipiens* | 100 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Lithobates sylvaticus* | 21 | 27 | 26 | 19 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 0.0 | 5.0 |
| *Plethodon cinereus* | 12 | 35 | 40 | 8.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 5.0 |
| *Ambystoma laterale* complex | 8.0 | 42 | 33 | 17 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Diadophis punctatus* | 0.0 | 50 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 50 |
| *Heterodon platirhinos* | 25 | 25 | 0.0 | 50 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Coluber constrictor foxii* | 0.0 | 0.0 | 33 | 33 | 0.0 | 0.0 | 0.0 | 0.0 | 33 | 0.0 | 0.0 |
| *Nerodia sipedon* | 0.0 | 0.0 | 0.0 | 100 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Chelydra serpentina* | 50 | 0.0 | 0.0 | 50 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Emydoidea blandingii* | 33 | 0.0 | 67 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Terrapene c. carolina* | 0.0 | 18 | 36 | 9.0 | 18 | 9.0 | 0.0 | 0.0 | 9.0 | 0.0 | 0.0 |
| *Graptemys geographica* | 67 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 33 |
| *Chrysemys picta* | 58 | 0.0 | 16 | 11 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 16 |
| *Apalone spinifera* | 0.0 | 0.0 | 50 | 50 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

Table 3.7: The Oak Openings Preserve has 14 unique land covers and here is the total amount of area (%) for each land cover type (based on Schetter and Root 2011).

| Habitat | % of Area |
|---|---|
| Turf | 0.20 |
| Residential | 47.8 |
| Asphalt | 0.00 |
| Pond | 0.00 |
| Savanna | 0.20 |
| Shrub | 0.00 |
| Swamp forest | 0.70 |
| Conifer | 1.10 |
| Upland forest | 1.50 |
| Floodplain forest | 1.30 |
| Barrens | 0.10 |
| Eurasian meadows | 0.20 |
| Prairie | 0.20 |
| Cropland | 46.7 |

Table 3.8: Multi-correlation table for average, minimum, maximum and median habitat variables: leaf litter (LL), coarse woody debris (CDW), logs, plant (Pl), tree (Tr), moist soil (MS), grass (Gr), dry soil (DS), conifer needles (CN), water (Wa), sand (Sa) and wet leaf litter (WLL).

| Average | LL | CDW | Logs | Pl | Tr | MS | Gr | DS | CN | Wa | Sa | WLL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| LL | 1.0 | -0.2 | -0.1 | -0.3 | -0.1 | -0.2 | -0.1 | -0.3 | -0.7 | 0.0 | 0.0 | -0.2 |
| CWD | -0.2 | 1.0 | -0.1 | -0.1 | -0.1 | -0.1 | -0.2 | -0.1 | 0.3 | 0.0 | -0.1 | -0.1 |
| Logs | -0.1 | -0.1 | 1.0 | 0.0 | 0.0 | -0.1 | 0.0 | 0.0 | -0.1 | 0.0 | -0.1 | 0.0 |
| Plant | -0.3 | -0.1 | 0.0 | 1.0 | 0.0 | 0.2 | 0.3 | -0.1 | -0.1 | 0.0 | -0.1 | 0.1 |
| Tree | -0.1 | -0.1 | 0.0 | 0.0 | 1.0 | 0.0 | 0.0 | -0.1 | 0.0 | 0.4 | -0.1 | 0.1 |
| Mo so | -0.2 | -0.1 | -0.1 | 0.2 | 0.0 | 1.0 | 0.1 | 0.0 | -0.1 | 0.1 | 0.0 | 0.1 |
| Grass | -0.1 | -0.2 | 0.0 | 0.3 | 0.0 | 0.1 | 1.0 | 0.1 | -0.2 | 0.0 | 0.0 | 0.0 |
| Dry so | -0.3 | -0.1 | 0.0 | -0.1 | -0.1 | 0.0 | 0.1 | 1.0 | -0.2 | 0.0 | 0.1 | -0.1 |
| CN | -0.7 | 0.3 | -0.1 | -0.1 | 0.0 | -0.1 | -0.2 | -0.2 | 1.0 | -0.1 | -0.1 | -0.1 |
| Water | 0.0 | 0.0 | 0.0 | 0.0 | 0.4 | 0.1 | 0.0 | 0.0 | -0.1 | 1.0 | 0.0 | 0.3 |
| Sand | 0.0 | -0.1 | -0.1 | -0.1 | -0.1 | 0.0 | 0.0 | 0.1 | -0.1 | 0.0 | 1.0 | 0.0 |
| W LL | -0.2 | -0.1 | 0.0 | 0.1 | 0.1 | 0.1 | 0.0 | -0.1 | -0.1 | 0.3 | 0.0 | 1.0 |
| Minimum | LL | CDW | Logs | Pl | Tr | MS | Gr | DS | CN | Wa | Sa | WLL |
| LL | 1.0 | -0.1 | -0.1 | -0.1 | 0.0 | -0.1 | -0.1 | -0.1 | -0.4 | 0.1 | 0.1 | -0.1 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CWD | -0.1 | 1.0 | 0.0 | 0.1 | 0.0 | 0.0 | -0.1 | -0.1 | 0.3 | 0.0 | 0.0 | -0.1 |
| Logs | -0.1 | 0.0 | 1.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Plant | -0.1 | 0.1 | 0.0 | 1.0 | 0.0 | 0.2 | 0.2 | -0.1 | 0.0 | 0.0 | 0.0 | -0.1 |
| Tree | 0.0 | 0.0 | 0.0 | 0.0 | 1.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Mo so | -0.1 | 0.0 | 0.0 | 0.2 | 0.0 | 1.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Grass | -0.1 | -0.1 | 0.0 | 0.2 | 0.0 | 0.0 | 1.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Dry so | -0.1 | -0.1 | 0.0 | -0.1 | 0.0 | 0.0 | 0.0 | 1.0 | -0.1 | 0.0 | 0.0 | 0.0 |
| CN | -0.4 | 0.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | -0.1 | 1.0 | 0.0 | 0.0 | 0.0 |
| Water | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 1.0 | 1.0 | 0.0 |
| Sand | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 1.0 | 1.0 | 0.0 |
| Wet LL | -0.1 | -0.1 | 0.0 | -0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 1.0 |
| Maximum | LL | CDW | Logs | Pl | Tr | MS | Gr | DS | CN | Wa | Sa | WLL |
| LL | 1.0 | 0.0 | -0.1 | -0.3 | 0.0 | 0.0 | 0.0 | 0.0 | -0.6 | 0.1 | 0.1 | 0.0 |
| CWD | 0.0 | 1.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.1 | 0.1 | 0.0 | 0.2 | 0.1 | 0.0 |
| Logs | -0.1 | 0.0 | 1.0 | 0.1 | 0.0 | 0.0 | 0.0 | -0.1 | -0.2 | -0.1 | -0.1 | 0.0 |
| Plant | -0.3 | 0.1 | 0.1 | 1.0 | 0.0 | 0.3 | 0.4 | 0.1 | -0.1 | 0.0 | 0.1 | 0.1 |
| Tree | 0.0 | 0.0 | 0.0 | 0.0 | 1.0 | 0.1 | -0.1 | 0.0 | 0.0 | 0.2 | -0.1 | 0.1 |
| Mo so | 0.0 | 0.0 | 0.0 | 0.3 | 0.1 | 1.0 | 0.1 | 0.0 | -0.2 | 0.3 | 0.1 | 0.4 |
| Grass | 0.0 | 0.1 | 0.0 | 0.4 | -0.1 | 0.1 | 1.0 | 0.2 | -0.2 | 0.0 | 0.1 | 0.0 |
| Dry so | 0.0 | 0.1 | -0.1 | 0.1 | 0.0 | 0.0 | 0.2 | 1.0 | -0.3 | 0.0 | 0.2 | -0.1 |
| CN | -0.6 | 0.0 | -0.2 | -0.1 | 0.0 | -0.2 | -0.2 | -0.3 | 1.0 | -0.1 | 0.0 | -0.2 |
| Water | 0.1 | 0.2 | -0.1 | 0.0 | 0.2 | 0.3 | 0.0 | 0.0 | -0.1 | 1.0 | 0.0 | 0.4 |
| Sand | 0.1 | 0.1 | -0.1 | 0.1 | -0.1 | 0.1 | 0.1 | 0.2 | 0.0 | 0.0 | 1.0 | 0.1 |
| Wet LL | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.4 | 0.0 | -0.1 | -0.2 | 0.4 | 0.1 | 1.0 |
| Median | LL | CDW | Logs | Pl | Tr | MS | Gr | DS | CN | Wa | Sa | WLL |
| LL | 1.0 | -0.1 | -0.1 | -0.3 | -0.1 | -0.2 | -0.1 | -0.3 | -0.7 | 0.1 | 0.1 | -0.2 |
| CDW | -0.1 | 1.0 | -0.1 | -0.1 | -0.1 | -0.1 | -0.2 | -0.1 | 0.3 | 0.0 | 0.0 | -0.1 |
| Logs | -0.1 | -0.1 | 1.0 | 0.0 | 0.1 | 0.0 | -0.1 | 0.0 | -0.1 | 0.0 | 0.0 | 0.0 |
| Plant | -0.3 | -0.1 | 0.0 | 1.0 | 0.2 | 0.1 | 0.2 | -0.1 | -0.1 | 0.1 | 0.1 | 0.1 |
| Tree | -0.1 | -0.1 | 0.1 | 0.2 | 1.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Mo so | -0.2 | -0.1 | 0.0 | 0.1 | 0.0 | 1.0 | 0.0 | 0.0 | -0.1 | 0.0 | 0.0 | 0.0 |
| Grass | -0.1 | -0.2 | -0.1 | 0.2 | 0.0 | 0.0 | 1.0 | 0.0 | -0.2 | 0.0 | 0.0 | 0.0 |
| Dry so | -0.3 | -0.1 | 0.0 | -0.1 | 0.0 | 0.0 | 0.0 | 1.0 | -0.2 | 0.0 | 0.0 | 0.0 |
| CN | -0.7 | 0.3 | -0.1 | -0.1 | 0.0 | -0.1 | -0.2 | -0.2 | 1.0 | 0.0 | 0.0 | -0.1 |
| Water | 0.1 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 1.0 | 1.0 | 0.0 |
| Sand | 0.1 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 1.0 | 1.0 | 0.0 |
| Wet LL | -0.2 | -0.1 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | -0.1 | 0.0 | 0.0 | 1.0 |

Table 3.9: Model results, including degrees of freedom (DF), chi-square ($X^2$), significance (p-value), correlation ($R^2$ ) and Akaike's Information Criterion adjusted for small sample size (AICc) for each model that examined environmental factors affecting presence or absence for nine herpetofauna species in the Oak Openings Preserve. Abbreviations used for type of data values used are average (avg), minimum (min), maximum (max) and median (med) and for environmental factors are: plants (p), dry soil (ds), moist soil (ms), trees (tr), coarse woody debris (cwd), wet leaf litter (w ll), leaf litter (ll), conifer needles (cn), water (wa), sand (sa) and grass (g).

| Species | Model | DF | $X^2$ | P < 0.05 | $R^2$ | AICc |
|---|---|---|---|---|---|---|
| Amphibians | Max: logs, p, ds, wa | 4 | 19.4 | 0.0007 | 0.080 | 228 |
| Anura | Max: logs, ms | 2 | 17.7 | 0.0001 | 0.070 | 236 |
| *Anaxyrus americanus* | Avg: logs, p, tr, ms, wa, sa | 6 | 27.5 | 0.0001 | 0.190 | 131 |
| *Anaxyrus fowleri* | Max: cwd, logs, p, ds, w ll | 5 | 15.5 | 0.0083 | 0.400 | 35.6 |
| *Hyla versicolor* | Avg: ms | 1 | 7.10 | 0.0077 | 0.320 | 19.1 |
| *Pseudacris crucifer* | Avg: logs, w ll | 2 | 13.7 | 0.001 | 0.090 | 144 |
| *Pseudacris triseriata* | Min: ll, cwd, p, ds, cn | 5 | 16.4 | 0.0058 | 0.530 | 26.8 |
| *Lithobates sylvaticus* | Avg: cwd, logs, ds | 3 | 12.9 | 0.0049 | 0.080 | 157 |
| Urodela | Max: p, ms, ds | 3 | 26.1 | <0.0001 | 0.100 | 243 |
| *Ambystoma laterale* complex | Max: cwd, ms, ds, cn | 4 | 12.2 | 0.0161 | 0.210 | 36.9 |
| *Plethodon cinereus* | Min: ll, g, cn | 3 | 27.4 | <0.0001 | 0.110 | 241 |
| *Diadophis punctatus* | Med: cn | 1 | 4.90 | 0.0268 | 0.390 | 11.6 |

*Figures*

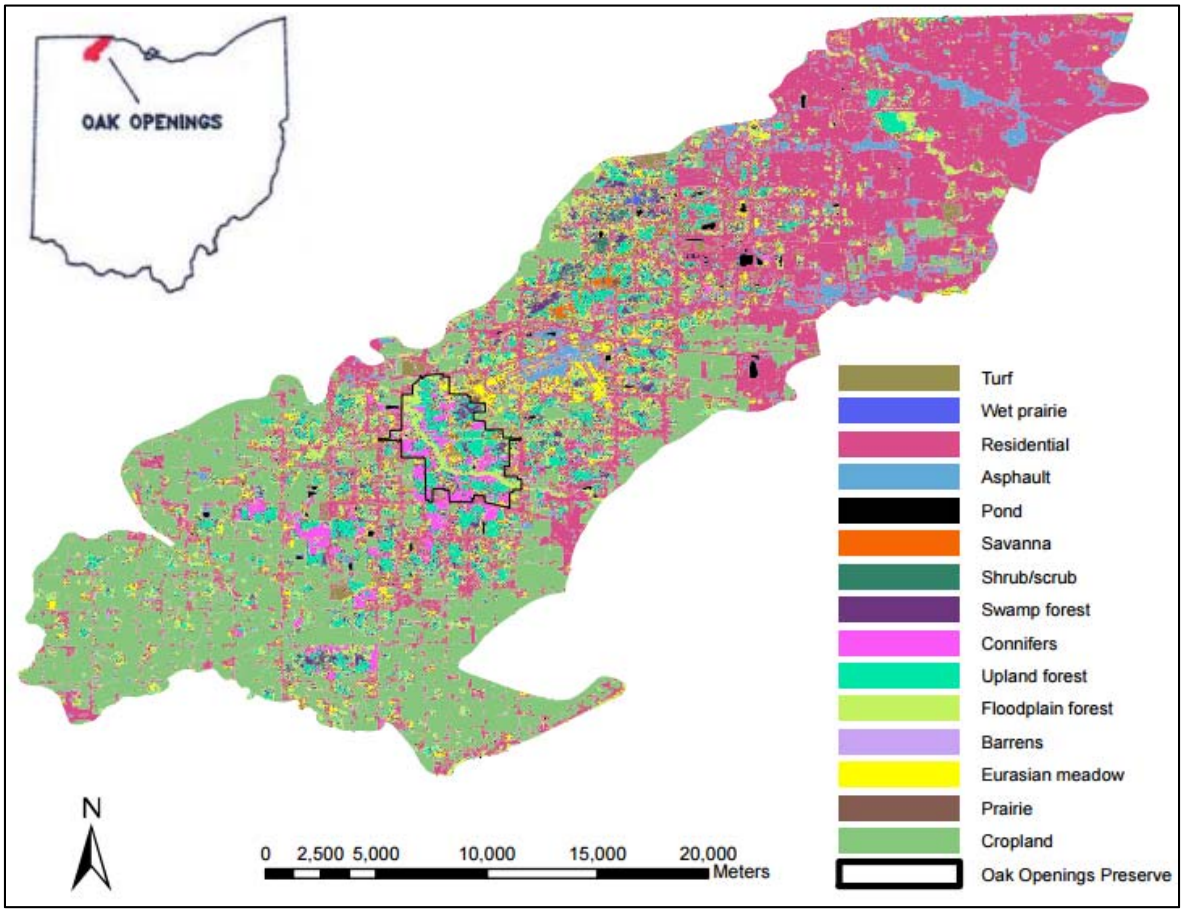


Figure 3.1: Map of the Oak Openings Region with land cover, (Schetter and Root 2011).

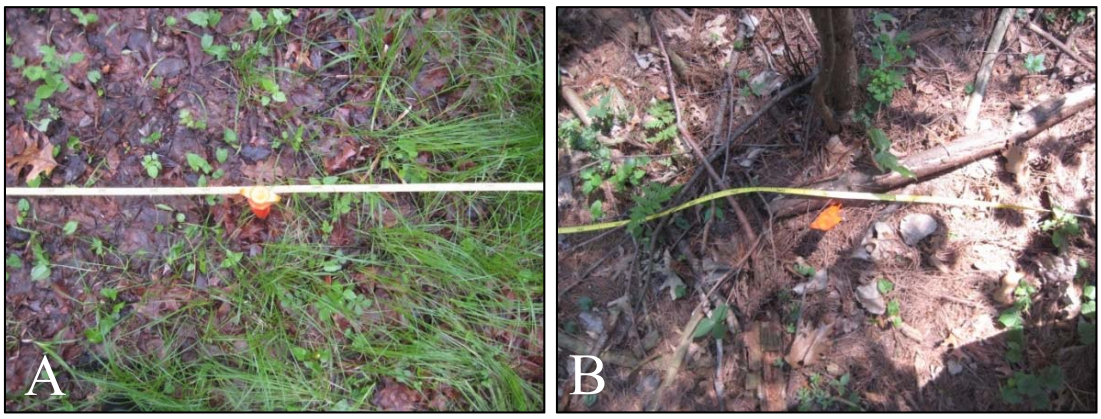


Figure 3.2: Vegetation survey for quadrat A14, east 4 m (A) and quadrat 298 (B), east 8 m. A14's survey for ground cover vegetation proportions were grass (0.30), plants (0.10) and wet leaf litter (0.60) and the proportions of ground cover vegetation for 298 were leaf litter (0.05), coarse woody debris (0.25), plants (0.15) and conifer needles (0.53).

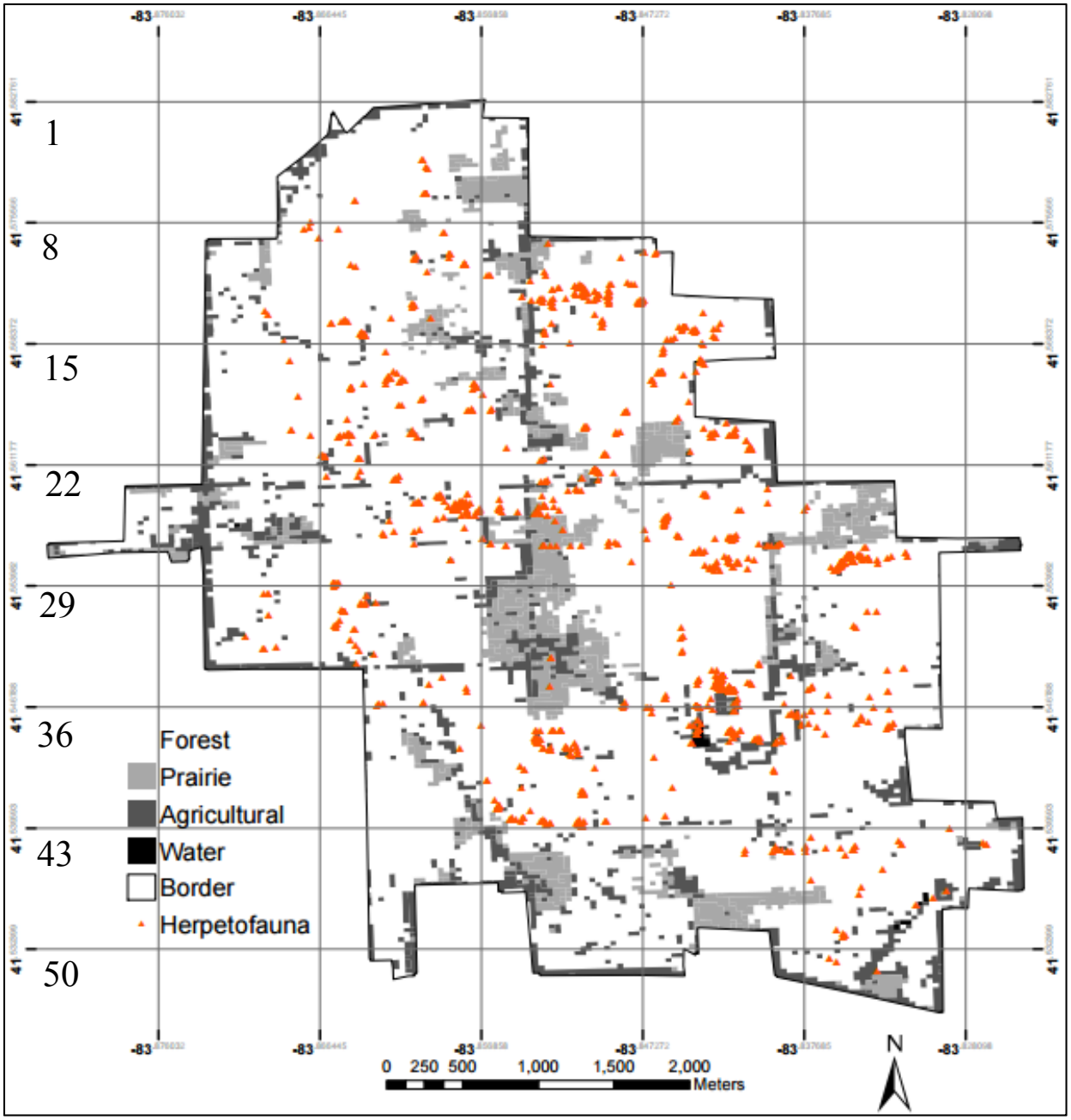


Figure 3.3: The Oak Openings Preserve with all of the herpetofauna detected with an 800 m by 800 m grid overlaid on top. The land cover is categorized into four types: forest, prairie, agricultural and water. The left grid cells are labeled and the top row contains grid cells 1-7, followed by 8-14, 15-21, 22-28, 29-35, 36-42, 43-49, and 50-57.

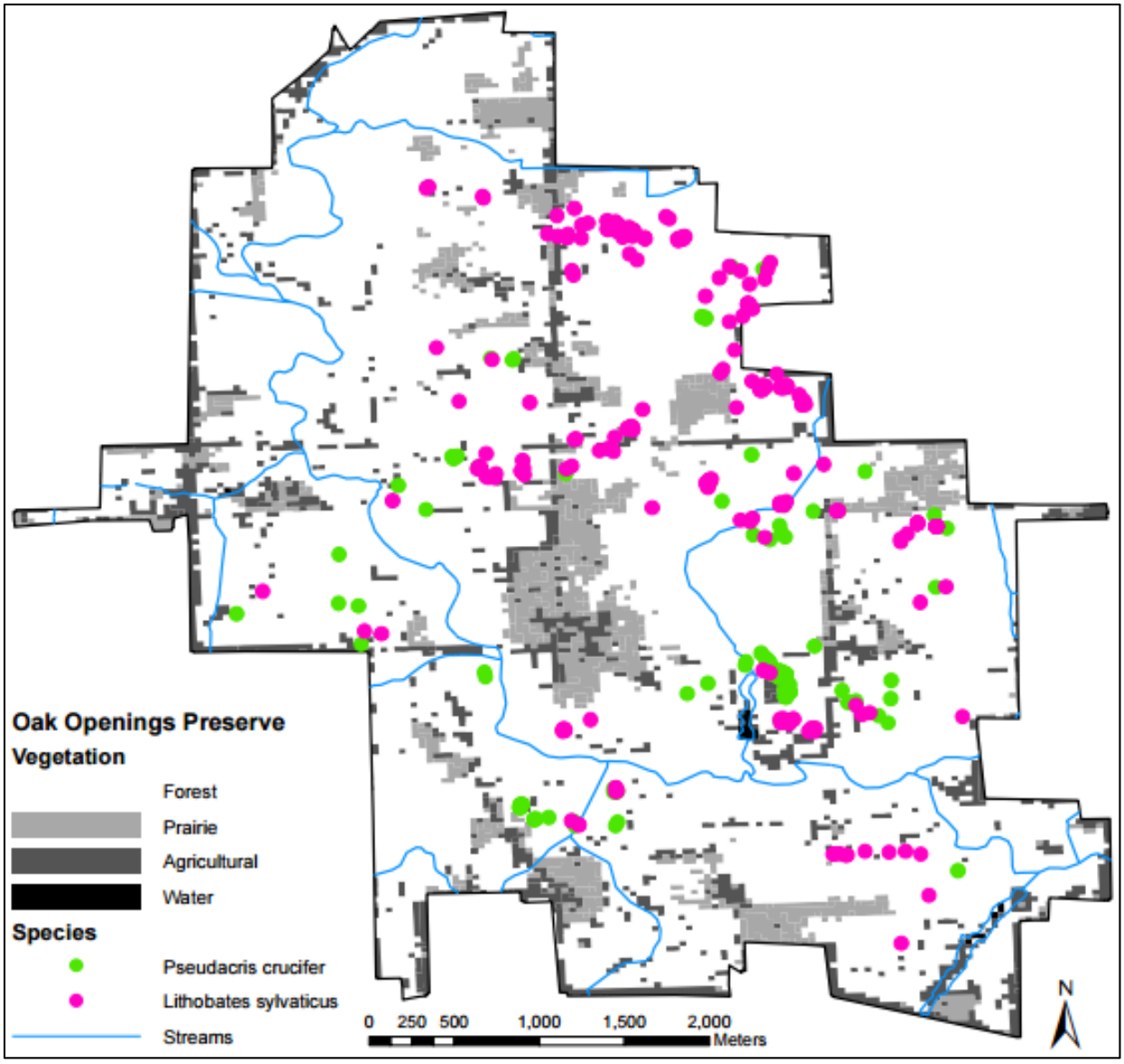


Figure 3.4: Spatial locations of *Pseudacris crucifer* and *Lithobates sylvaticus* sampled within the Oak Openings Preserve with streams and land cover categorized into four types: forest, prairie, agricultural and water.

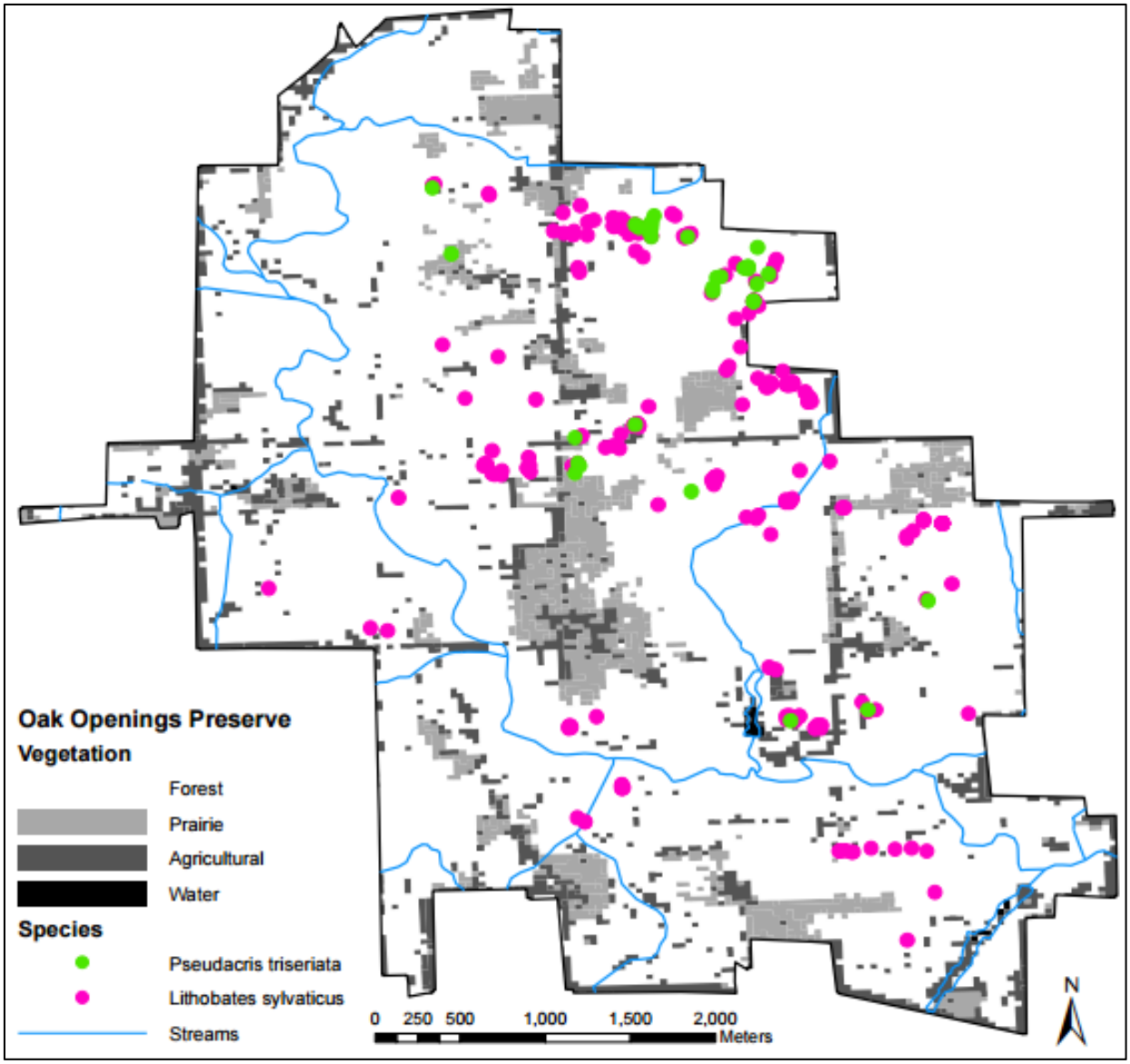


Figure 3.5: Spatial locations of *Pseudacris triseriata* and *Lithobates sylvaticus* sampled within the Oak Openings Preserve with streams and land cover categorized into four types: forest, prairie, agricultural and water.

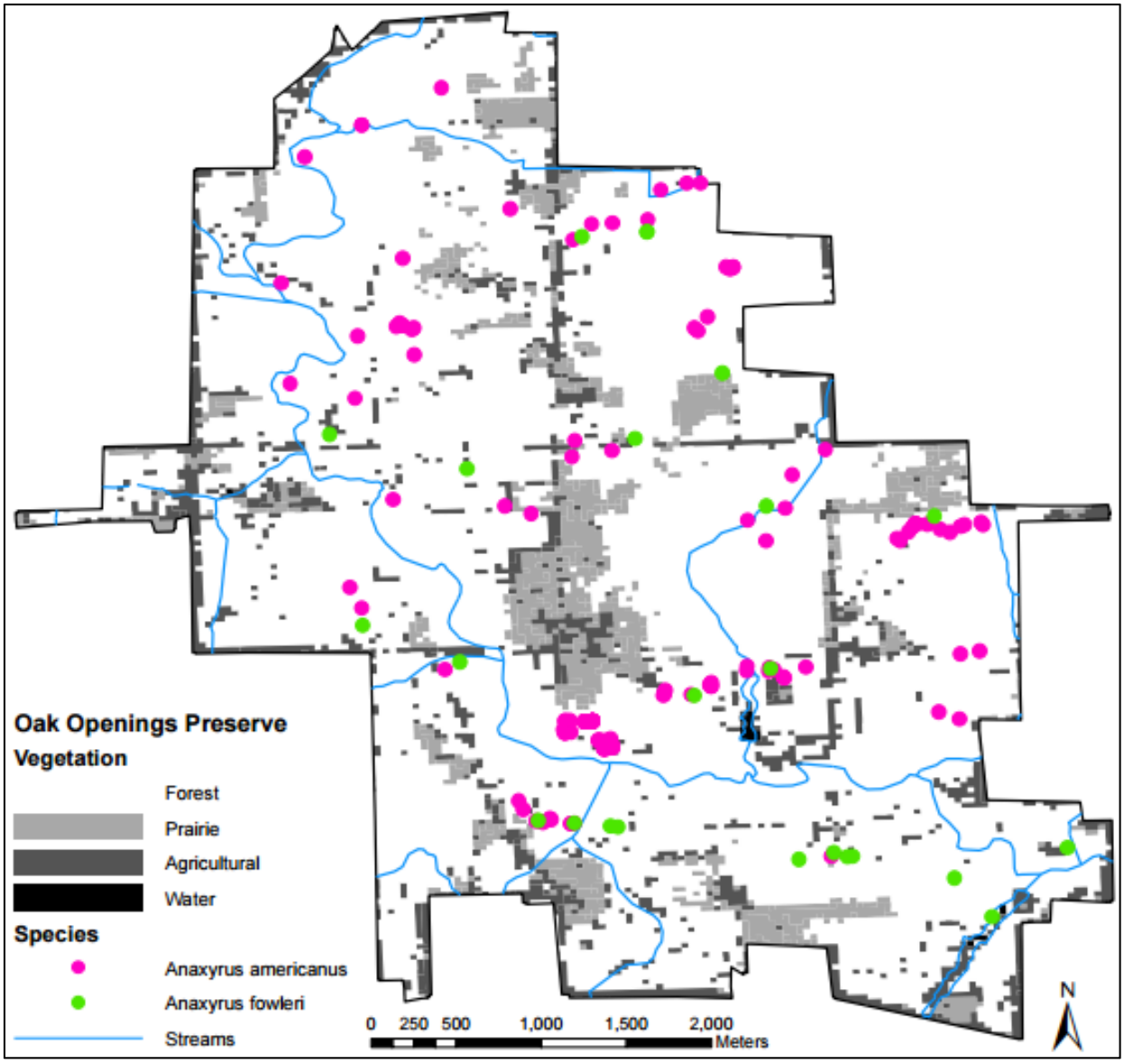


Figure 3.6: Spatial locations of *Anaxyrus americanus* and *Anaxyrus fowleri* sampled within the Oak Openings Preserve with streams and land cover categorized into four types: forest, prairie, agricultural and water.

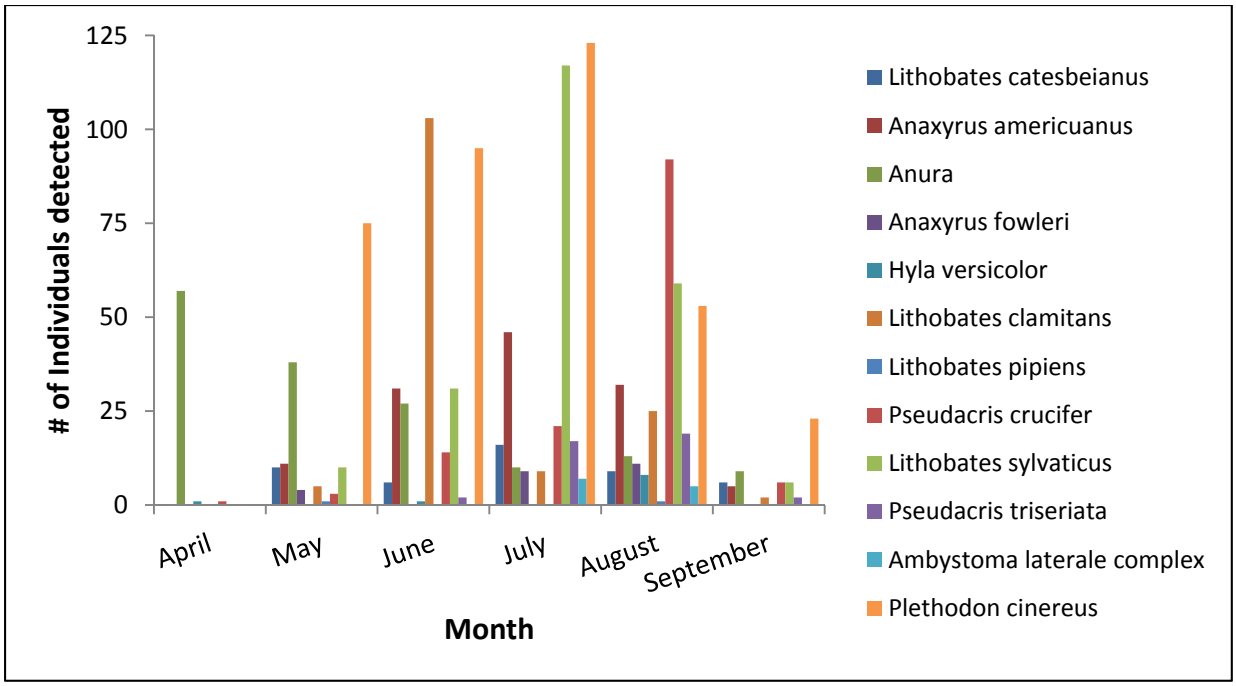


Figure 3.7: Amphibian species abundances that were detected by month (April-September) in 2014.

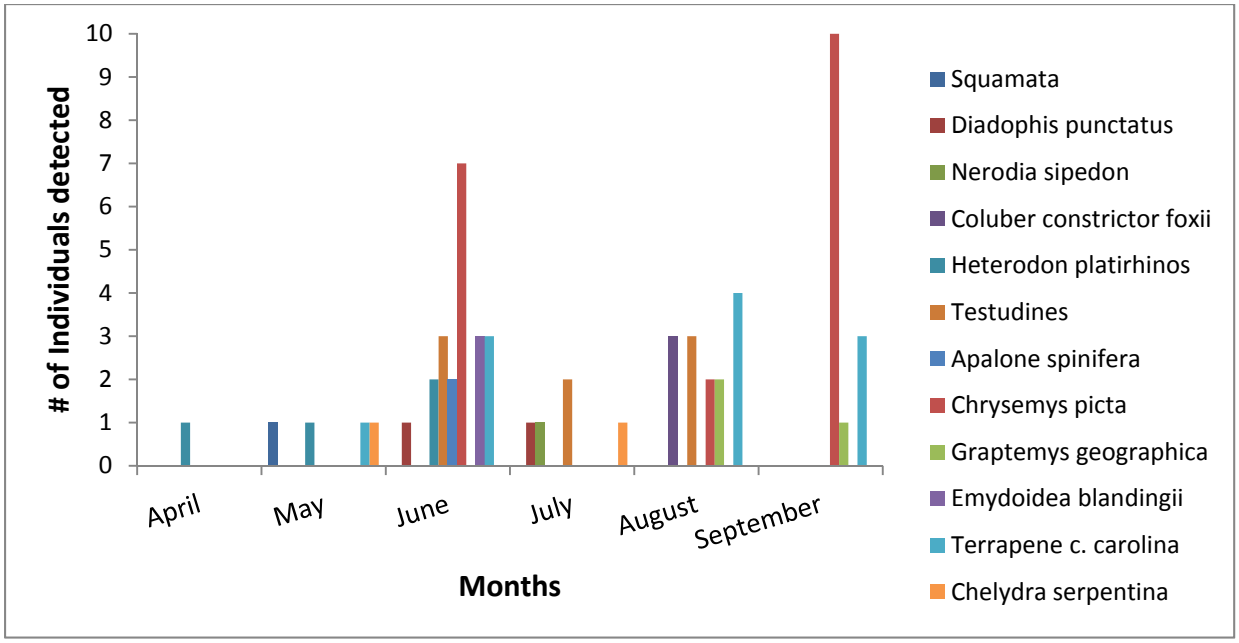


Figure 3.8: Reptile species abundances that were detected by month (April-September) in 2014.

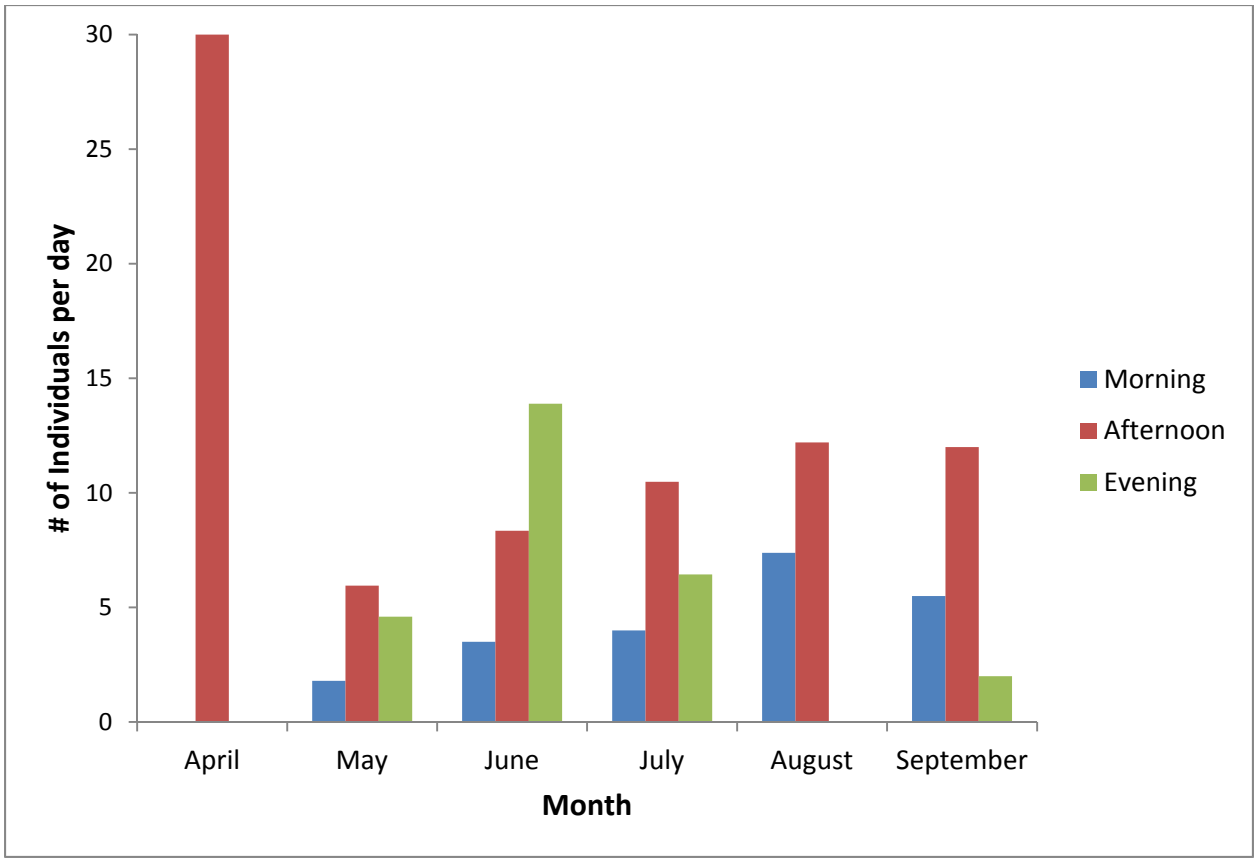


Figure 3.9: The number of individuals detected in the morning, afternoon and evening per day for each month sampled.

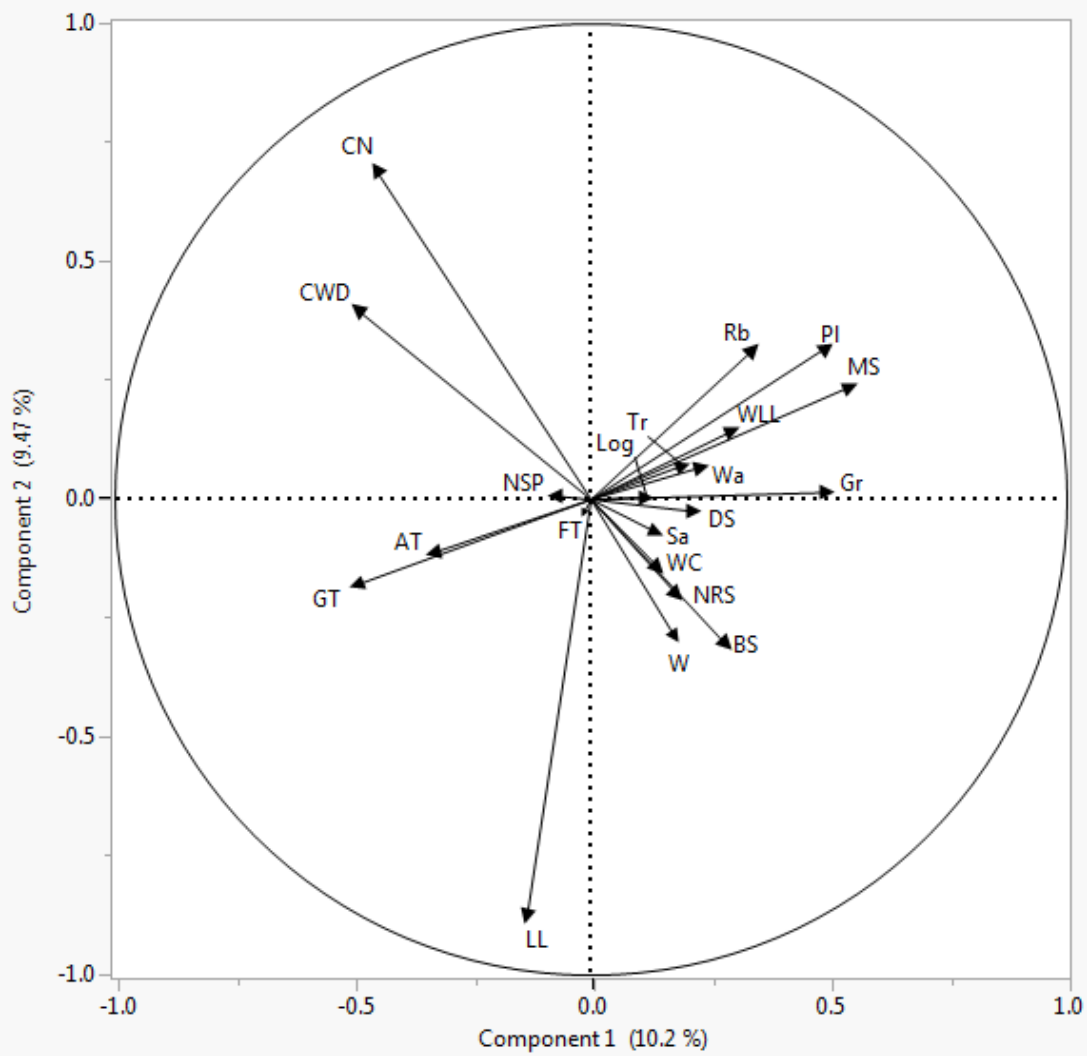


Figure 3.10: Principal Components Analysis shows vectors of species and habitat variables for herpetofauna detected within quadrats in the Oak Openings Preserve. The species variables are: *Anaxyrus americanus* (AT), *Anaxyrus fowleri* (FT), *Hyla versicolor* (GT), *Pseudacris crucifer* (NSP), *Pseudacris triseriata* (WC), *Lithobates sylvaticus* (W), *Ambystoma laterale* complex (BS), *Plethodon cinereus* (Rb), and *Diadophis punctatus* (NRS). The habitat variables are: the proportion of leaf litter (LL), coarse woody debris (CWD), logs (log), plants (Pl), trees (Tr), moist soil (MS), grass (Gr), dry soil (DS), conifer needles (CN), water (Wa), sand (Sa), and wet leaf litter (WLL) for the average proportion of each variable. Association of each variable is represented by the orientation of the lines.

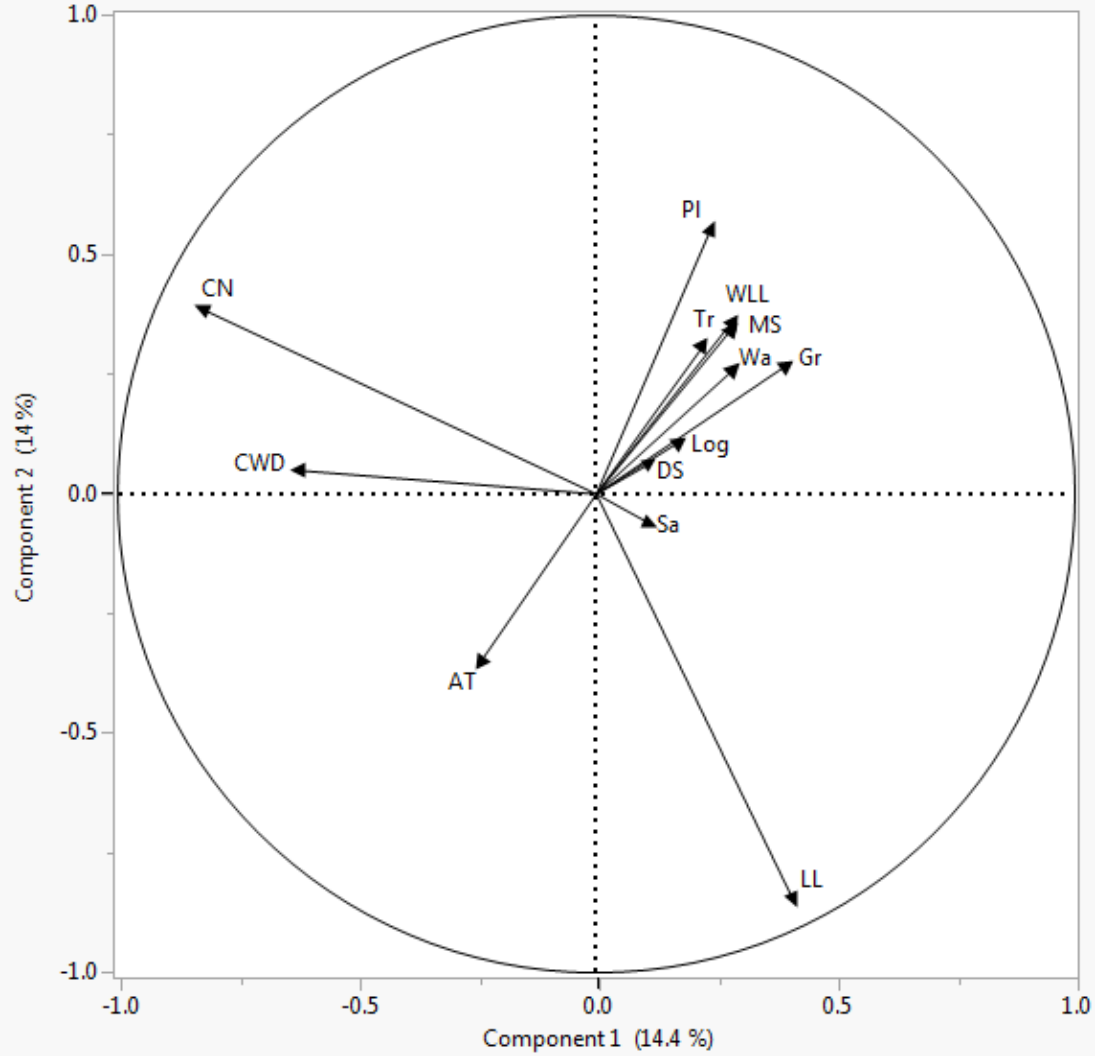


Figure 3.11: Principal Components Analysis shows vectors for *Anaxyrus americanus* (AT) and habitat variables for herpetofauna detected within quadrats in the Oak Openings Preserve. The habitat variables are: the proportion of leaf litter (LL), coarse woody debris (CWD), logs (log), plants (Pl), trees (Tr), moist soil (MS), grass (Gr), dry soil (DS), conifer needles (CN), water (Wa), sand (Sa), and wet leaf litter (WLL) for the average proportion of each variable. Association of each variable is represented by the orientation of the lines.

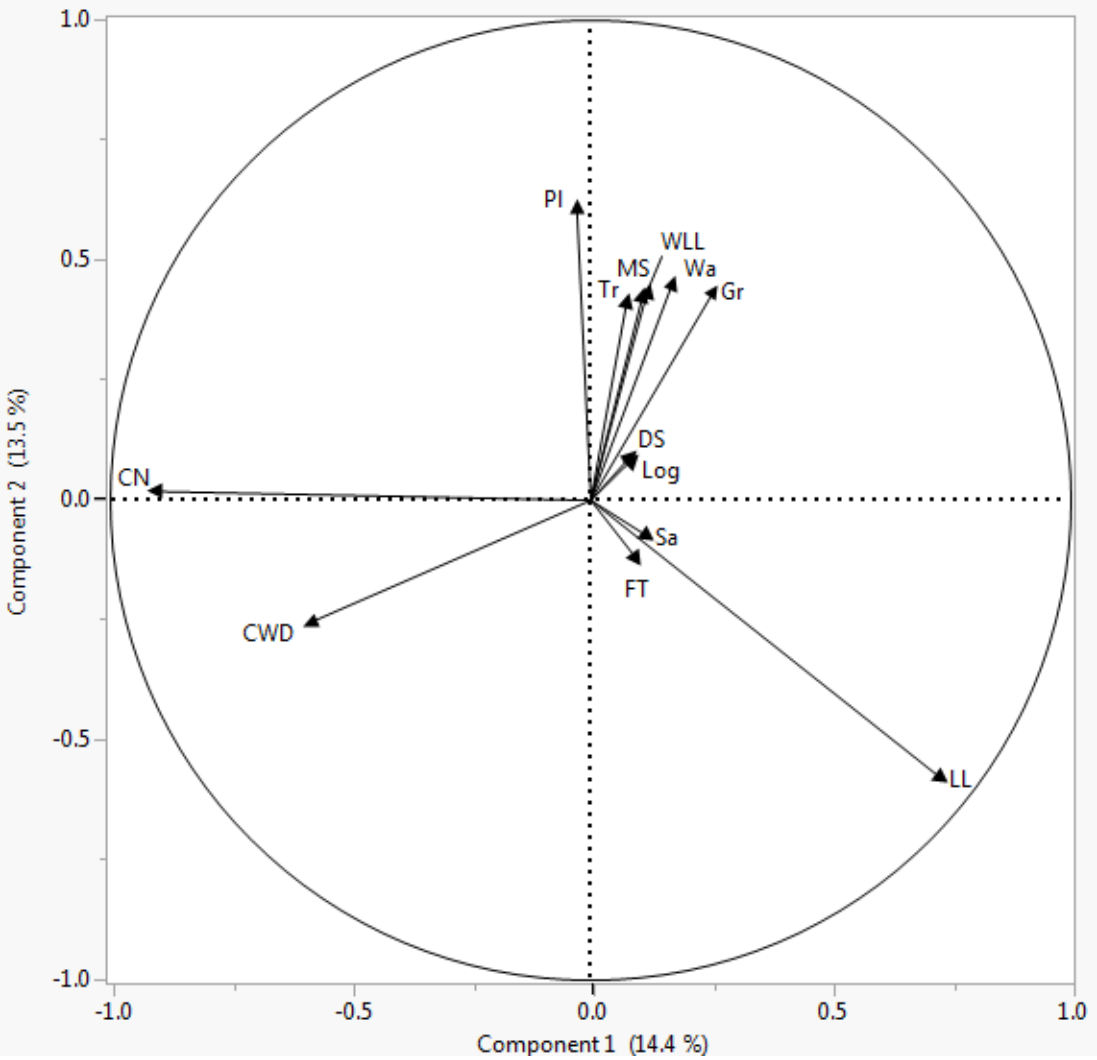


Figure 3.12: Principal Components Analysis shows vectors for *Anaxyrus fowleri* (FT) and habitat variables for herpetofauna detected within quadrats in the Oak Openings Preserve. The habitat variables are: the proportion of leaf litter (LL), coarse woody debris (CWD), logs (log), plants (Pl), trees (Tr), moist soil (MS), grass (Gr), dry soil (DS), conifer needles (CN), water (Wa), sand (Sa), and wet leaf litter (WLL) for the average proportion of each variable. Association of each variable is represented by the orientation of the lines.

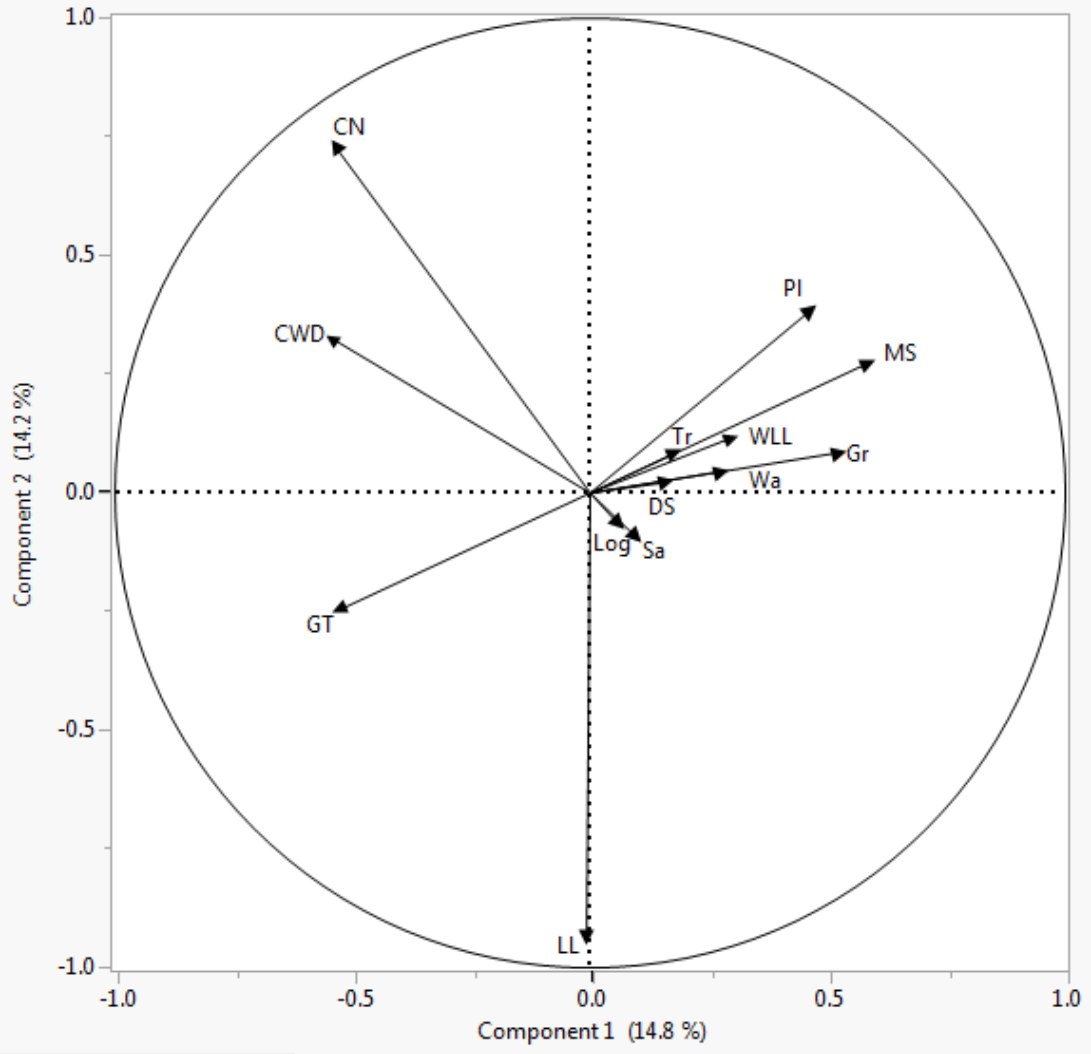


Figure 3.13: Principal Components Analysis shows vectors for *Hyla versicolor* (GT) and habitat variables for herpetofauna detected within quadrats in the Oak Openings Preserve. The habitat variables are: the proportion of leaf litter (LL), coarse woody debris (CWD), logs (log), plants (Pl), trees (Tr), moist soil (MS), grass (Gr), dry soil (DS), conifer needles (CN), water (Wa), sand (Sa), and wet leaf litter (WLL) for the average proportion of each variable. Association of each variable is represented by the orientation of the lines.

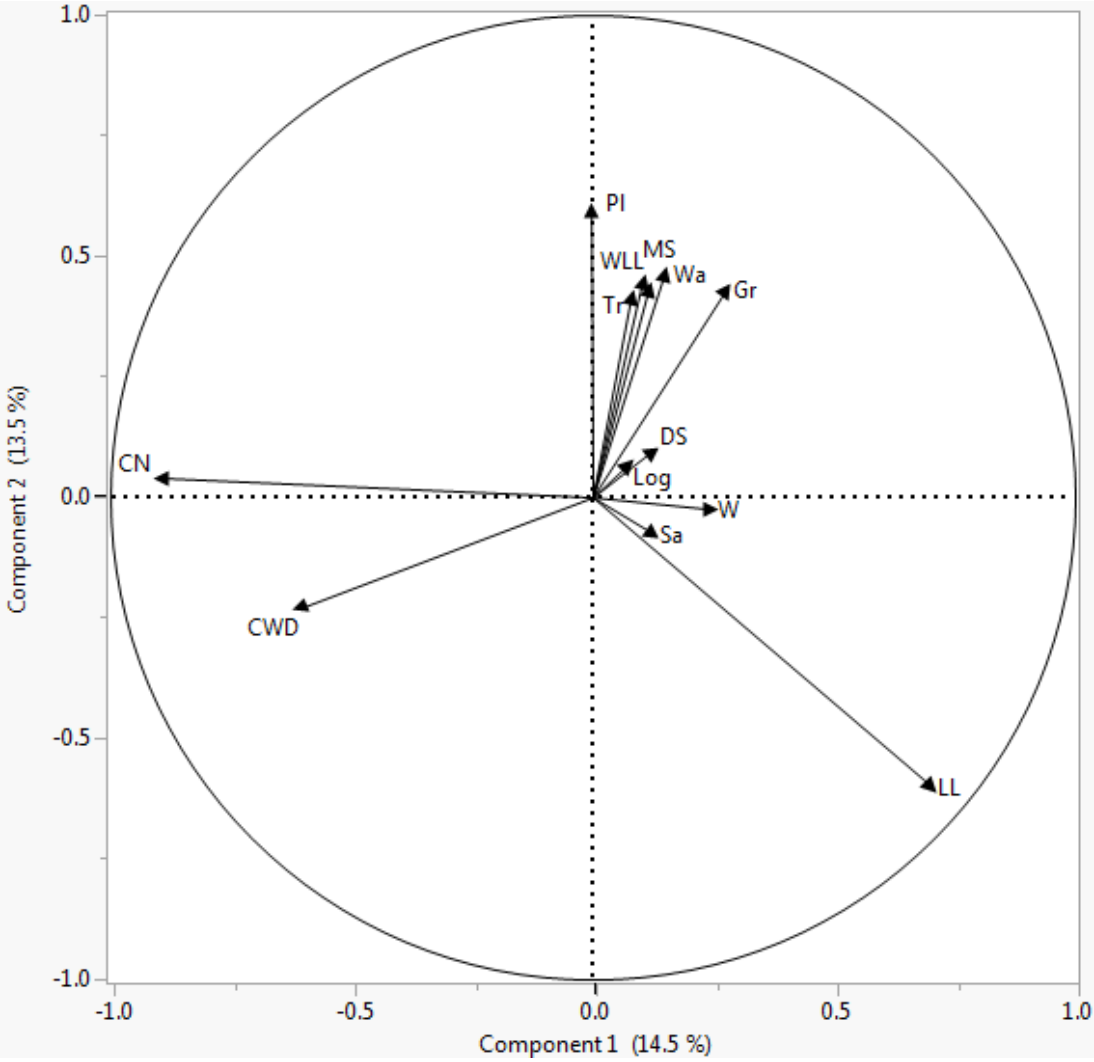


Figure 3.14: Principal Components Analysis shows vectors for *Lithobates sylvaticus* (W) and habitat variables for herpetofauna detected within quadrats in the Oak Openings Preserve. The habitat variables are: the proportion of leaf litter (LL), coarse woody debris (CWD), logs (log), plants (Pl), trees (Tr), moist soil (MS), grass (Gr), dry soil (DS), conifer needles (CN), water (Wa), sand (Sa), and wet leaf litter (WLL) for the average proportion of each variable. Association of each variable is represented by the orientation of the lines.

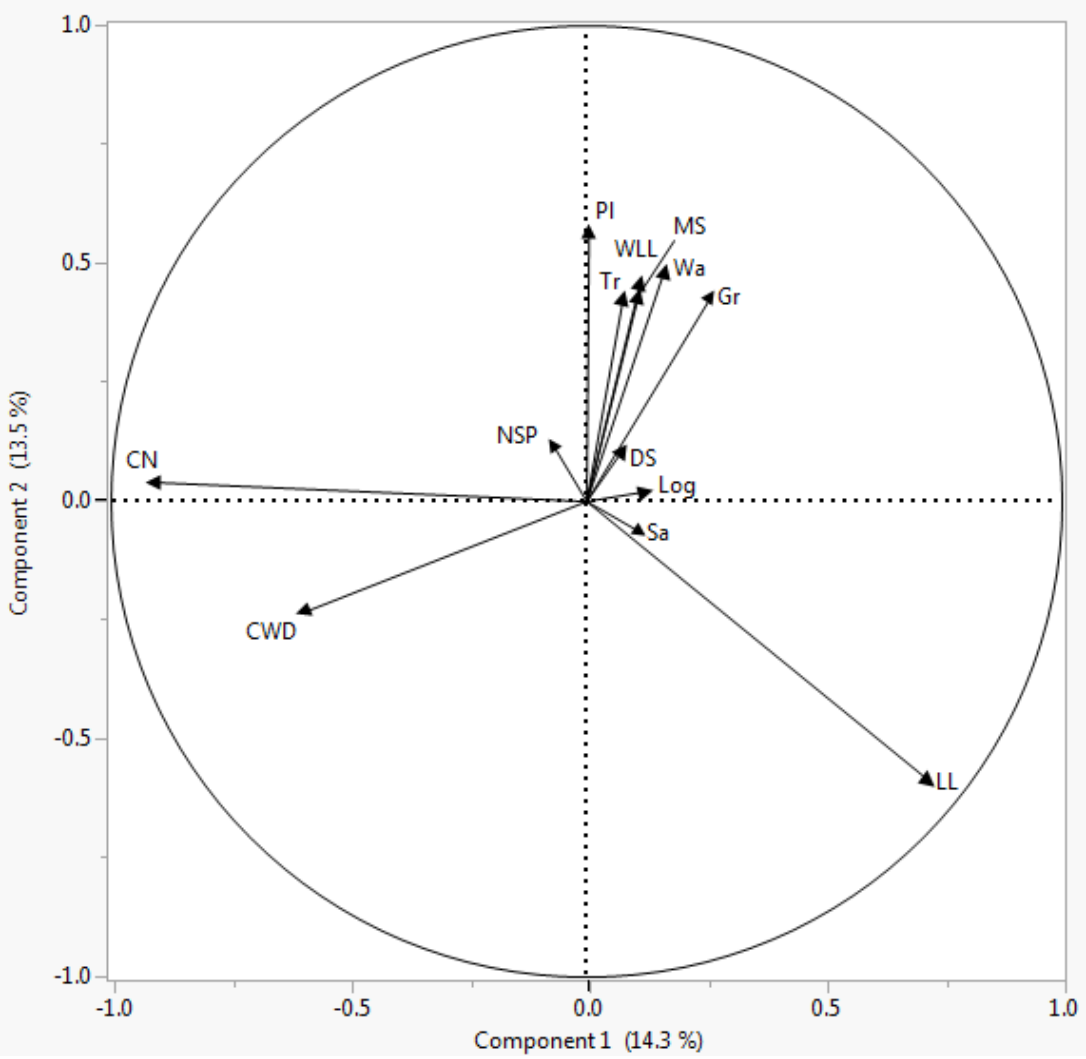


Figure 3.15: Principal Components Analysis shows vectors for *Pseudacris crucifer* (NSP) and habitat variables for herpetofauna detected within quadrats in the Oak Openings Preserve. The habitat variables are: the proportion of leaf litter (LL), coarse woody debris (CWD), logs (log), plants (Pl), trees (Tr), moist soil (MS), grass (Gr), dry soil (DS), conifer needles (CN), water (Wa), sand (Sa), and wet leaf litter (WLL) for the average proportion of each variable. Association of each variable is represented by the orientation of the lines.

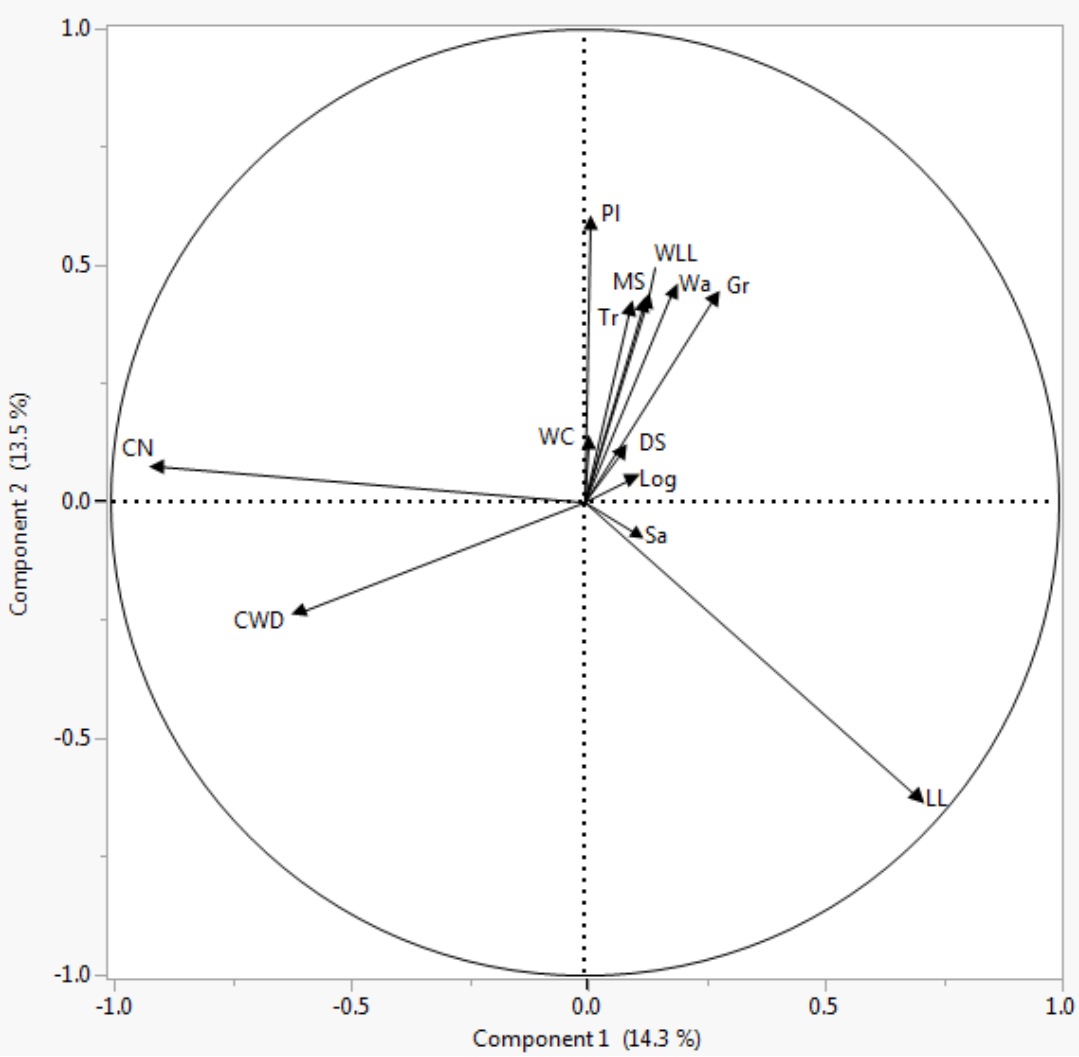


Figure 3.16: Principal Components Analysis shows vectors for *Pseudacris triseriata* (WC) and habitat variables for herpetofauna detected within quadrats in the Oak Openings Preserve. The habitat variables are: the proportion of leaf litter (LL), coarse woody debris (CWD), logs (log), plants (Pl), trees (Tr), moist soil (MS), grass (Gr), dry soil (DS), conifer needles (CN), water (Wa), sand (Sa), and wet leaf litter (WLL) for the average proportion of each variable. Association of each variable is represented by the orientation of the lines.

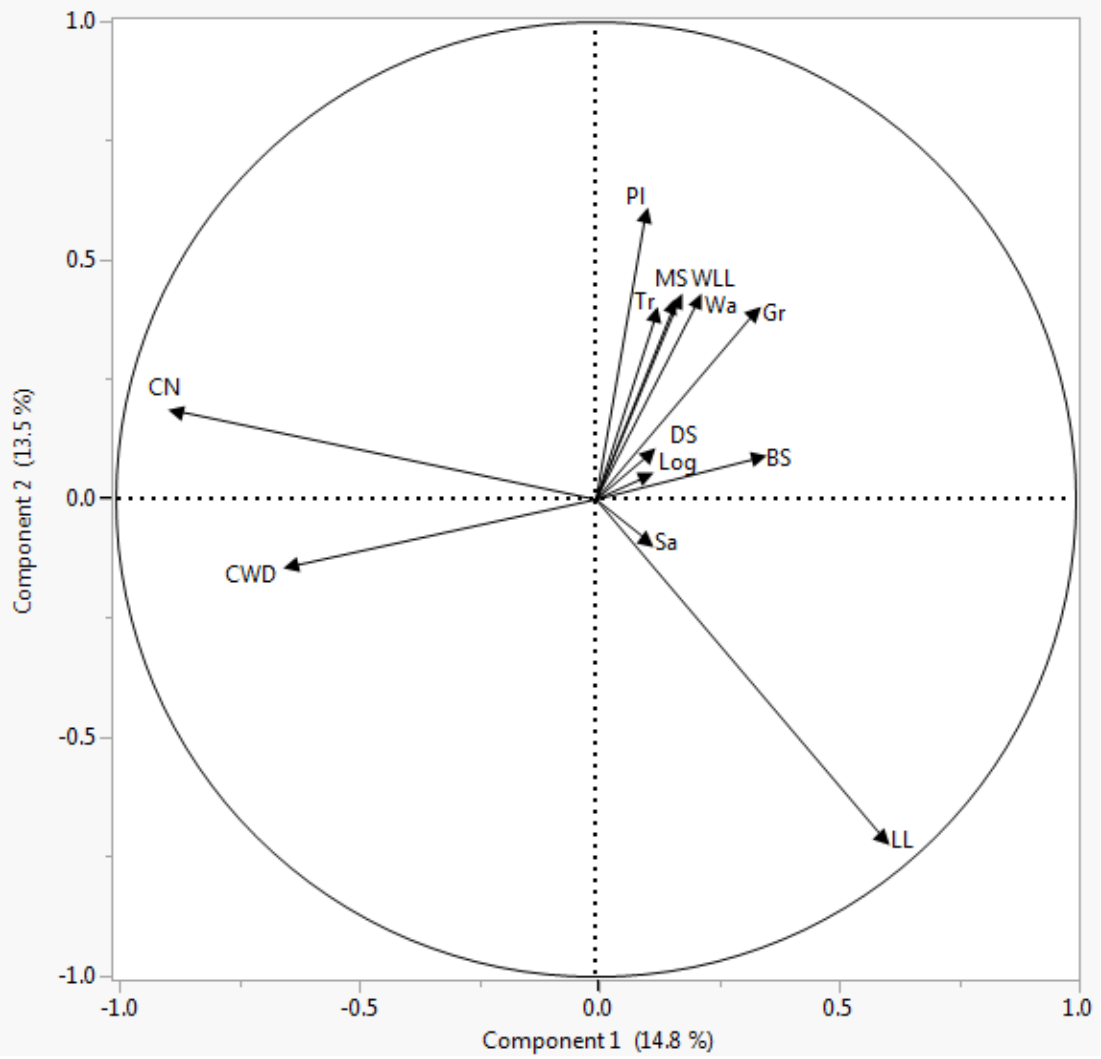


Figure 3.17: Principal Components Analysis shows vectors for *Ambystoma laterale* complex (BS) and habitat variables for herpetofauna detected within quadrats in the Oak Openings Preserve. The habitat variables are: the proportion of leaf litter (LL), coarse woody debris (CWD), logs (log), plants (Pl), trees (Tr), moist soil (MS), grass (Gr), dry soil (DS), conifer needles (CN), water (Wa), sand (Sa), and wet leaf litter (WLL) for the average proportion of each variable. Association of each variable is represented by the orientation of the lines.

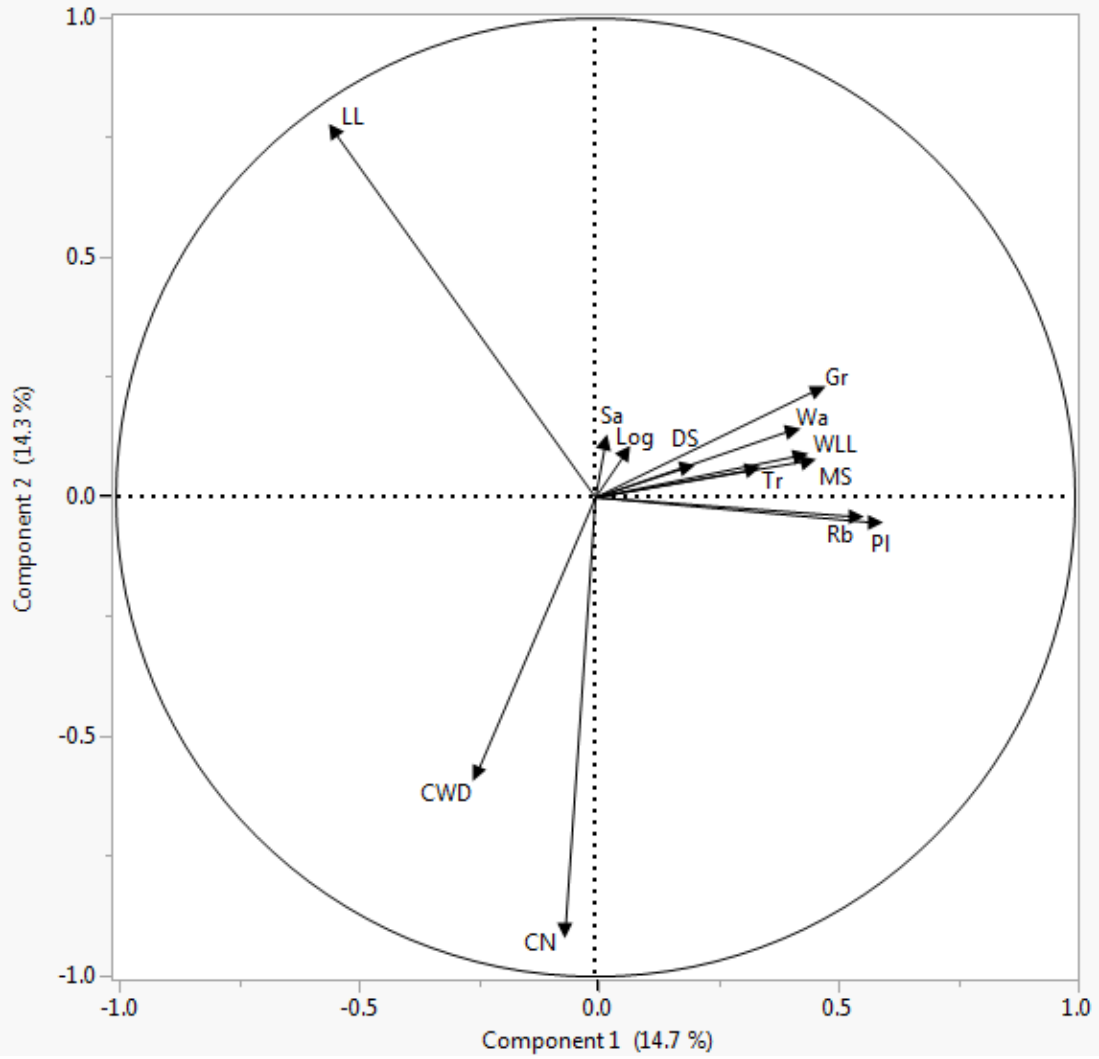


Figure 3.18: Principal Components Analysis shows vectors for *Plethodon cinereus* (Rb) and habitat variables for herpetofauna detected within quadrats in the Oak Openings Preserve. The habitat variables are: the proportion of leaf litter (LL), coarse woody debris (CWD), logs (log), plants (Pl), trees (Tr), moist soil (MS), grass (Gr), dry soil (DS), conifer needles (CN), water (Wa), sand (Sa), and wet leaf litter (WLL) for the average proportion of each variable. Association of each variable is represented by the orientation of the lines.

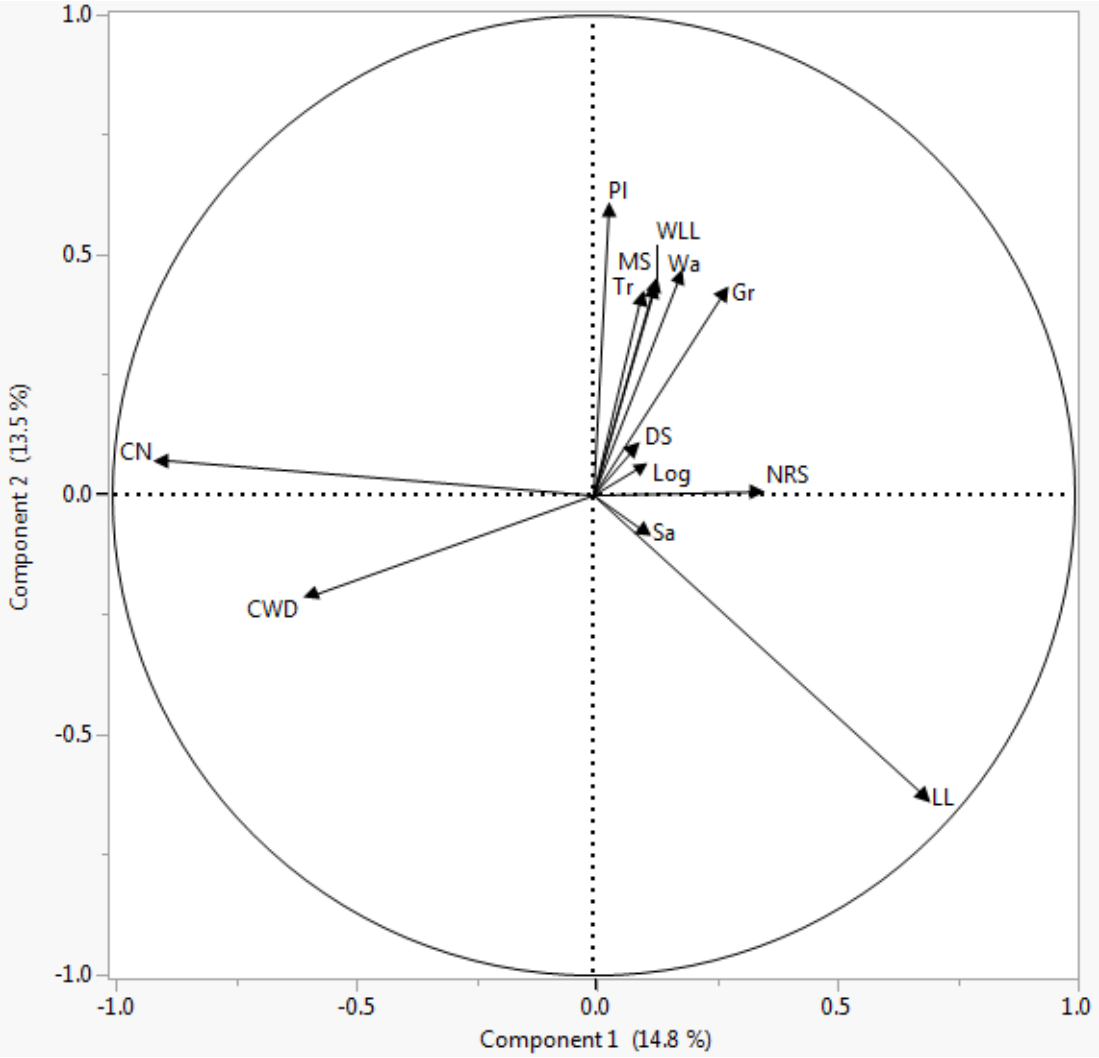


Figure 3.19: Principal Components Analysis shows vectors for *Diadophis punctatus* (NRS) and habitat variables for herpetofauna detected within quadrats in the Oak Openings Preserve. The habitat variables are: the proportion of leaf litter (LL), coarse woody debris (CWD), logs (log), plants (Pl), trees (Tr), moist soil (MS), grass (Gr), dry soil (DS), conifer needles (CN), water (Wa), sand (Sa), and wet leaf litter (WLL) for the average proportion of each variable. Association of each variable is represented by the orientation of the lines.

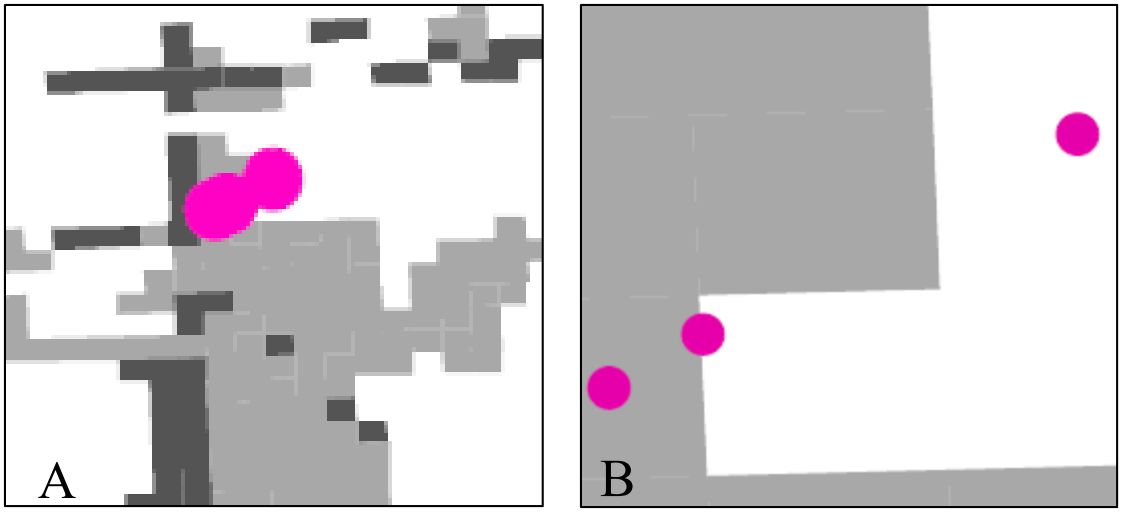


Figure 3.20: Map A shows three *Coluber constrictor foxii* GPS coordinates in a large spatial extent and Map B shows the same three individuals' GPS coordinates at a smaller spatial extent.

# CHAPTER 4: COMPARING COMPLMEMNTARY TRACKING METHODS FOR EASTERN BOX TURTLES

*Abstract*

We examined three methods of tracking eastern box turtles (*Terrapene carolina carolina*): thread trailing, fluorescent powder and radio telemetry. Previous studies have examined turtle movements with thread trailing and radio telemetry; however fluorescent powder has not been used to track adult box turtles. We found that thread trailing and radio telemetry underestimated movement patterns when compared to the complementary fluorescent powder trail. Our box turtles traveled 28.4 m for thread trailing, 46.0 m for fluorescent powder, and 17.68 m for radio telemetry. Thread trailing traced more linear pathways while fluorescent powder delineated more curves. Fluorescent powder and thread trailing provides similar results, which suggests using fluorescent powder is a more useful tool for examining movement patterns because it is less invasive. Radio telemetry was the best method for relocating turtles; however it does not allow for direct analysis of fine-scale movement patterns. Our results can be generalized to other box turtle populations within this region and these methods are applicable for monitoring of other species.

*Introduction*

Eastern box turtles (*Terrapene carolina carolina*) are considered species of concern within Ohio and are listed as vulnerable by the IUCN Red List of Threatened Species (IUCN Red List of Threatened Species, Version 2014.3; http://www.iucnredlist.org/). There has been a long-term radio telemetry study within the Oak Openings Preserve to identify if the population is at risk using Passive Integrated Transponders (PIT) to uniquely mark box turtles that are encountered and record how many box turtles are detected (Cross, unpublished data; Wilson

2012). Radio telemetry was first used in the 1960s (Habib et al. 2014) and has since been used to study many different animals such as: western toads (*Anaxyrus boreas*), Browne & Paszkowski 2014; pond turtles (*Actinemys marmorata*) Pilliod, Welty & Stafford 2013, boreal toads (*Bufo boreas*), Goates, Hatch & Eggett 2007); eastern massasauga (*Sistrurus catenatus catenatus*), Moore & Gillingham 2006; and Blanding's turtles (*Emydoidea blandingii*), Refsnider & Linck 2012. This method has been shown to be successful to study home ranges and habitat use; however actual movements between radio telemetry relocations are unknown. We wanted to bridge the gap between these radio telemetry relocations to identify the type of movement patterns that occur between relocations. Examining spatial patterns will help us understand eastern box turtle ecology by examining the individual's needs at certain points in time or identifying the resources available to the individual (Moore & Gillingham 2006). We used thread trailing, a well-established method for monitoring turtle movements, developed by Breder (1927), and fluorescent powder, a relatively new method, first used on mammals in Lemen & Freeman's (1985) study and on reptiles in Fellers & Drost's (1989) study. There has been little previous research for monitoring turtle movements with fluorescent powder and our study appears to be the first to examine fine-scale movement patterns in eastern box turtles (*Terrapene c. carolina*) with this method. Our study is also the first study to compare thread trailing, fluorescent powder and radio telemetry.

We chose the three methods because they have different levels of invasiveness (i.e., handling time). If the least invasive fluorescent powder method has similar results as the most invasive thread trailing method, then we would suggest that future studies should use the least invasive method to reduce stress on the subject. Our goals were to: (1) compare tracking methods, (2) examine daily movement patterns, and (3) examine habitat occupancy. We

expected that thread trailing would underestimate the distance that eastern box turtles traveled because the thread trail would be more linear in its movement pattern. We expected that fluorescent powder would show the greatest distances traveled because the fluorescent powder trail would be very accurate and include curvatures within the trail. We expected that radio telemetry would give us general distances traveled, but it will underestimate finer scale (e.g., daily) movements and not indicate the types of movement patterns eastern box turtles were making. We would like to note that this is a pilot study which has taken the first steps at examining how these three different methods compare to one another to study eastern box turtle spatial patterns. Our study is likely to be applicable to other animals that have been tracked using radio telemetry in order to understand their fine-scale movements.

*Methods*

***Ethics Statement***

All animals in this study were quickly processed in order to reduce handling time and distress. All research conducted was in accordance with Bowling Green State University IACUC approval 14-001, see Appendix B.

***Study Area***

The Oak Openings Region is a biodiversity hotspot, which contains an abundance of diverse species. The heterogeneous area hosts five globally significant communities: Great Lakes Twig-rush Wet Meadow (Wet Prairie), Great Lakes White Oak-Pin Oak Flatwoods, Mesic Sand Prairie, Midwest Sand Barrens, and Black Oak/Lupine Barrens (Oak Savanna) (EPA 2012). The region supports a variety of species, like the endangered Karner blue butterfly, *Lycaeides melissa samuelis*, and has 177 rare species, along with other organisms from different taxa (EPA 2012). It encompasses approximately 40,000-ha and it extends from northwestern Ohio to parts of

southern Michigan. It was shaped by glaciation and subsequent anthropogenic influences (e.g., water drainage and fire suppression) and alterations (e.g., urban expansion and agricultural intensification). There are several protected areas within this region, but our study focused on the largest preserve for which there has been an ongoing eastern box turtle (*Terrapene carolina* carolina) survey (Cross, unpublished data; Wilson 2012). The 1618-ha preserve is the largest contiguous protected area that contains a high amount of biodiversity for all taxa (The Ohio Ornithological Society 2014).

We searched the Oak Openings Preserve, Figure 4.1, in Swanton, Ohio, from 26 April 2014 to 27 September 2014 for eastern box turtles in order to investigate fine-scale movements and compare tracking methods. The preserve contains many different land cover types; we used a land cover map (Schetter & Root 2011) to combine similar land covers into four main groups: forests (swamp forest, conifers, upland forest, floodplain forest, and shrub), prairies (Eurasian meadows, prairie, barrens, savanna, and wet prairie), agricultural (cropland, residential, turf, and asphalt) and water (pond). For our study, we focused on forested areas to prevent disturbing ground nesting birds.

***Study Species***

We completed visual searches in order to locate eastern box turtles within the Oak Openings Preserve. Turtles located without a radio transmitter were used for the study to monitor fine-scale movements. When located, turtles were handled with rubber gloves (to reduce disease transmission risk). We recorded basic information for each turtle which included sex, weight, carapace measurements and behavior at the location point (Somers & Matthews 2006). We measured mass using a tubular spring scale (grams), an electronic digital caliper was used to measure carapace measurements (millimeters), sex was determined by plastron shape (concave

for males and flat for females) with notation of eye color (red for males and brown for females), and behavior was visually identified such as feeding, basking, sitting in leaf litter, or traveling. On encounter, we first scanned each turtle with a PIT tag reader in order to identify if they were tagged or not. We marked each turtle that was over four inches in length with a PIT tag (AVID®MicroChip ID Systems, Folsom, Louisiana, USA), unless the turtle already had one. The AVID PIT tag is very small (12 mm in length) and each PIT tag has a unique identification number. The PIT tag was injected using a 12-gauge needle under the skin of the lower abdomen just in front of the rear leg, which allowed for unique turtle identification. The PIT tags have minimal effects on behavior as a result of their small size and weight and each insertion was done with care to prevent improper insertion (Mellor, Beausoleil & Stafford 2004). The turtles were monitored for any behavioral effects from marking them with a PIT tag. Turtles were released from the capture location after they were tagged, data was collected and the tracking device was applied.

Turtles were exposed to two tracking methods at one time: either a thread trailer and fluorescent powder mixture or fluorescent powder mixture and a radio transmitter. One turtle was tracked using only thread trailing (turtle 6). Three of the turtles were tracked with both thread trailing and fluorescent powder (turtle's 2, 3 and 5) and one turtle was tracked using both fluorescent powder and radio telemetry (turtle 4).

***Thread Trailing***

When a turtle was located, male or female, we collected basic measurements and pit tagged if necessary. Afterwards, we attached a thread trailer (Figure 4.2) onto the dorsal area of the carapace on the left fourth costal scute using a non-toxic adhesive five minute epoxy, (Devcon, Danvers, MA, USA) to decrease the possibility of the trailer interfering with daily

activities or mating (Iglay et al. 2007). We used a Nyquil® dose cup as the thread trailer because it is light-weight, capable of containing a spool of thread and easy to poke a hole for the thread to exit. We wrapped sewing thread around a plastic bobbin until it could not contain any more thread and was still able to fit within the thread trailer. We then poked a circular hole into the side of the dose cup to allow the thread to exit the trailer. The dose cup was then glued to the turtle's scute with the exit hole facing downwards using the five minute epoxy. When the epoxy dried, we then took the loose thread and put it through the hole and placed the rest of the spool in the dose cup. We placed a flag marker next to the turtle and tied the loose thread to the flag marker which indicated the beginning of the trail. We placed paraffin film over the top of the dose cup and wrapped it around the rim of the dose cup to seal it and decrease the chance that the spool of thread would pop out of the dose cup. When the thread trailer did not stay attached to the turtle's shell, we modified it by cutting the dose cup in half and repeated the same steps. However, we also had to wrap a rubber band around the paraffin film to prevent it from falling off. When the thread trailer was successfully attached to the turtle, the thread unwound within the dose cup as the turtle traveled which left a trail behind the turtle, without interfering with its ability to walk (Stickel 1950). A previous three year study has shown that thread trailers do not alter the turtle's behavior and movement patterns are not different from non-trailer turtles (Stickel 1950).

The turtles were released at their initial encounter point, marked with a flag marker, and remained undisturbed until the next day. If relocated, the thread trailer was refilled with new thread (Donaldson & Echternacht 2005) and that detection spot was marked with a flag marker. We then went back to the original starting point and marked the thread every three meters with a flag marker. We photographed each flag marker for vegetation data and recorded the Global

Positioning System (GPS) coordinates using a handheld GPS unit (Garmin eTrex). The thread trail itself was drawn, videotaped and photographed. The thread trailers are lightweight and will fall off with exposure to the elements as time passes and any turtle whose thread ran out or broke before relocation should not be hindered by the continued attachment of the thread trailer until it naturally falls off.

Time investment for thread trailing will vary based on how long it takes to relocate the turtle and how far the turtle traveled. Set up took ~30 minutes for which each bobbin takes ~15 minutes to wrap the string and time relocating turtles took from ~15 minutes. Applying the thread trailer took ~35 minutes to ensure that the trailer was properly attached. Afterwards, it took about five minutes to exchange bobbins. Data collection time varies on how long the trail is, but generally it took 30 to 60 minutes to measure the trail and place flag markers every 3 m, ~5 minutes to collect GPS coordinates and photographs for a flag marker, ~20 minutes to photograph and video tape the entire trail and ~5 minutes to remove thread trail. Using a minimum estimate, it takes approximately three hours per day for one person to use thread trailing as a tracking technique.

***Fluorescent Powder***

When a turtle was located, male or female, we collected basic measurements and pit tagged if necessary. Afterwards, we painted the plastron with a non-toxic fluorescent powder (Radiant Color, Richmond, California, USA) and parafilm mineral oil (Fisher Scientific Company, Fair Lawn, New Jersey, USA) mixture in a 1:3 gram ratio (Kappler 2009) (see Figure 4.3). Turtles were handled with care while wearing rubber gloves. A light coating of fluorescent powder mixture was painted on the plastron using a small paintbrush. We did not paint the turtle's feet because box turtles are capable of closing their shell tightly and we did not want to

stress the turtle further by trying to pry open the shell and pull the feet out to paint. After the turtle was painted, we set it back in its original detection point and we placed a flag marker to mark the beginning of the trail. The turtle was then left alone and we returned either that night or the next night to locate the trail. At night, around 9:00 pm, we returned to the original GPS coordinates and used an ultraviolet (UV) black light (366 nm) to light up the fluorescent powder mixture trail left by the turtle. As the trail was lit up, we placed a rope trail over the powder marks in order to physically see the trail without the UV black light, see Figure 4.4. If the turtle was relocated, we did not disturb it. The next day, we returned to the marked trail. If the turtle was relocated, then we repainted the plastron with the fluorescent powder mixture and then released it at the new detection point. We returned to the trail and marked every three meters with a flag marker (Nicolas & Colyn 2007) and for each flag marker we recorded the GPS coordinates and photographed for vegetation data. The trail itself was then drawn, videotaped and photographed. Fluorescent powder is highly vulnerable to heavy rainfalls and turtles that were not recaptured before wiping away any remnants of fluorescent powder should not be negatively affected because the fluorescent powder will be washed away after several heavy rainstorms. We used two different fluorescent powder colors: orange and red.

Time investment for fluorescent powder will vary based on how long it takes to relocate the turtle and how far the turtle traveled. Set up took ~20 minutes for which mixing the fluorescent powder and mineral oil takes ~5 minutes and relocating the turtle takes ~15 minutes. When the turtle has been detected, it takes ~5 minutes to paint the plastron and place a flag marker. Night tracking varies based on the distance traveled, but it took ~1 to 2 hours to lay string over the fluorescent powder trail with the UV light's assistance for one person. Data collection time varies on how long the trail is, but generally it took ~5 minutes to place flag

markers at marked 3 m increments on the string and ~5 minutes to collect GPS coordinates and photographs for a flag marker. It took about ~20 minutes to photograph and video tape the entire trail and one minute to remove the flag markers. Using a minimum estimate, it takes approximately two hours per day for one person to use fluorescent powder as a tracking technique.

***Radio Telemetry***

When the male turtle was located, we collected basic measurements. We used a TRX-1000s receiving unit and a Yagi antenna to track the radio transmitter (Wilson 2012). The radio transmitter (9 g) was glued onto the dorsal area of the carapace on the fourth left costal scute using a five-minute epoxy (Devcon, Danvers, MA, USA), see Figure 4.5. The radio transmitter with epoxy weighed between 3-8% of the turtle's body weight. The turtle was monitored for at least half an hour to ensure that the transmitter was adequately glued onto the shell. After the initial encounter, the turtle was then released from its original detection point. The next day we relocated the turtle using radio telemetry and collected the GPS coordinates of its new location point. This was continued until the transmitter fell off and we were unable to relocate the turtle.

Time investment for radio telemetry will vary based on how long it takes to relocate the turtle. Set up took ~15 minutes to relocate the turtle, however, it can take up to 45 minutes to relocate turtles using radio telemetry, but our turtle stayed within the same area and it did not take long to relocate him. Applying the radio transmitter takes ~30 minutes to ensure the transmitter is properly attached. Relocating the turtle took us ~15 minutes at most and data collection took ~2 minutes to record the GPS coordinates and take a photograph. Using a minimum estimate, it takes approximately 50 minutes per day for one person to use radio telemetry as a tracking technique.

### *Data Analysis*

We analyzed the distances traveled for each method based on the number of flag markers used to mark the trail. We calculated the tortuosity ratio for each turtle's path to determine the linearity of the path; the distance traveled each day was divided by the total number of days to find the average distance traveled. The range used the minimum and maximum distance traveled for each day. We mapped the GPS coordinates for each flag marker using ArcGIS and overlaid the trail with the land cover features. We identified which specific land covers each flag marker point was found in. The percent found within each land cover was calculated by dividing the total number of flag markers within the specified land cover by the total number of flag markers. For the one turtle that we tracked using radio telemetry, we calculated the straight-line distance between radio telemetry points using ArcGIS in order to estimate the distance traveled (Martino et al. 2012). The total distance traveled by each turtle for each method was calculated by summing the total distance traveled per day. Using video footage of each trail, we observed whether the turtles made linear, curvy or linear-curvy movements.

## *Results*

### *Turtle Detections And Tracking*

Eastern box turtles were very difficult to detect, we detected six box turtles. Turtle one's thread trailer fell off at the original capture location and no data was collected beyond basic measurements (see Table 4.1). Turtle 2 was tracked for two days with fluorescent powder and lost on day 2; thread trailer was retrieved on location site. Turtle 3 was tracked with fluorescent powder and thread trailer for one day and lost, thread trailer was not recovered, but should have fallen off. Turtle 4 was tracked with fluorescent powder for 10 days and with radio telemetry for 21 sightings before the radio transmitter fell off and was recovered. Turtle 5 was tracked with

fluorescent powder for four days and with thread trailing for three days; thread trailer not recovered, but should have fallen off. Turtle 6 was tracked for one day with thread trailer, heavy rainstorm washed away fluorescent powder trail, and thread trailer was not recovered, but should have fallen off.

***Movements And Habitat Occupancy***

Eastern box turtles tracked with thread trailing, on average, traveled 28 m per day and ranged from 9 m to 34 m. The average distance traveled per day for each turtle was 9 m, 34 m, 42 m and 29 m. Of the turtles tracked with thread trailing two turtles had a high tortuosity ratio, 0.77 and 0.80, which indicates that the turtles traveled shorter paths than the other two turtles that had tortuosity ratios of 0.34 and 0.36, more twists and turns. On average, the tortuosity ratio for thread trail turtles was 0.57, which indicates that the path had both twists/turns and linearity. All thread trails were located within forested habitat.

Eastern box turtles tracked with fluorescent powder, on average, traveled 46 m per day and ranged from 11 m to 87 m. The average distance traveled per day for each turtle was 55 m, 39 m, 41 m and 50 m. All four turtles tracked with fluorescent powder had tortuosity ratios less than 0.50 which indicates that their paths were had more twists and turns making it a longer path traveled. Two turtles' fluorescent powder trails were completely located in forested habitat, one turtle's trail was located 43% in forested habitat and 57% in agricultural habitat and another turtle's trail was located 71% in forested habitat and 29% in prairie habitat.

The eastern box turtle tracked with radio telemetry, on average, traveled 18 m per day and ranged from 3 m to 47 m. Its tortuosity ratio was 0.31 which indicates that it took a long path with twists and turns to travel from flag marker 1 to flag marker 134. The turtle was relocated

70% of the time in forested habitat and 30% in prairie habitat. Refer to Appendix C for individual turtle tracking data.

***Comparison***

Tracks using only the thread trailing method yielded a total of 28.7 m and the thread trail tended to be very linear, some curves could be seen, but generally the trail left behind was linear (personal observation). Tracks using thread trailing (TT) and fluorescent powder (FP) methods yielded a total of 9 m (TT) and 109 m (FP) for turtle 2, 34 m (TT) and 39 m (FP) for turtle 3 and 126 m (TT) and 199 m (FP) for turtle 5 (as shown in Table 4.2). Tracks using thread trailing tended to have smaller total distance traveled than fluorescent powder; however the thread trailer tended to break or fall off before the fluorescent powder trail ended. Thread trails tended to be straighter than fluorescent powder trails which tended to be curvy, following the turtle's movement exactly (as in Figure 4.3). Tracks using fluorescent powder and radio telemetry (RT) methods yielded a total of 406.5 m (FP) and 371.25 m (RT) for turtle 4. Fluorescent powder tracked turns between flag markers which led to larger estimates of the distance traveled in contrast to radio telemetry which usually assumes a straight-line distance between relocations. All of the turtles tracked traveled using both linear and curvy movements, some examples: straight-line movements traveling alongside logs, curvy movements going around small trees, or traveling over small logs based on video footage of trails.

*Discussion*

Analyzing movement patterns is essential to understanding how an organism interacts with their environment. There are many reasons why animals move through their environment: find food or shelter, to escape predation, and find mates. We looked at the movement patterns of eastern box turtles in the Oak Openings Preserve using three methods with varying degrees of

invasiveness (handling time). We found that box turtles were difficult to detect as a result of their camouflage, however using these tracking methods can help us to relocate box turtles within this heterogeneous landscape.

When a box turtle was located, we used thread trailing, fluorescent powder or radio telemetry. We sampled the Oak Openings Preserve using quadrats and opportunistic surveys, however box turtles were only detected opportunistically which suggests that they are more mobile individuals. We determined that thread trailing was the most invasive handling technique because the turtle needed to be monitored daily to change the thread and they carried the thread trailer. Fluorescent powder was considered a less invasive handling technique than thread trailing because individuals only had to be repainted each day with the fluorescent powder. Finally, radio telemetry was considered the least invasive handling technique because after the first encounter to glue the transmitter onto the carapace, we did not have to handle the turtle again when tracking it with radio telemetry.

We found that thread trailing and fluorescent powder collected similar data, however thread trailing tracks tended to be more linear and extend a smaller distance than fluorescent powder. This suggests that thread trailing underestimates distance traveled because it does not completely account for nonlinear movements. Fluorescent powder provided the most accurate representation of the turtle's movement pathway while radio telemetry was successful at relocating turtles and our thread trailing was less successful for tracking box turtles. Our study showed two general types of movements, direct routes and meandering, which have different biological reasons. Direct routes allow turtles to travel quickly to where they are going this expends less energy and occur when females are moving to nesting sites (Iglay et al. 2006). Meandering is ecologically beneficial by allowing the turtle to explore new areas to locate

resources, find mates or find new habitats. Both movement types can occur in small or large areas and radio telemetry would be the best method for measuring direct routes, while thread trailing and fluorescent powder are better for delineating detailed meandering movements.

We had a number of issues using thread trailing and our results were not as successful in monitoring turtle movements. The original thread trailer was modified by cutting the dose cup in half, see Figure 4.2 A and B, because it fell off the first two turtles, turtle 1 at the detection point and turtle 2 after the turtle traveled 9 m. When the modified trailer was implemented and the appropriate time given for the glue to dry fixed the problem of the trailer falling off. However, we ran into issues with the thread. Turtle 3 and 6's thread holder popped out of the trailer device and turtle 5's thread ran out before we were able to change thread spools. The thread holder popping out was most likely a result of the paraffin film ripping (turtle 6) or falling off (turtle 3). After turtle 3's issue, we added rubber bands to hold the paraffin film tightly in place, however turtle 6's issue may only have been resolved if we used different materials that were less likely to break. On average, we were able to track box turtles with thread trailing for 1.5 days with a range of 1-3 days. This suggests that our attempts at creating an inexpensive thread trailer are not sufficient for tracking box turtles.

Thread trailing has been successful in previous studies for: spiny bandicoot (*Echymipera kalubu*), Anderson et al. 1988; ornate box turtles (*Terrapene ornata*), Claussen, Finkler, & Smith 1997; eastern box turtles (*Terrapene c. carolina*), Donaldson & Echternacht 2005, Iglay, Bowman, & Nazdrowicz 2006 and Iglay, Bowman, & Nazdrowicz 2007; wood turtles (*Glyptemys insculpta*), Saumure, Herman, & Titman 2010; and giant bullfrogs (*Pyxicephalus adspersus*), Yetman & Ferguson 2011. Our thread trailing method may need improvement because we were unsuccessful in tracking turtles for more than three days. Our first two turtles

were lost because we did not spend the appropriate amount of time gluing the apparatus to the carapace. We lost two other turtles because we did not seal the dose cup with appropriate material. These problems occurred because of poor implementation of the method rather than problems with the method itself. However, we did lose one turtle because the thread broke and another because the thread ran out. When comparing our thread trailer to other studies, Claussen, Finkler, & Smith (1997) duct taped a 35-mm film canister to the turtle's carapace with 300 m of white cotton thread inside the canister; the container itself is a stronger trailer than our dose cup and the use of duct tape may have secured the canister to prevent it from dislodging. This same method worked for Iglay, Bowman & Nazdrowicz (2006), but they did have instances where the thread trailers failed, such as falling off, the thread breaking or entanglement of the thread. However, their study was more successful because it was part of a larger ecology study for which they had 80 radio tagged box turtles to use and whenever a thread trailer failed, they used a different radio tagged box turtle. Donaldson & Echternacht (2005) also used a 35 mm film canister bound to the box turtle's carapace using duct tape and PC-11 epoxy, with a screw with two washers fastened around it as the spool. They had two sizes for their 20 tracked turtles, juvenile and adult, for which the spool either contained 180 m or 250 m of extra-strong cotton thread. A film canister allowed these other studies to contain more thread than our dose cup and small bobbin could hold, which may have resulted in the loss of turtle 5. Our method could be improved by using a film canister instead of our dose cup and using a combination of epoxy and duct tape to keep the container in place. This would allow us to have more thread to record longer distances traveled. Thread breaking is still a possibility when using this method, but it is necessary in order to decrease any chances that the turtle could get stuck. If this study is done again using the dose cup, the researchers should spend a minimum of 30 minutes making sure

the thread trailer is firmly attached and change thread spools every morning before the turtle begins its daily movements. Turtle 5 was lost because we were unable to return in the morning the following day, and the turtle had already left its resting place when we returned in the afternoon and found that the thread ran out.

We found that fluorescent powder was more useful examining fine-scale movements than thread trailing; however it was not as useful relocating a turtle after it had been painted. We had trouble relocating box turtles using fluorescent powder because as the turtle traveled through its environment, the trail became less discernible and in most instances, the powder wore off before the box turtles reached their evening shelter. Using the same fluorescent powder color for one turtle caused an issue for turtle 4's final trail; he went around in a small circle (about 3 $m^2$ area) and we were unable to identify where he left the circle. Box turtles tend to have high site fidelity and when tracking an individual within the same area my cause problems when using one fluorescent powder color as a result of overlapping trails. In order to reduce this issue, more than one color can be used if the individual is staying in the same area for a large period of time. On average, we were able to track box turtles for 4.25 days with a range of 1-10 days; however the range should be 1-4 days because the turtle tracked for 10 days was relocated with radio telemetry.

Several studies have successfully used fluorescent powder to track: small mammals and lizards, Brehme et al. 2013; snakes, Furman, Scheffers, & Paskowski 2011; rodents, Lemen & Freeman 1985; small mammals, Nicolas & Colyn 2007; red-spotted newts, (*Notophthalmus viridescens*), Roe & Grayson, and wood turtles (*Glyptemys insculpta*), Tuttle & Carroll 2005. Fluorescent powder has not been used to track eastern box turtles before, and based on our results; we have shown that it is a practical method for examining their fine-scale movements.

Some concerns are the fluorescent powder trail will increase mortality rates through predation (Dodd 1992); however we did not find any mortality in our study. We used two colors, orange and red, and found that both are susceptibility to heavy rainfall. Turtle 6 was lost because the thread trailer fell off and after ~30 minutes of rainfall, the red fluorescent powder was washed away and the trail was gone. Dodd (1992) also found that rainfall eliminated trails, even a light 5-10 minute rainfall led to partial trail tracking. For future studies that use fluorescent powder, using a second tracking method such as radio telemetry and monitoring rainfall before applying the technique will help reduce losing turtles.

Radio telemetry was the most successful method for relocating box turtles; it took 24 days before the transmitter fell off. However, radio telemetry does not show the detail of movement between relocations (Roe & Grayson 2008), which suggests that fluorescent powder is more successful for examining detailed movements and habitat actually used (Furman, Scheffers & Paszkowski 2011). We were able to estimate distance traveled by using a straight-line distance between two points, however this tends to underestimate the distance actually traveled because organisms typically do not travel in a straight line all of the time (Iglay, Bowman, & Nazdrowicz 2006). On average, turtle 4 traveled 17.68 m with a range of 3.43 m to 47.04 m. When the radio telemetry results were compared with the fluorescent powder results for turtle 4, we found that radio telemetry underestimated distance traveled by 35.25 m.

Comparing all three methods, we found that radio telemetry had the smallest distance traveled of 3.43 m, fluorescent powder had the largest distance traveled of 87 m and the average for all distances traveled per day was 30.06 m. Many times we were unable to relocate the turtles, which suggest that each method underestimated actual distance traveled. The maximum distance traveled for our study is lower than other studies: 208 m for *Terrapene c. carolina*

(Iglay, Bowman & Nazdrowicz 2007), 219 m for *Terrapene c. carolina* (Donaldson & Echternacht 2005), and 314.8 m for *Terrapene ornata* (Claussen, Finkler & Smith 1997), which supports our conclusion that we underestimated travel distances.

Our box turtles occupied both forested (conifer forests, floodplain forests, upland forests, shrubs and swamp forests) prairies (Eurasian meadows, prairie, barrens, savanna, and wet prairie) and agricultural areas (residential area). We found for all turtles tracked with thread trailing, their trails only occupied forested areas (100%) and none of the trails were found in the agricultural, prairie or water land covers. We found for all turtles tracked with fluorescent powder, their trails occupied forested areas 75% of the time, agricultural areas 5% of the time and prairie areas 15% of the time and none of the trails were found in the water land cover. Finally, we found for the turtle tracked with radio telemetry, his trail occupied forested areas 73% of the time and agricultural areas 27% of the time. It appears that our box turtles were spending more time in forested areas than open areas as has been found in other studies (e.g., Fredericksen 2014). This may have occurred because box turtles often maintain their body temperature by either seeking cover under leaf litter or find areas for basking (Fredericksen 2014). Box turtles may also decrease predation risks by utilizing thick vegetation in forested areas rather than open areas, which can have higher risk of avian predation.

Our study has shown that fluorescent powder is a practical method for studying box turtle movements. We suggest that a secondary tracking method such as radio telemetry be used with fluorescent powder in order to increase relocation reliability. For areas with consistent rainfall, thread trailers may be preferable; however, it should be noted that the movements tracked are most likely underestimating the actual distance traveled. We have provided preliminary results in

order to facilitate future research on tracking daily movements and examining movement patterns for eastern box turtles within the Oak Openings Preserve.

Our results should be generalizable to other turtle species, which are excellent models for thread trailing and radio telemetry because they have a shell for easy attachment (Claussen, Finkler, & Smith 1997). Although, there may be some problems if aquatic turtles are used. Future research can examine other species using each of these methods. Thread trailing can be modified into a backpack that can be hooked onto other animals such as lizards, salamanders and frogs or toads or small mammals. Thread trailing is not an applicable method for tracking snakes, but fluorescent powder can be used to track their fine-scale movements, especially as a replacement method to radio telemetry. Fluorescent powder is light-weight (< 0.5 g) and is less than the smallest bobbin (~1.7 g) and greatly less than radio transmitters (~0.65 g) which will help decrease any impact on speed or movement by the organism (Furman, Scheffers, & Paszkowski 2011). Radio-tracked snakes require the implantation of the radio transmitter by removing them from the field and surgically implanting a radio transmitter and finally returning them to the field (Harvey & Weatherhead 2006). This can cause a large amount of stress and may alter their behaviors. Using fluorescent powder is a quick process and can minimize stress by capturing the snake and only applying a coating of fluorescent powder by using a bag. Fluorescent powder has an advantage over thread trailing and radio telemetry because it can monitor vertical movements and microhabitat use by organisms (Nicolas & Colyn 2007). Any vertical movements with thread trailing would increase entanglement problems and radio telemetry would only record location points and may miss vertical ascensions. Radio telemetry has been used with a variety of organisms, but complex environments can confuse the transmission signals making it difficult to relocate individuals, such as signal bouncing in

forested areas. This can increase the amount of time spent searching for an individual as compared to thread trailing where you locate the beginning of the trail and follow the string.

Radio telemetry has been the main method for examining animal movements because it allows researchers to relocate cryptic animals on a regular basis (Iglay, Bowman, & Nazdrowicz 2006). It is a costly method which requires expensive tracking equipment where each transmitter can cost $150 or more. Thread trailing and fluorescent powder are inexpensive alternatives and can provide more data for fine-scale movements; however they require more time investment. Radio telemetry allows researchers to monitor individuals with larger time gaps while thread trailing and fluorescent powder methods need to monitor individuals daily. This time gap may increase the chance of losing the individual completely if the signal is lost or transmitter falls off, while thread trailing and fluorescent powder gives the researcher a chance of relocating the individual before it travels too far away.

Each method had advantages and disadvantages based on the study species, study area and implementation of the techniques. Several studies have compared thread trailing and radio telemetry, but our research is the first that we have seen to compare thread trailing, fluorescent powder and radio telemetry. We found that fluorescent powder is another viable method for tracking box turtles in a heterogeneous habitat. We suggest that further work should be done, such as retrying thread trailing and using both radio telemetry and fluorescent powder together to consistently track box turtles.

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*Tables*

Table 4.1: Basic measurement data taken for each box turtle detected. Carapace length and width are in millimeters, age is in years, and weight is in grams. Abbreviations are: turtle 1 (1), turtle 2 (2), turtle 3 (3), turtle 4 (4), turtle 5 (5), turtle 6 (6), carapace length (CL), carapace width (CW), male (M), female (F), and weight (WT).

| | Date | Time | CL | CW | Sex | PIT tag ID | Age | WT | Behavior |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 6/2 | 9:01 AM | 152.78 | 113.01 | M | | 15 | >300 | Cross pavement |
| 2 | 6/21 | 12:08 PM | 114.92 | 116.94 | M | 031023850 | 15 | >300 | Cross trail |
| 3 | 6/29 | 1:48 PM | 114.88 | 90.13 | F | 031039864 | 10 | 275 | Sit by log |
| 4 | 8/13 | 10:37 AM | 153.56 | 112.80 | M | 011271605 | 10 | >300 | Sit in leaf litter |
| 5 | 8/15 | 11:58 AM | 126.1 | 111.59 | M | 031036585 | 12 | >300 | Sit in plants |
| 6 | 8/19 | 1:34 PM | 160.35 | 122.43 | M | 030888853 | 16 | >300 | Sit in leaf litter |

Table 4.2: The distance (in meters) traveled for each turtle per day for each tracking method. Radio telemetry data is the straight-line distance traveled between two data points. Data not collected is symbolized by -. Abbreviations are: turtle 1 (1), turtle 2 (2), turtle 3 (3), turtle 4 (4), turtle 5 (5), turtle 6 (6), and radio telemetry (RT).

| | Fluorescent Powder | | | | | | | | | | Thread Trailing | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 1 | 2 | 3 |
| T1 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| T2 | 22 | 87 | - | - | - | - | - | - | - | - | 9.0 | - | - |
| T3 | 39 | - | - | - | - | - | - | - | - | - | 34 | - | - |
| T4 | 87 | 72 | 12 | 53 | 27 | 11 | 39 | 13 | 54 | 39 | - | - | - |
| T5 | 15 | 63 | 54 | 67 | - | - | - | - | - | - | 24 | 57 | 45 |
| T6 | - | - | - | - | - | - | - | - | - | - | 29 | - | - |
| T4 RT | 12 | 47 | 38 | 15 | 20 | 10 | 12 | 5.0 | 5.0 | 4.0 | 9.0 | 47 | 10 |
| T4 RT continued | | | | | | | | | 16 | 8.0 | 14 | 47 | 23 |

*Figures*

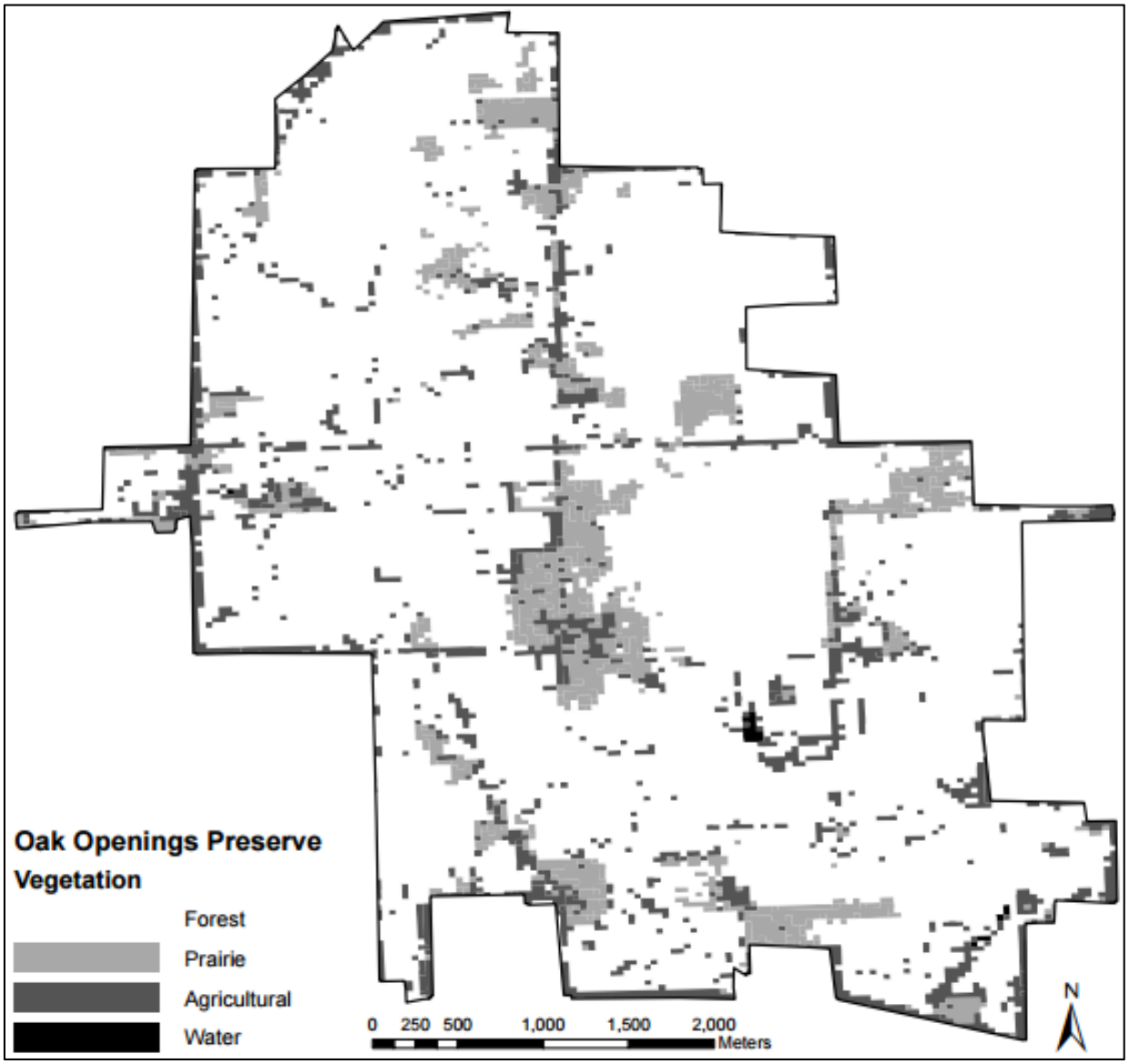


Figure 4.1: The Oak Openings Preserve with simplified land cover with four types: forest, prairie, agricultural and water based on Schetter & Root (2011).


Figure 4.2: Attachment of thread trailer onto turtle 2 (A) and modified thread trailer attached to turtle 3 (B).

Figure 4.3: Turtle 3 with orange fluorescent powder mixture applied to its plastron.

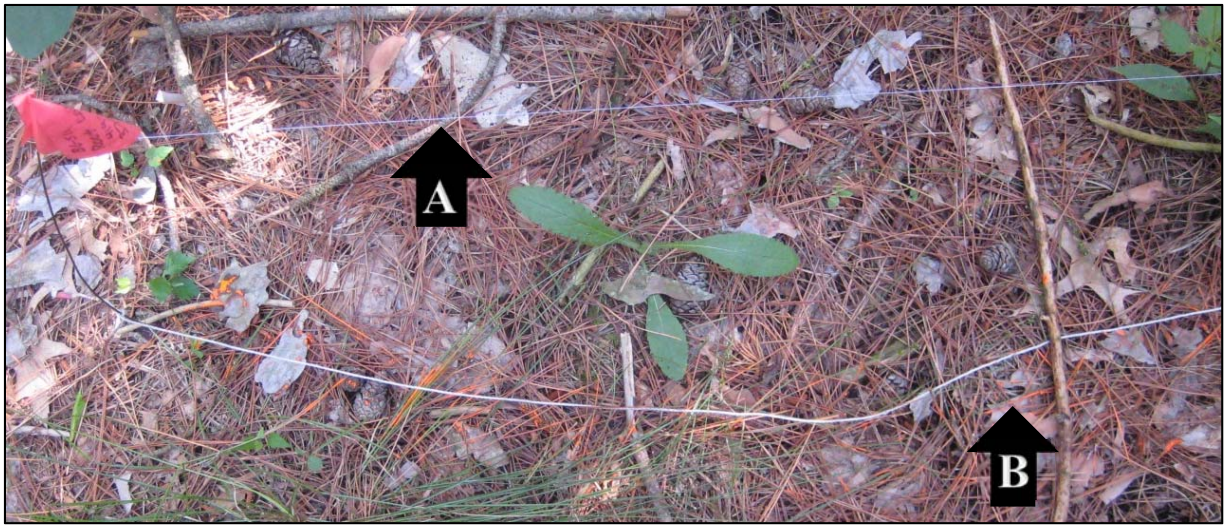


Figure 4.4: Thread (A) and fluorescent powder (B) trail for turtle 2 starting at flag marker one.

Figure 4.5: Turtle 3 with radio transmitter applied onto the left backside of its carapace.

# CONCLUSIONS

Herpetofauna are an essential part of the ecological environment and we have laid foundational work for future studies. Chapter 1 shows that the Oak Openings Preserve is diverse for both amphibians and reptiles, even though fewer reptiles were detected. We found that Urodela were widely distributed across the preserve and identified important informational gaps within our data. For example, we need greater research efforts to examine Urodela diversity, only two species were detected, and reptile abundances, only 11 Squamata were detected. Our data can be generalized to other parks within this diverse region because they share similar land cover features and herpetofauna diversity and distributions should follow similar spatial patterns.

Chapter 2 dives into the methodology behind our research efforts. Few studies examine how their methods influenced the results of the surveys. We used visual encounter surveys instead of invasive trapping methods in order to cover an extensive area. Our results support that visual encounter surveys are adequate for detecting herpetofauna, which can help reduce monitoring costs. Visual searches are inexpensive and can provide valuable data especially when used over a long period of time. Our quadrat method is easy to set up and observers can be trained quickly while detection data can be collected and the use of photographs can allow experts to identify the species and confirm species identification. With successive use, researchers can examine temporal and spatial dynamics of herpetofauna communities. However, we were unsuccessful in detecting reptiles using the quadrat method and there may be a need to employ other methods such as pitfall traps, drift fences or searching refugia in order to better understand reptile diversity. These visual encounter methods are very suitable for long-term monitoring programs and citizen science data collection.

Chapter 3 examined how the environment influenced the displayed spatial patterns within the Oak Openings Preserve. We found that each species varied and some species were more likely to be found in areas with other species and others did not spatially overlap with one another. We found that herpetofauna were considered to have a clustered distribution; however many of the individual species had dispersed distributions. These species with dispersed distributions had a small sample size ($n < 11$) except for two species (*Plethodon cinereus*, $n = 369$, and *Pseudacris crucifer*, $n = 136$). We may have seen more clustered distributions if we had a greater sample size for many of the herpetofauna species. This suggests that managers should consider the impacts for multiple species within a management area and can utilize our species maps to help identify which species may be impacted. We expected a large portion of amphibians to occupy areas near streams and few individuals to be found near roads; very few individuals were found near streams and roads. High temperatures were associated with Squamata and high humidity was associated with Anura. This may be as result of temperature being important for behavioral regulation in reptiles and humidity being important for hydration in amphibians. We found that herpetofauna tended to occupy forested areas and the important ground cover vegetation are leaf litter, coarse woody debris, conifer needles, moist soil and plants which can all be used for shelter. This suggests that managers should not remove ground cover vegetation to support herpetofauna biodiversity.

Chapter 4 examined the fine-scale movements of eastern box turtles using thread trailing, fluorescent powder and radio telemetry. We found that both thread trailing and radio telemetry underestimated distance traveled when compared to fluorescent powder. Movement patterns contained both linear and non-linear pathways, but thread trailing tracks were more linear than those found using fluorescent powder. We have determined that fluorescent powder is a useful

tool for examining movement patterns because it is less invasive, provides more detailed movement pathways and does not underestimate distances traveled. Although rainfall may make it difficult to use this method, applying both fluorescent powder and radio telemetry would be an effective approach to monitor box turtle or other species movements.

Overall, this research has addressed informational gaps for herpetofauna biodiversity. We have addressed important ecological questions using different scales (e.g. fine-scale, local, landscape, temporal, and spatial). This work goes beyond basic questions of what species are present and how many, but has examined the environmental influences driving species presence-absence. Not only have we described important diversity estimates, but our results can be directly related for management implications and be used to help conserve and protect herpetofauna biodiversity. Using these methods, we can work with managers to protect and monitor highly underappreciated taxa while still protecting charismatic mammals and birds.

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# APPENDIX A: SPECIES DISTRIBUTION MAPS

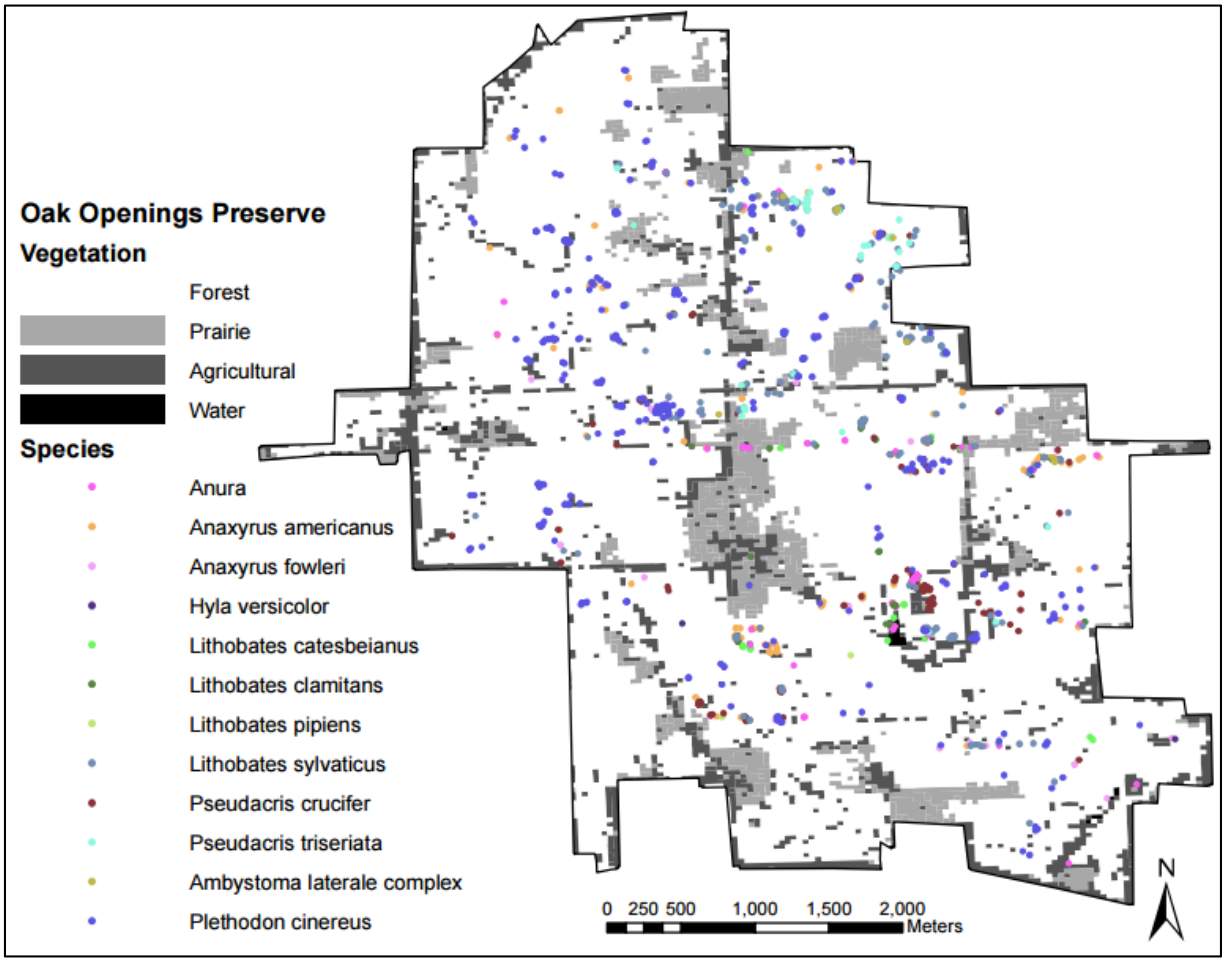



Figure A.1: Spatial locations of each amphibian individual sampled within the Oak Openings Preserve with land cover categorized into four types: forest, prairie, agricultural and water.

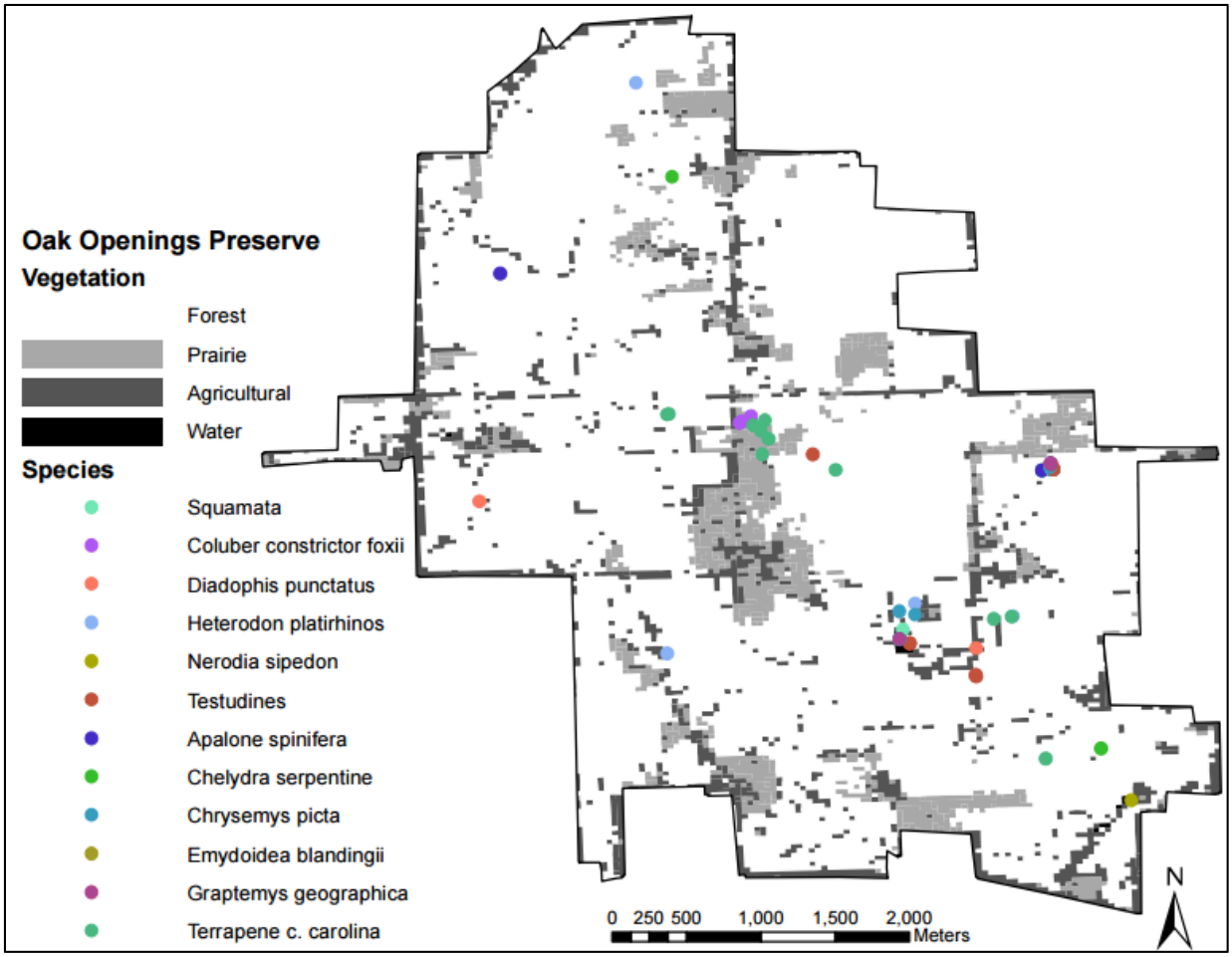



Figure A.2: Spatial locations of each reptile individual sampled within the Oak Openings Preserve with land cover categorized into four types: forest, prairie, agricultural and water.

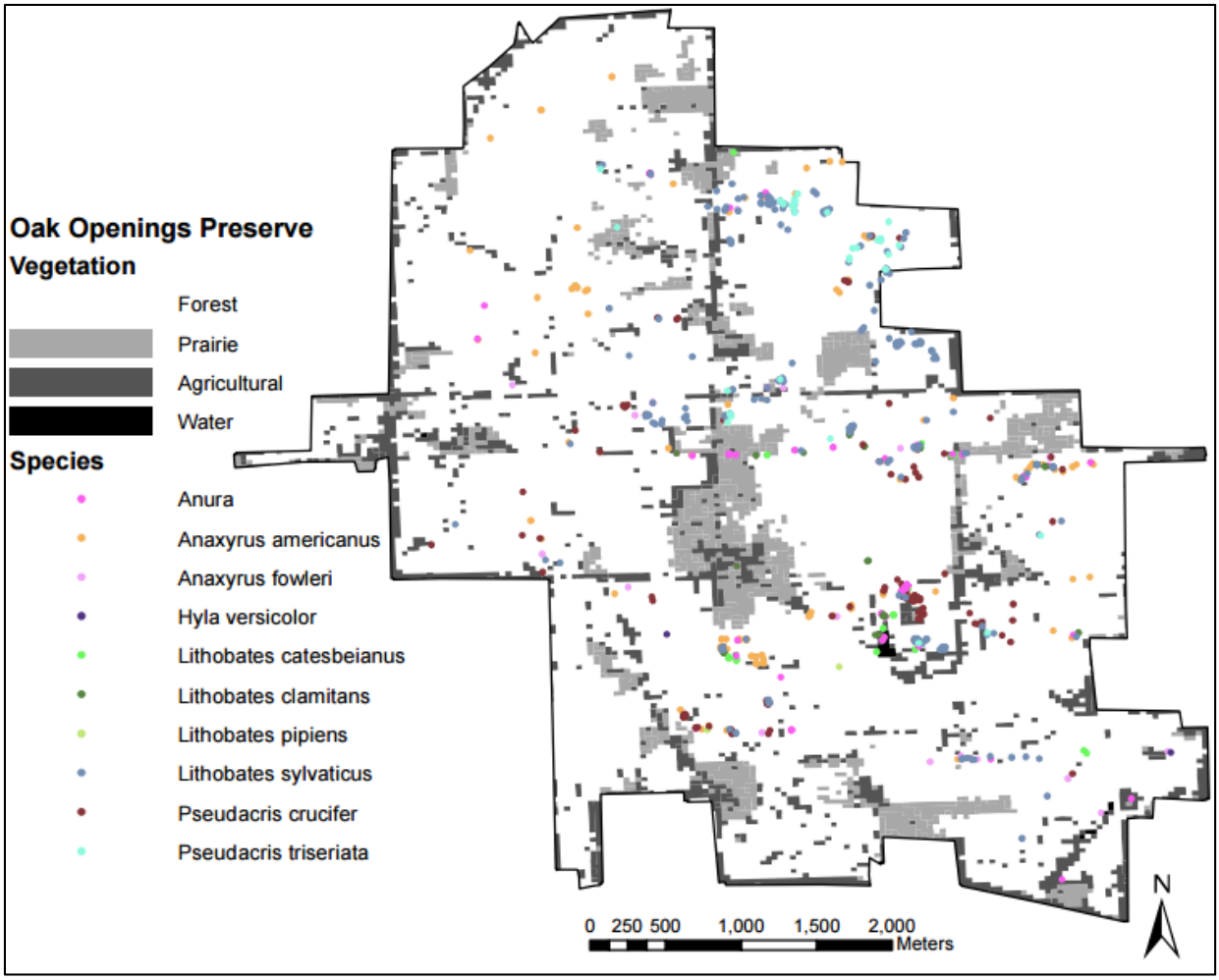



Figure A.3: Spatial locations of each Anura individual sampled within the Oak Openings Preserve with land cover categorized into four types: forest, prairie, agricultural and water.

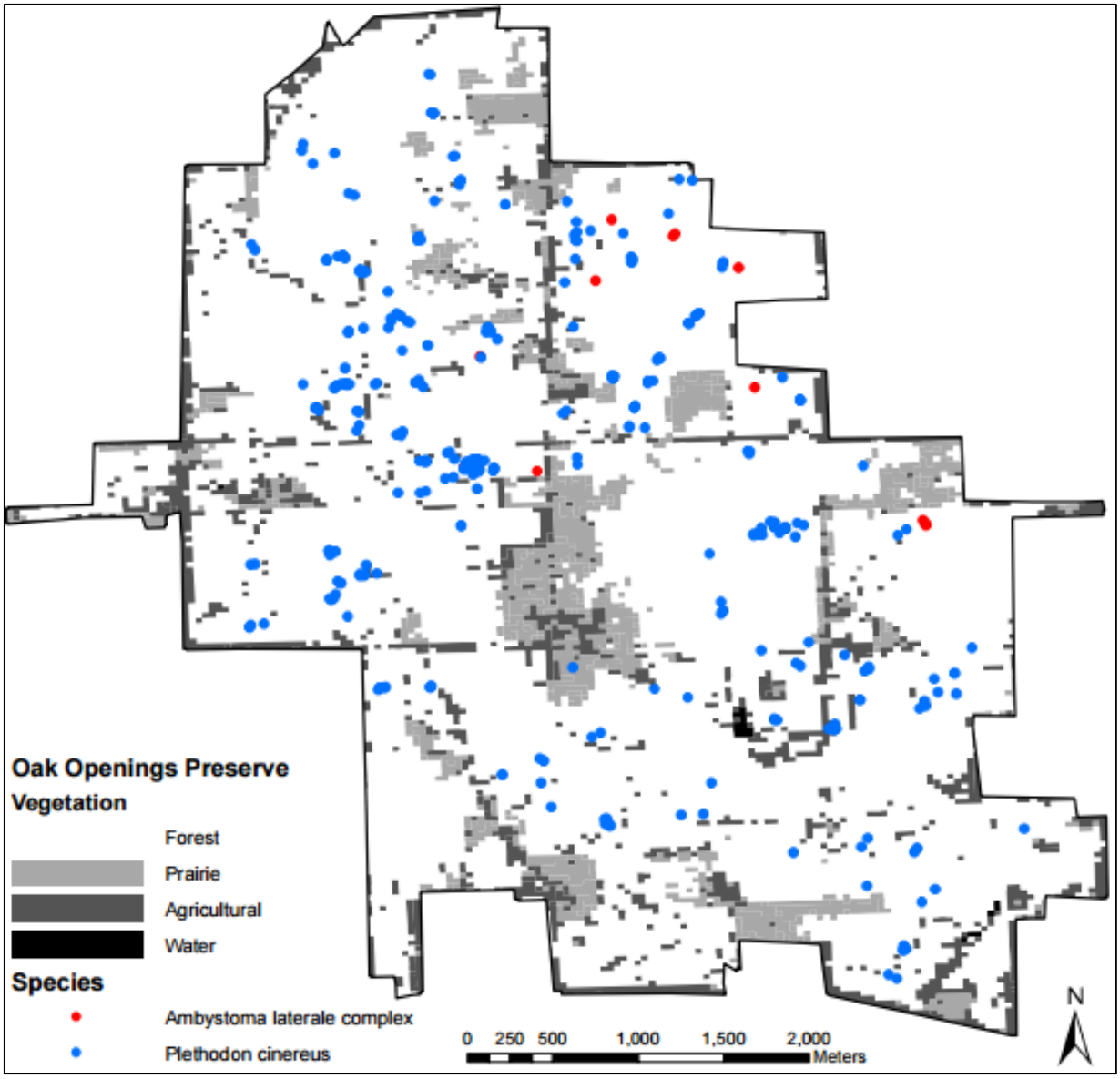



Figure A.4: Spatial locations of each Urodela individual sampled within the Oak Openings Preserve with land cover categorized into four types: forest, prairie, agricultural and water.

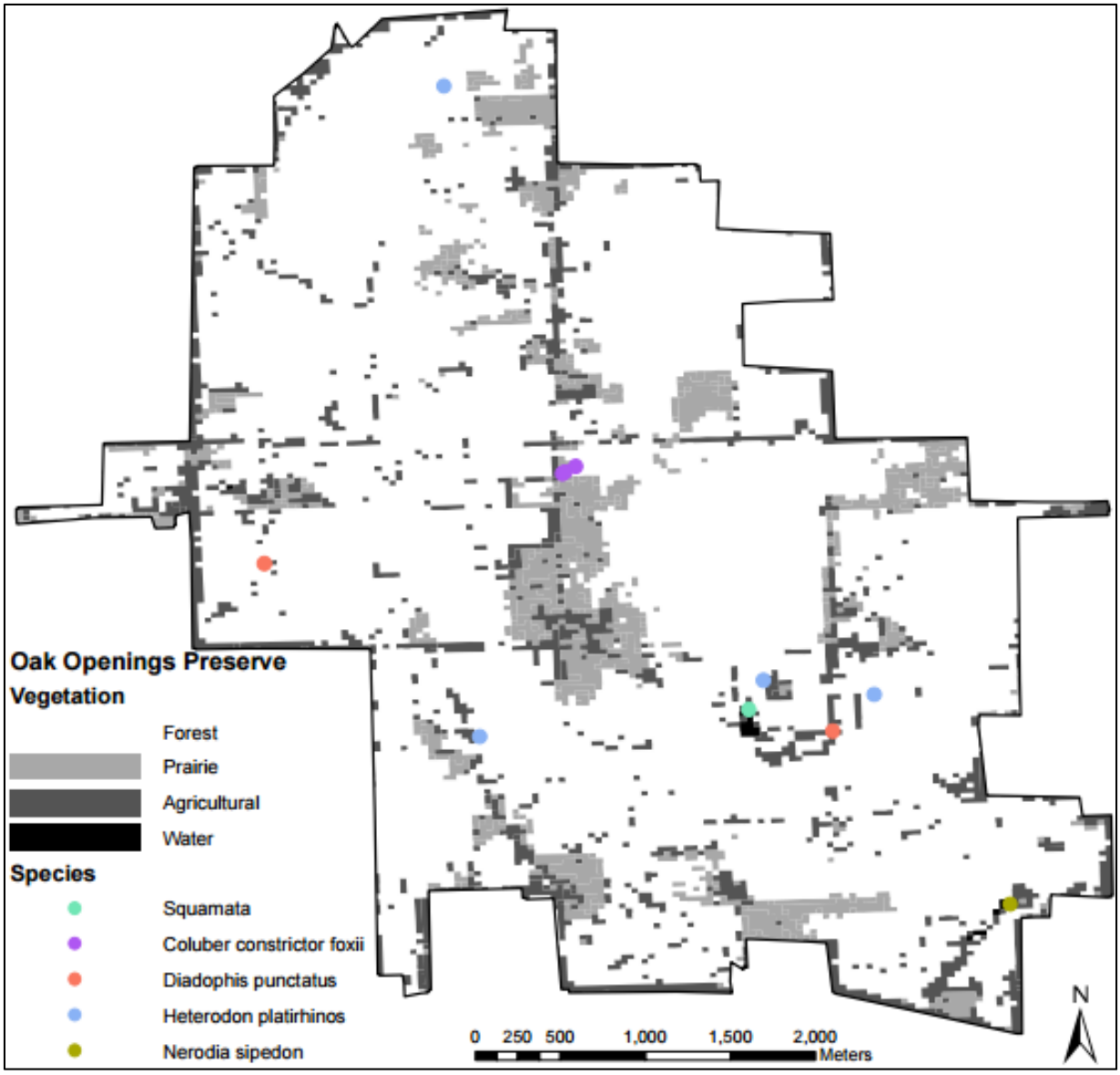



Figure A.5: Spatial locations of each Squamata individual sampled within the Oak Openings Preserve with land cover categorized into four types: forest, prairie, agricultural and water.

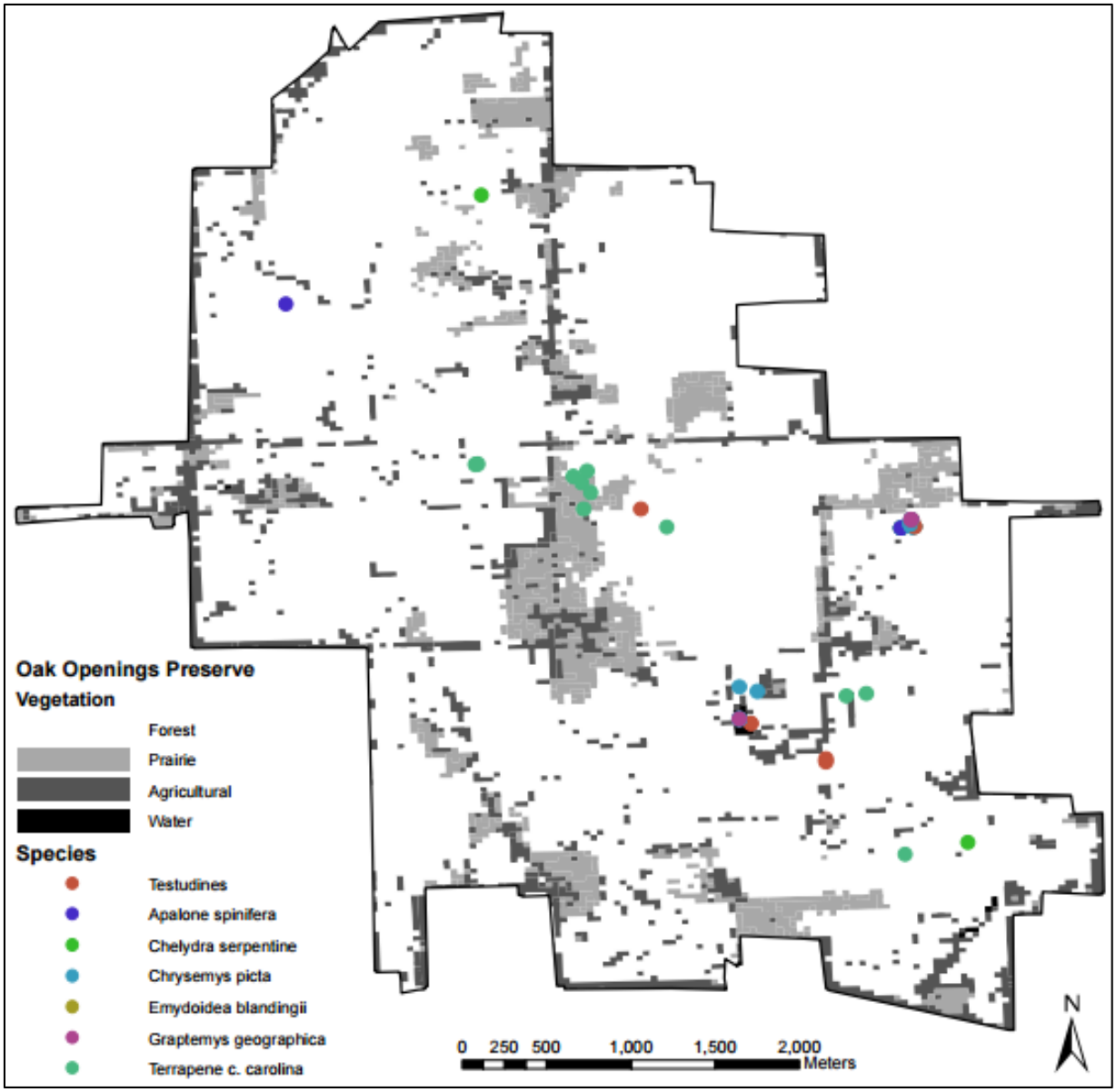



Figure A.6: Spatial locations of each Testudines individual sampled within the Oak Openings Preserve with land cover categorized into four types: forest, prairie, agricultural and water.

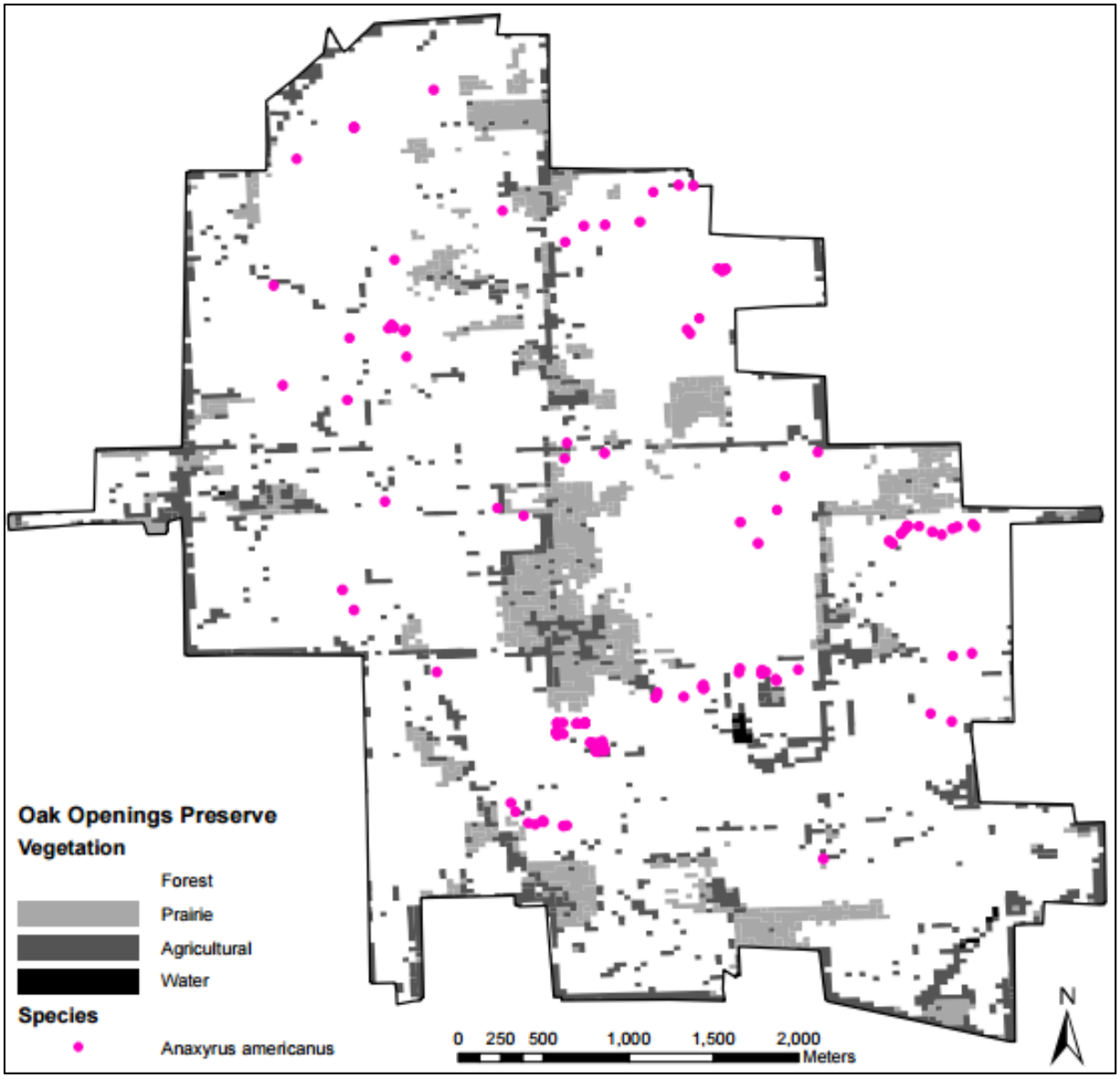



Figure A.7: Spatial locations of each *Anaxyrus americanus* individual sampled within the Oak Openings Preserve with land cover categorized into four types: forest, prairie, agricultural and water.

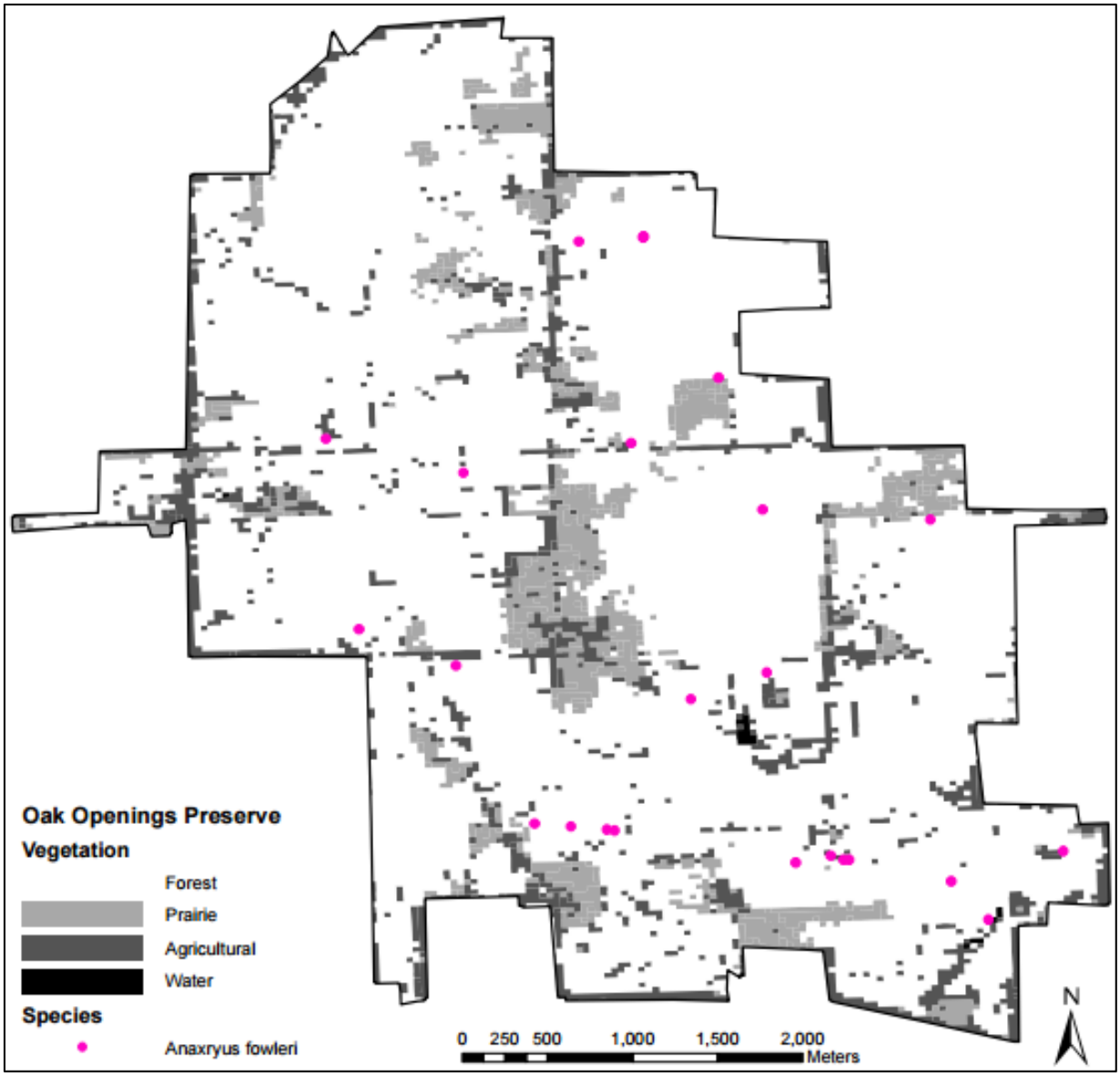



Figure A.8: Spatial locations of each *Anaxyrus fowleri* individual sampled within the Oak Openings Preserve with land cover categorized into four types: forest, prairie, agricultural and water.

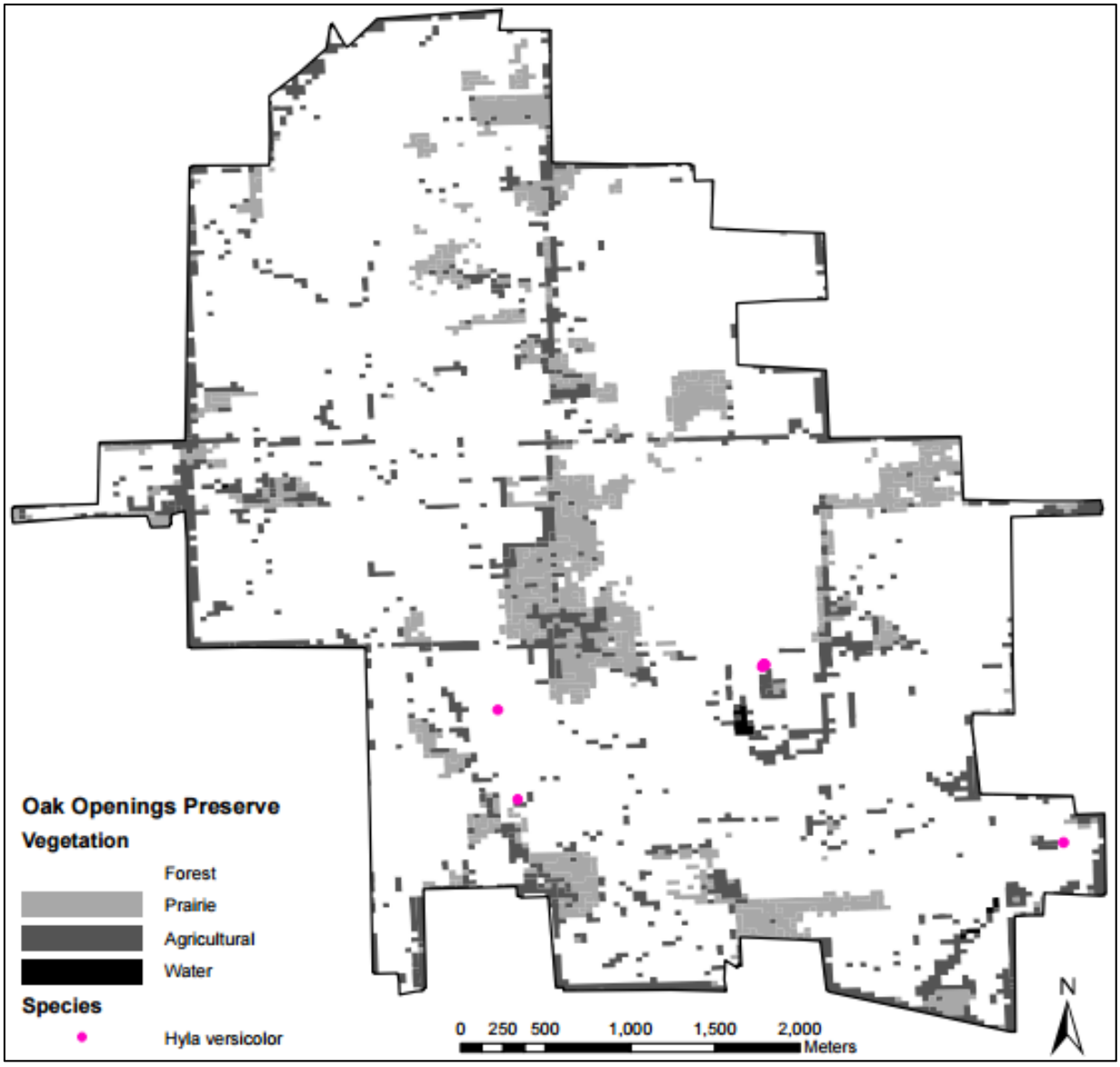



Figure A.9: Spatial locations of each *Hyla versicolor* individual sampled within the Oak Openings Preserve with land cover categorized into four types: forest, prairie, agricultural and water.

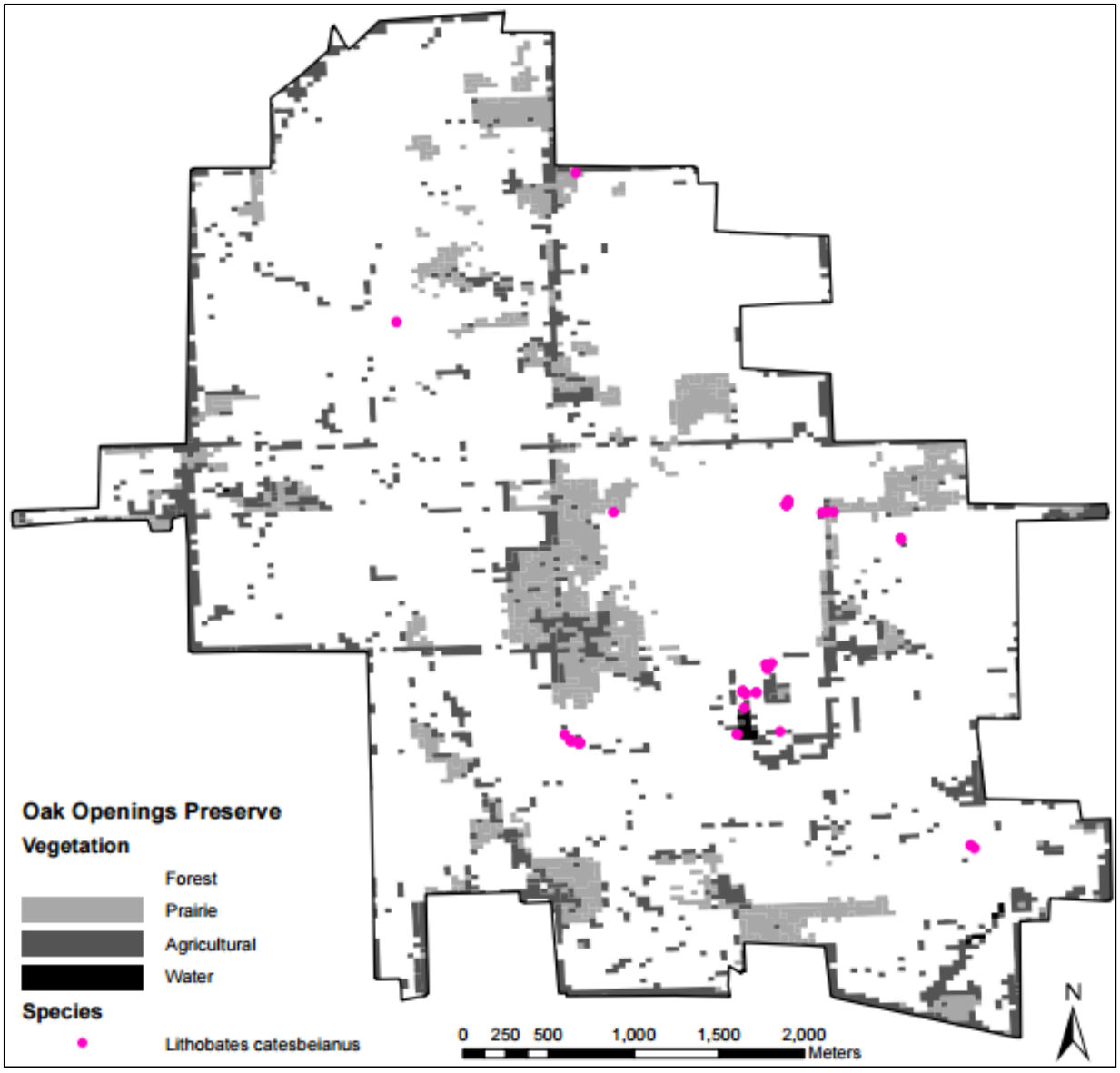



Figure A.10: Spatial locations of each *Lithobates catesbeianus* individual sampled within the Oak Openings Preserve with land cover categorized into four types: forest, prairie, agricultural and water.

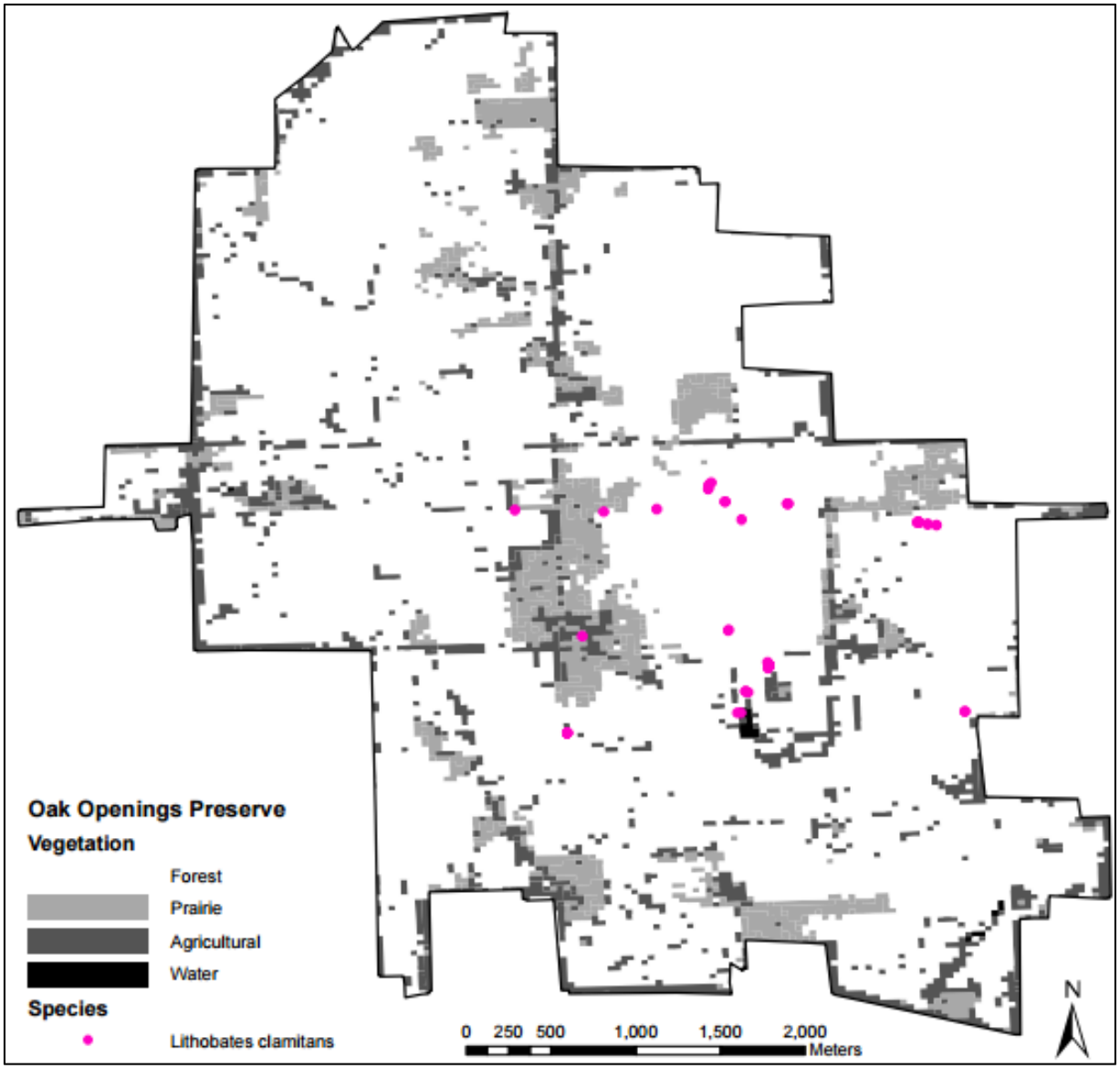



Figure A.11: Spatial locations of each *Lithobates clamitans* individual sampled within the Oak Openings Preserve with land cover categorized into four types: forest, prairie, agricultural and water.

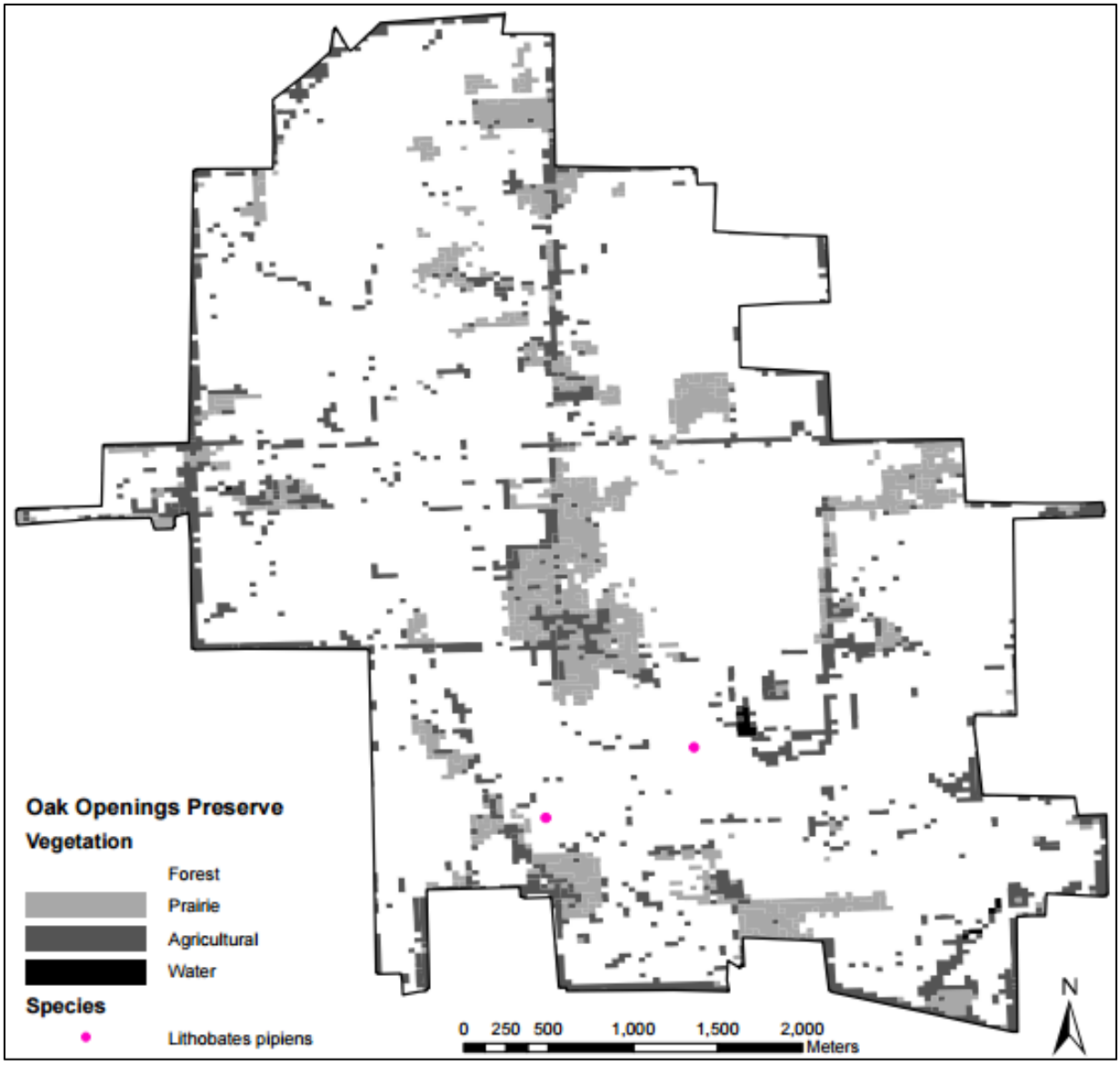



Figure A.12: Spatial locations of each *Lithobates pipiens* individual sampled within the Oak Openings Preserve with land cover categorized into four types: forest, prairie, agricultural and water.

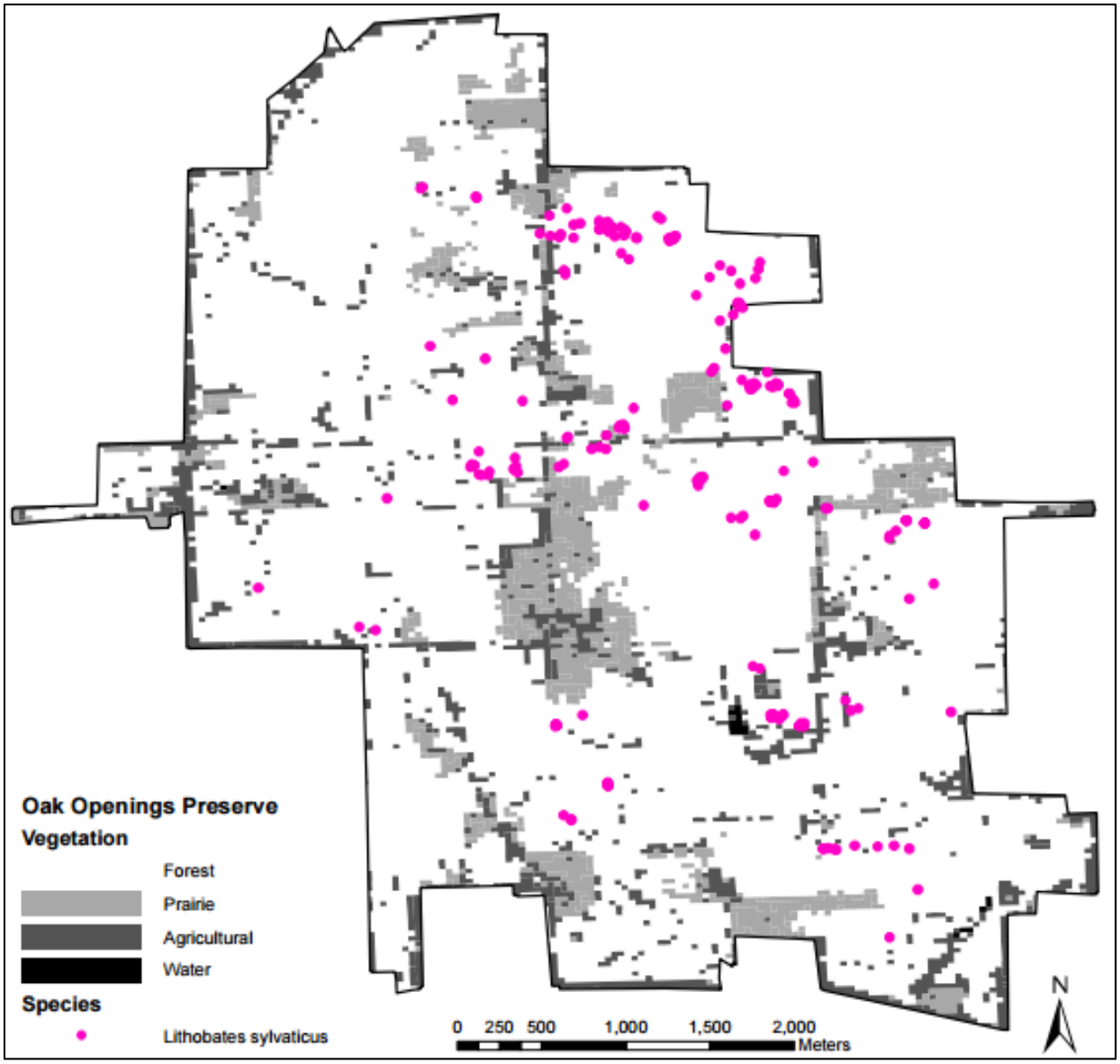



Figure A.13: Spatial locations of each *Lithobates sylvaticus* individual sampled within the Oak Openings Preserve with land cover categorized into four types: forest, prairie, agricultural and water.

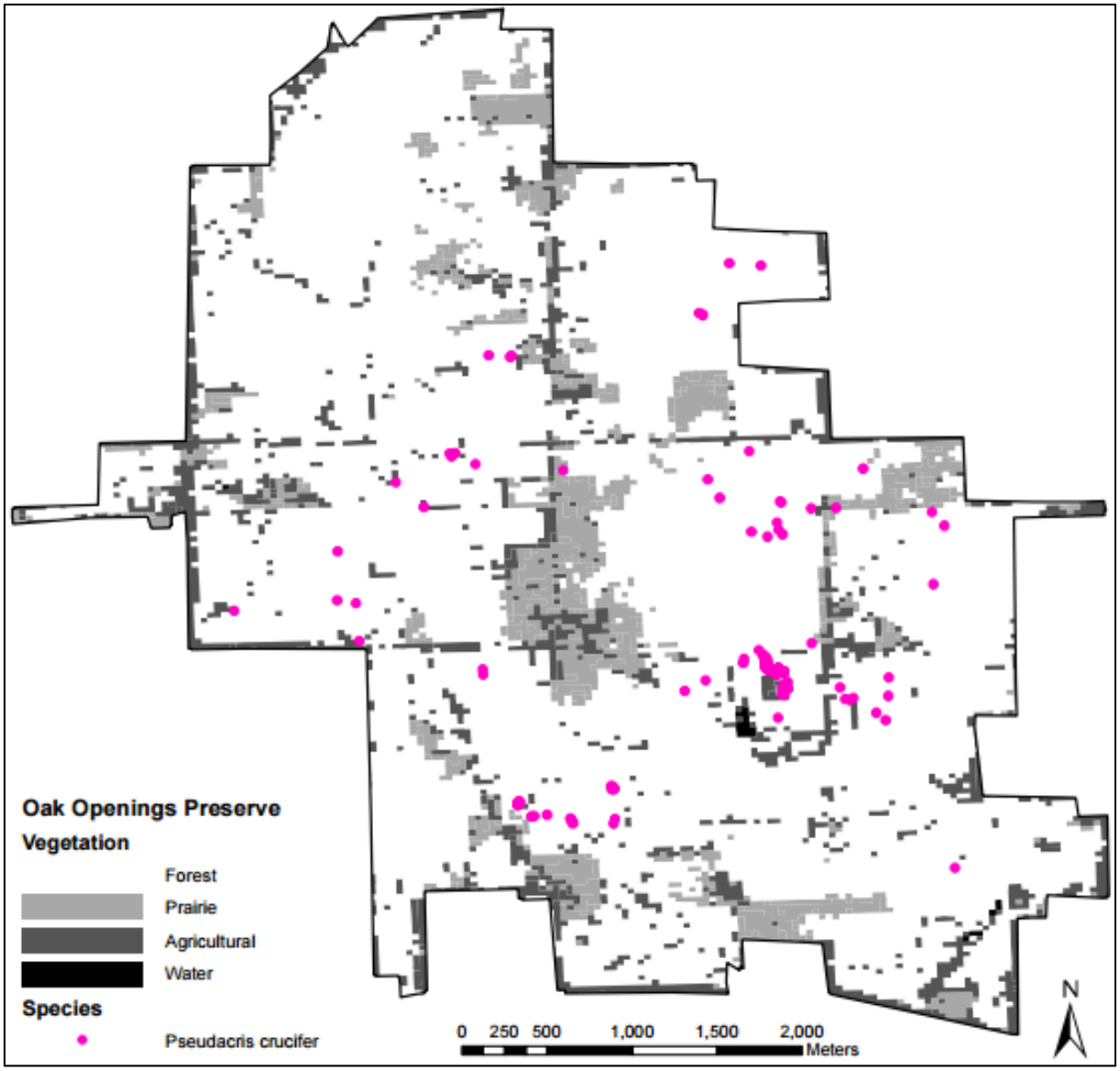



Figure A.14: Spatial locations of each *Pseudacris crucifer* individual sampled within the Oak Openings Preserve with land cover categorized into four types: forest, prairie, agricultural and water.

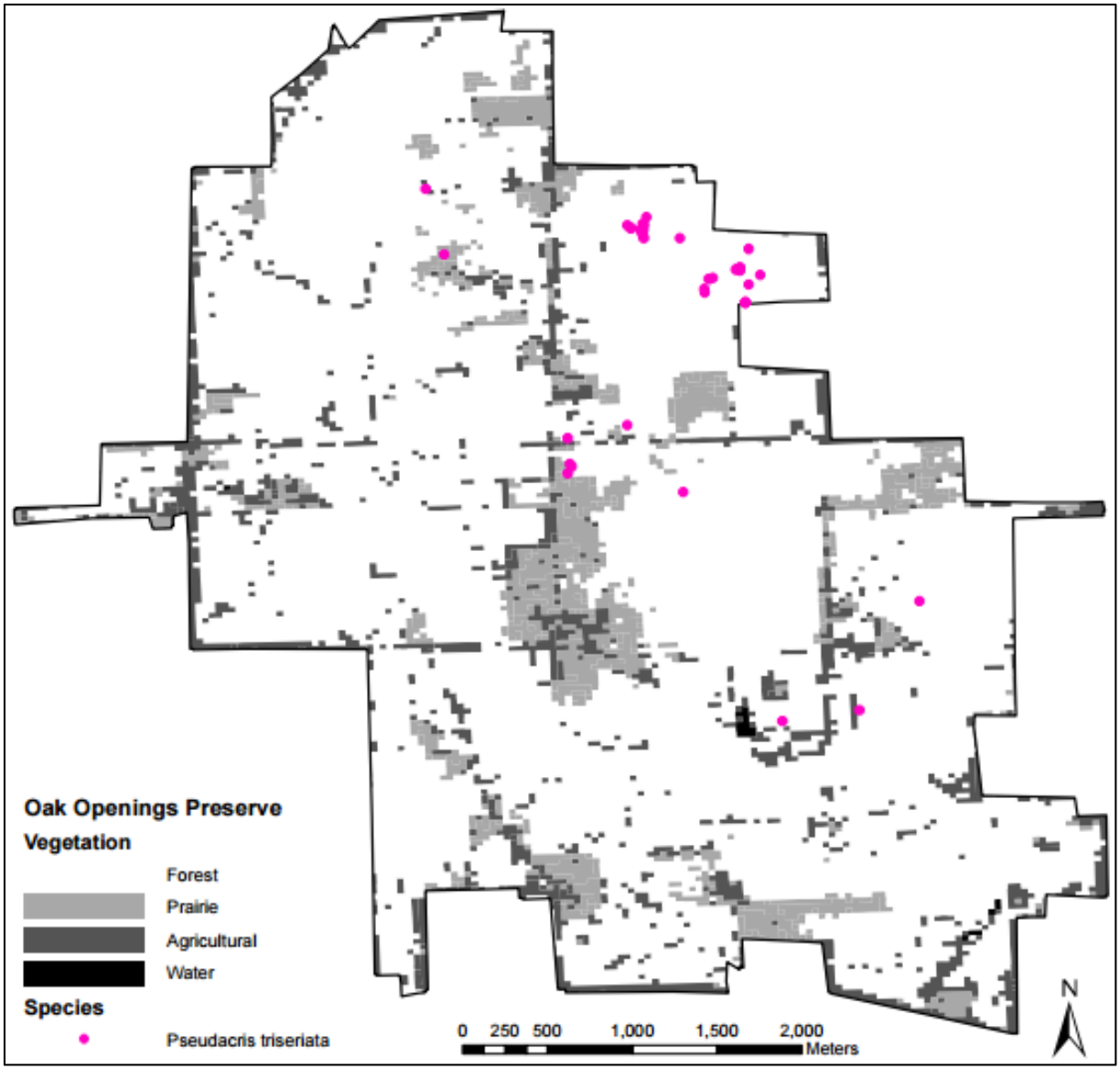



Figure A.15: Spatial locations of each *Pseudacris triseriata* individual sampled within the Oak Openings Preserve with land cover categorized into four types: forest, prairie, agricultural and water.

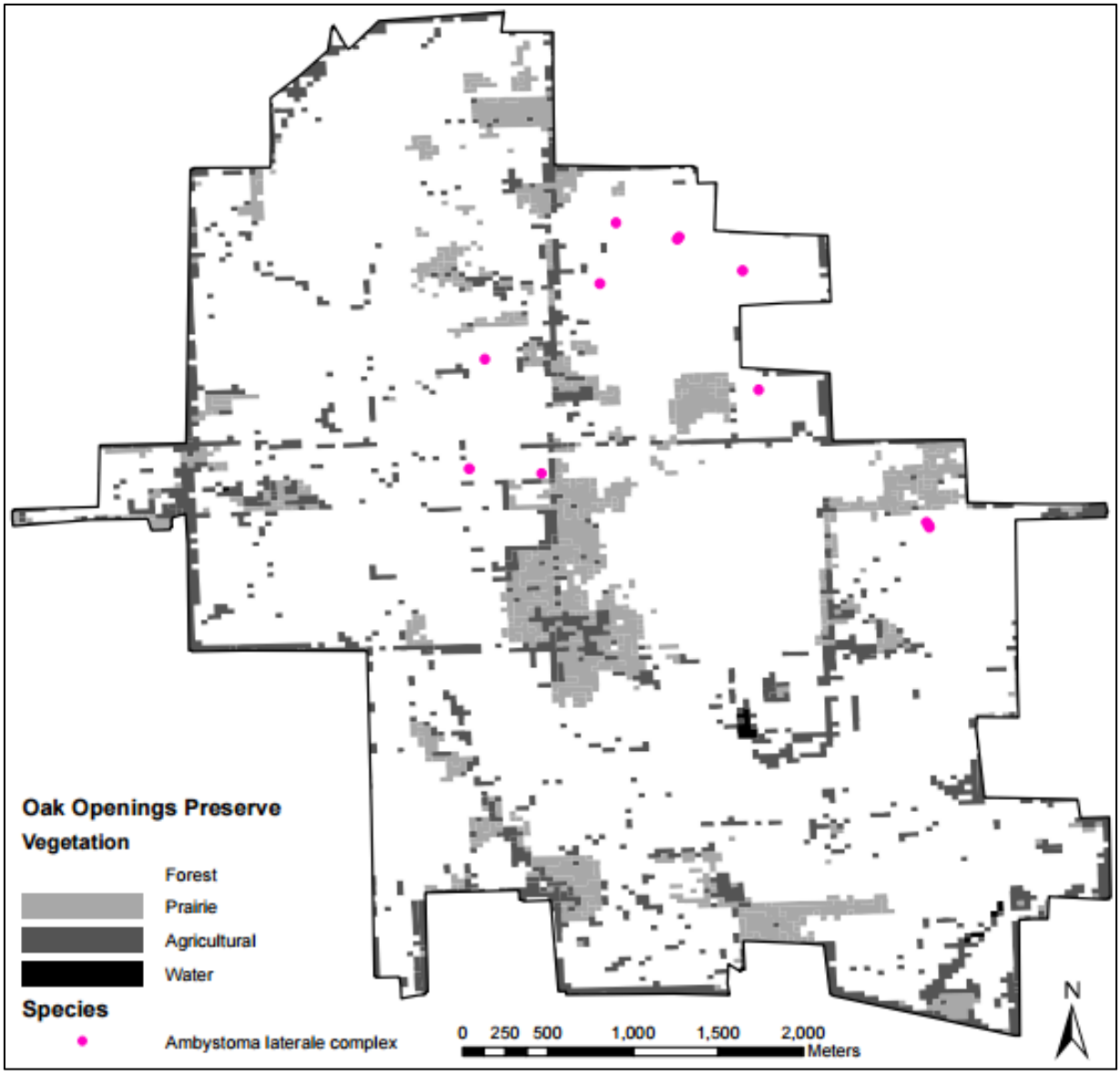



Figure A.16: Spatial locations of each *Ambystoma laterale* complex individual sampled within the Oak Openings Preserve with land cover categorized into four types: forest, prairie, agricultural and water.

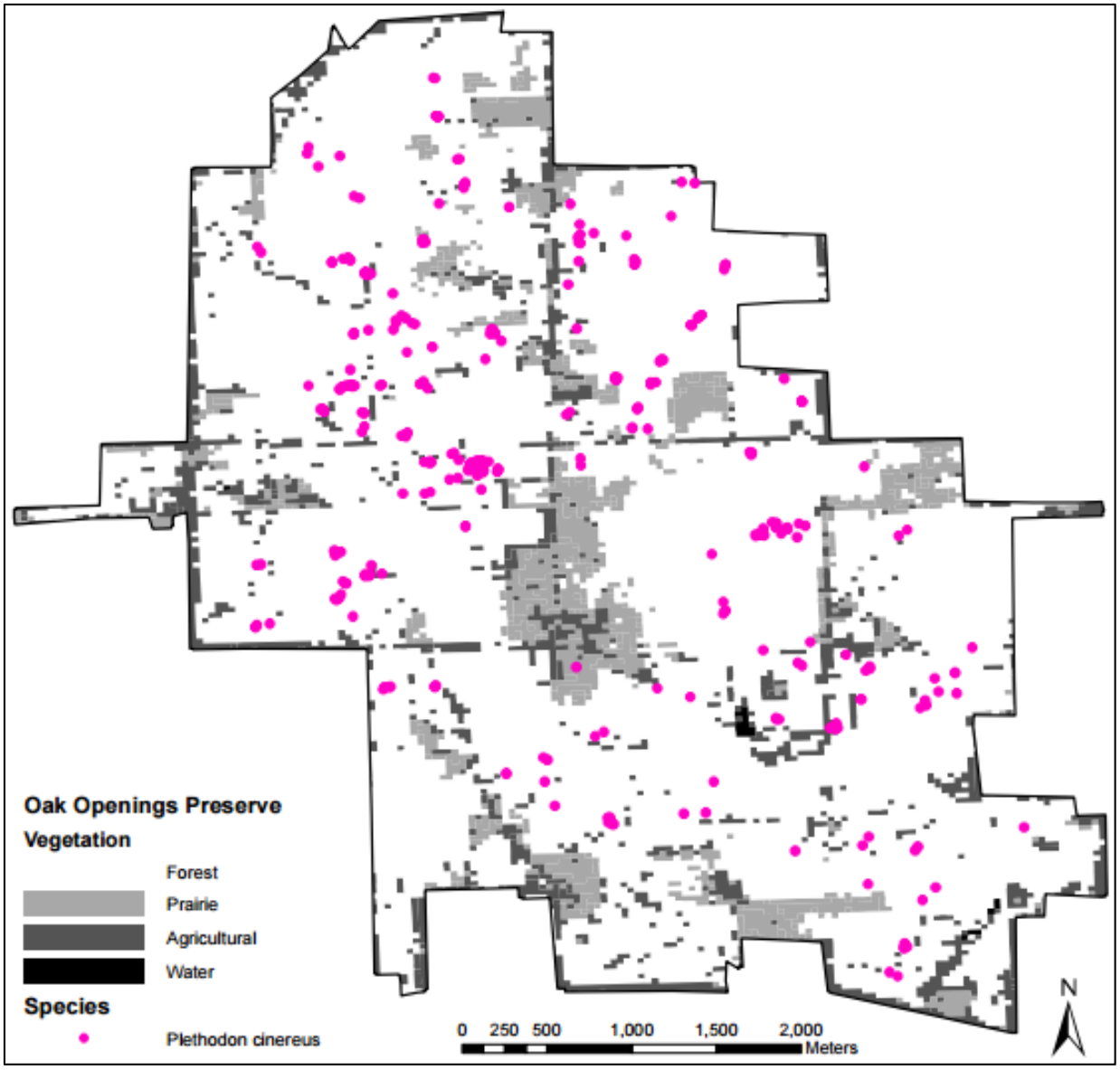



Figure A.17: Spatial locations of each *Plethodon cinereus* individual sampled within the Oak Openings Preserve with land cover categorized into four types: forest, prairie, agricultural and water.

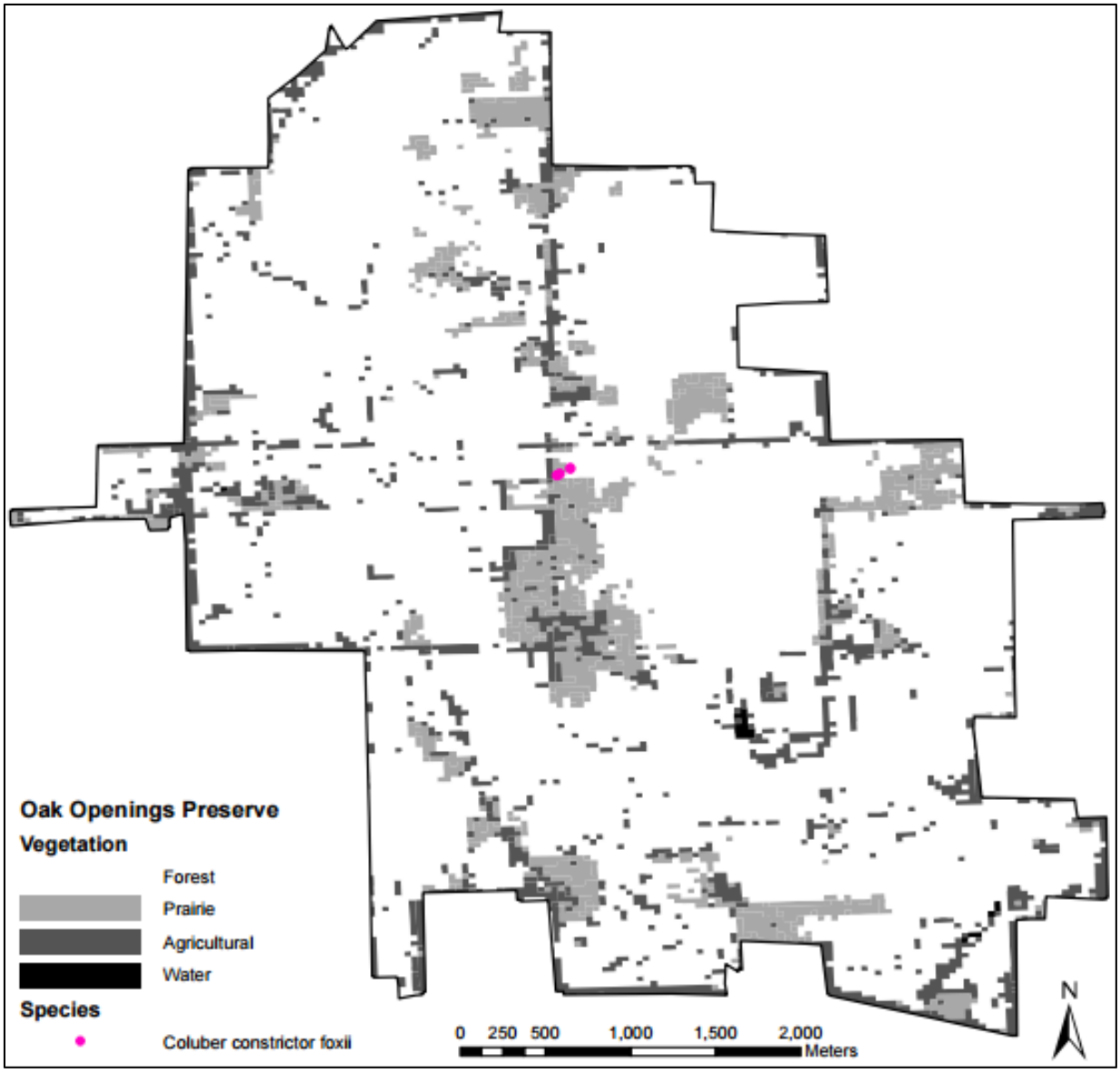



Figure A.18: Spatial locations of each *Coluber constrictor foxii* individual sampled within the Oak Openings Preserve with land cover categorized into four types: forest, prairie, agricultural and water.

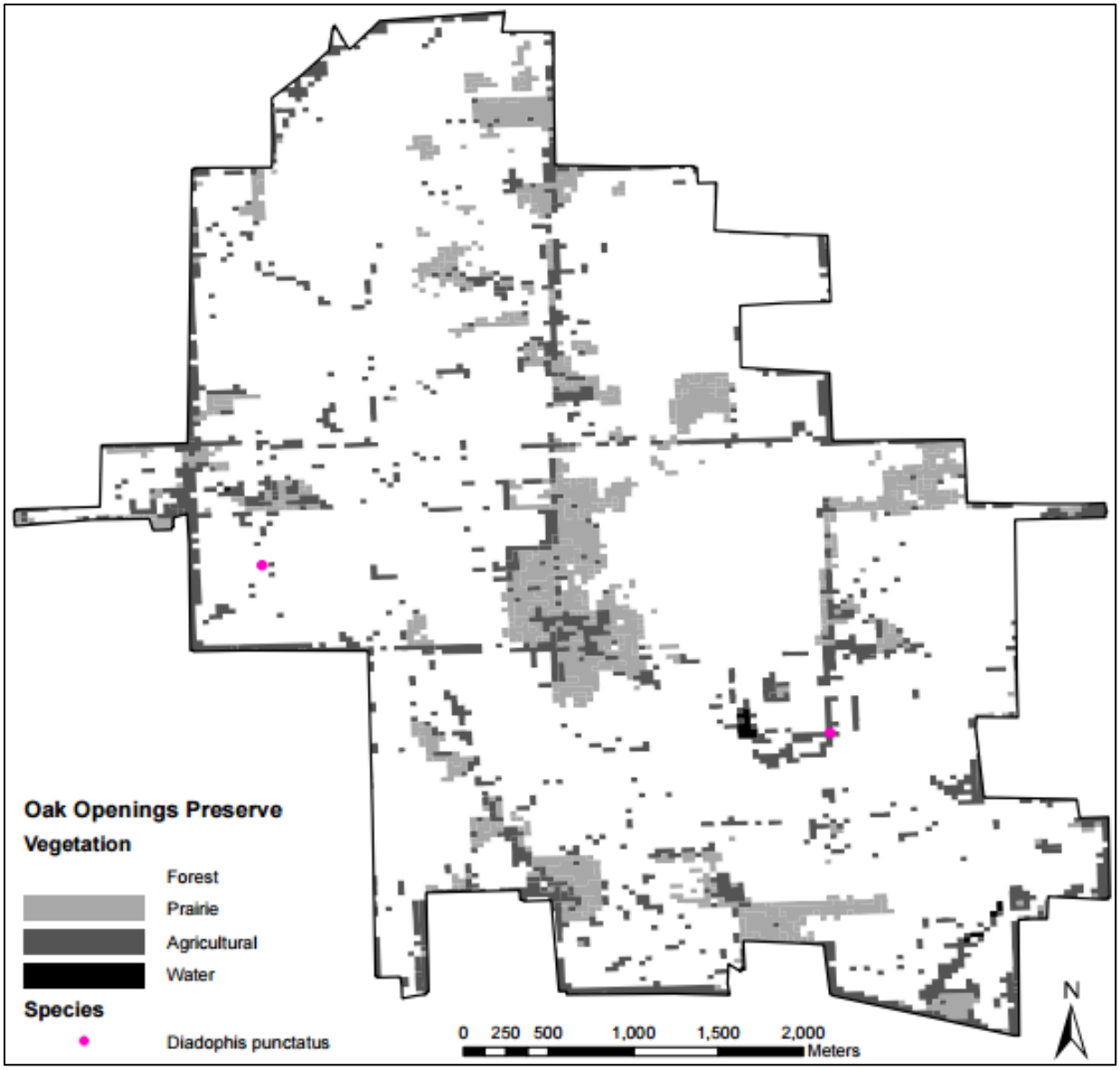



Figure A.19: Spatial locations of each *Diadophis punctatus* individual sampled within the Oak Openings Preserve with land cover categorized into four types: forest, prairie, agricultural and water.

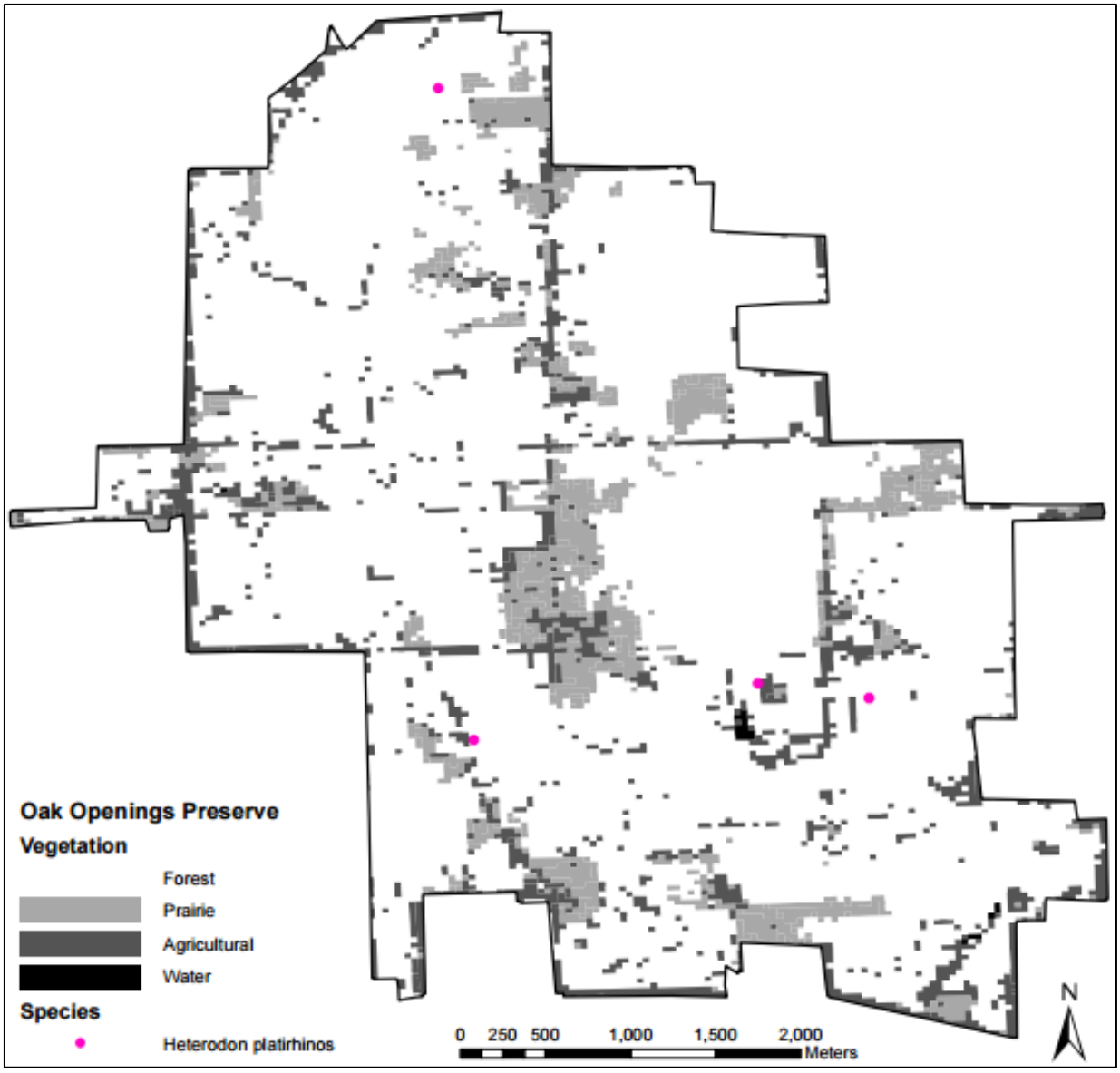



Figure A.20: Spatial locations of each *Heterodon platirhinos* individual sampled within the Oak Openings Preserve with land cover categorized into four types: forest, prairie, agricultural and water.

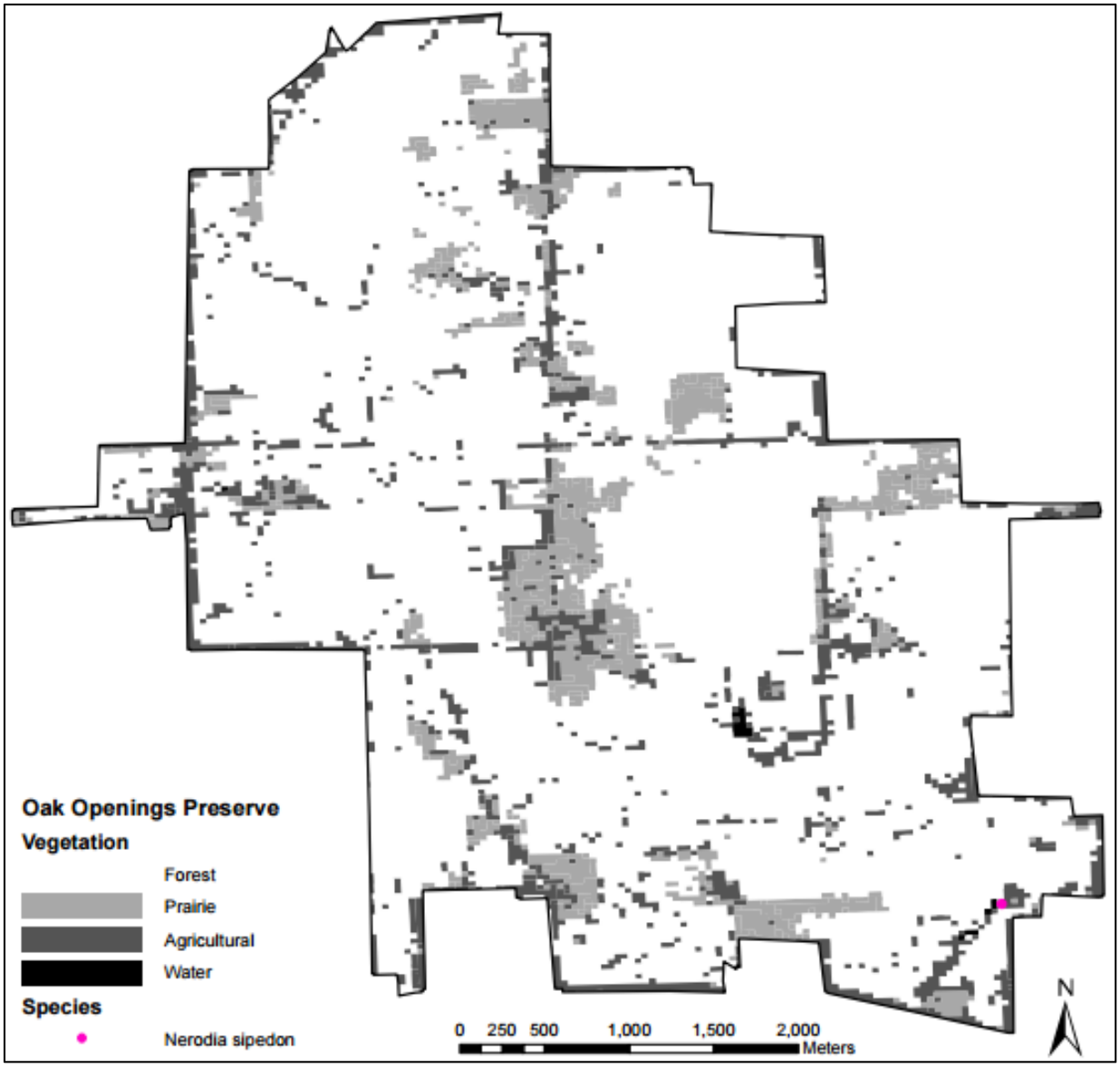



Figure A.21: Spatial locations of each *Nerodia sipedon* individual sampled within the Oak Openings Preserve with land cover categorized into four types: forest, prairie, agricultural and water.

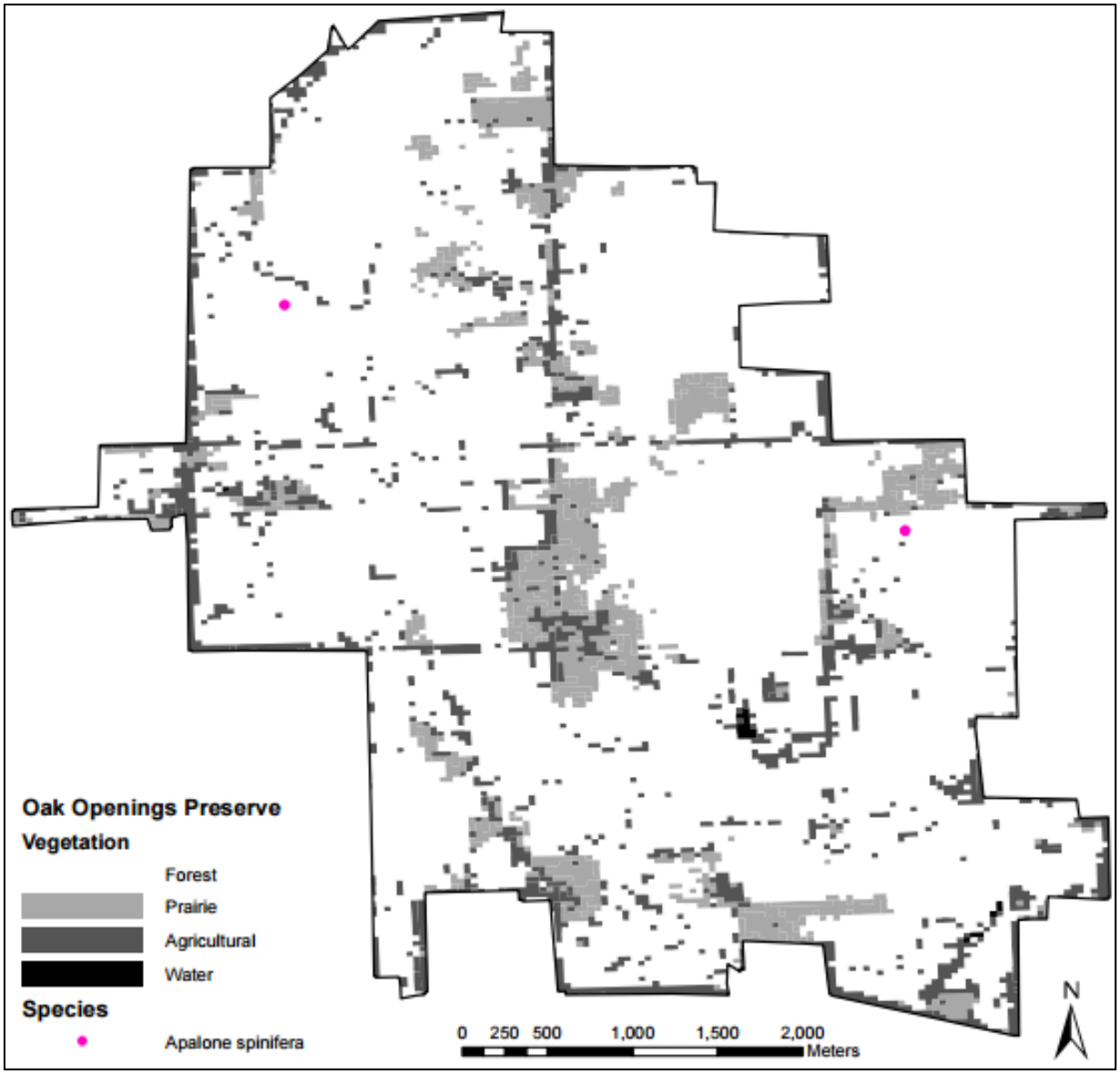



Figure A.22: Spatial locations of each *Apalone spinifera* individual sampled within the Oak Openings Preserve with land cover categorized into four types: forest, prairie, agricultural and water.

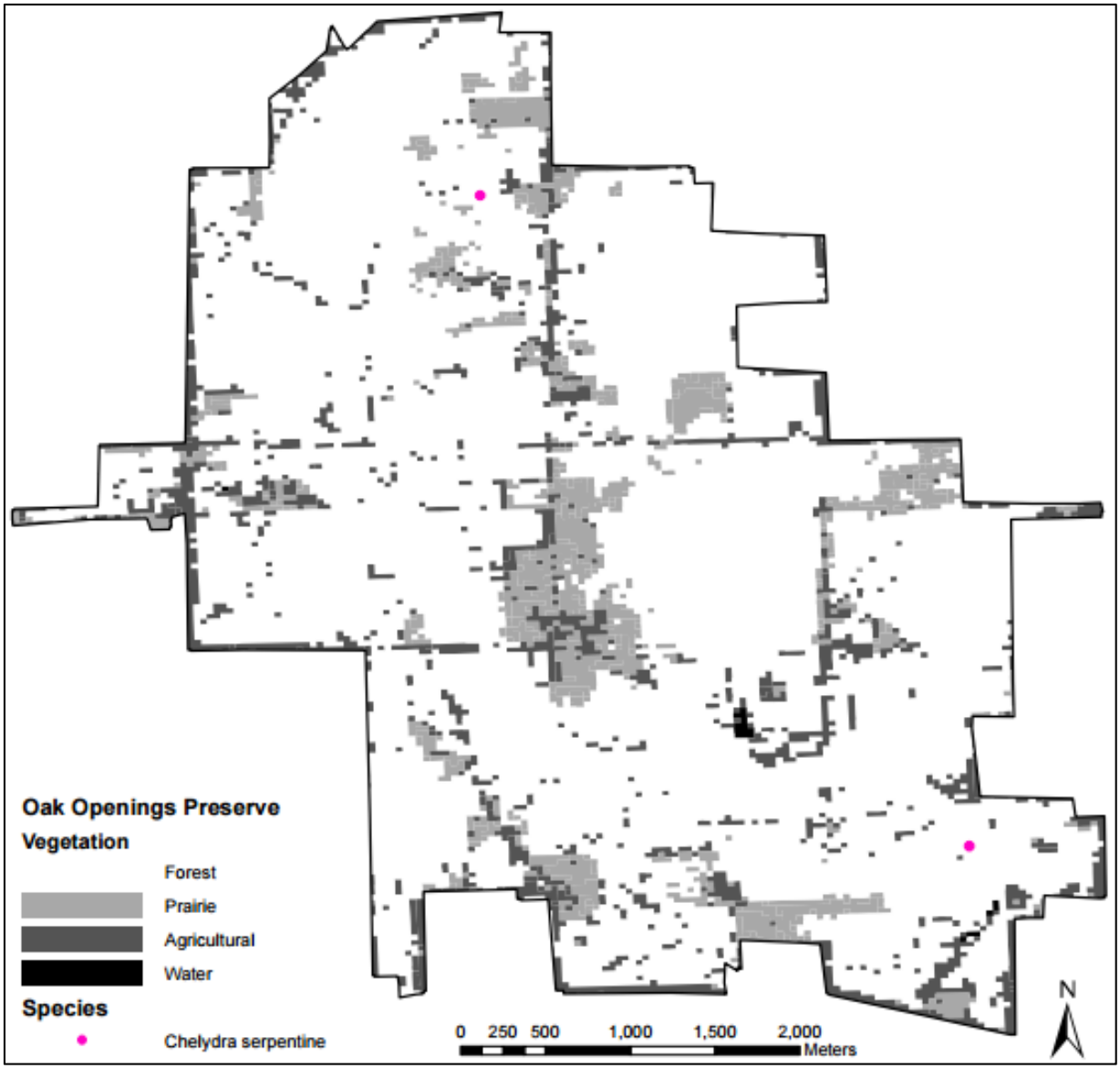



Figure A.23: Spatial locations of each *Chelydra serpentina* individual sampled within the Oak Openings Preserve with land cover categorized into four types: forest, prairie, agricultural and water.

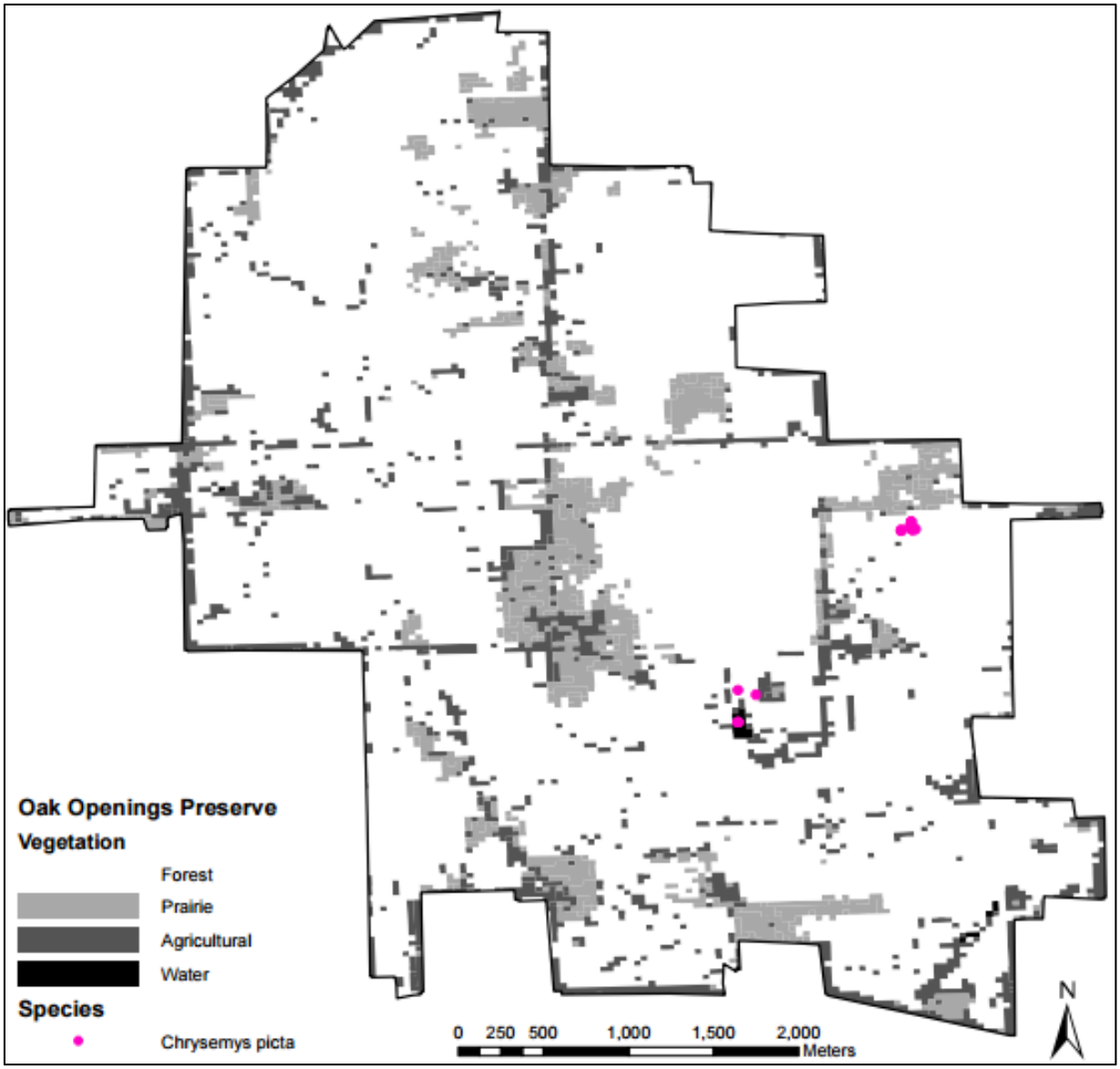



Figure A.24: Spatial locations of each *Chrysemys picta* individual sampled within the Oak Openings Preserve with land cover categorized into four types: forest, prairie, agricultural and water.

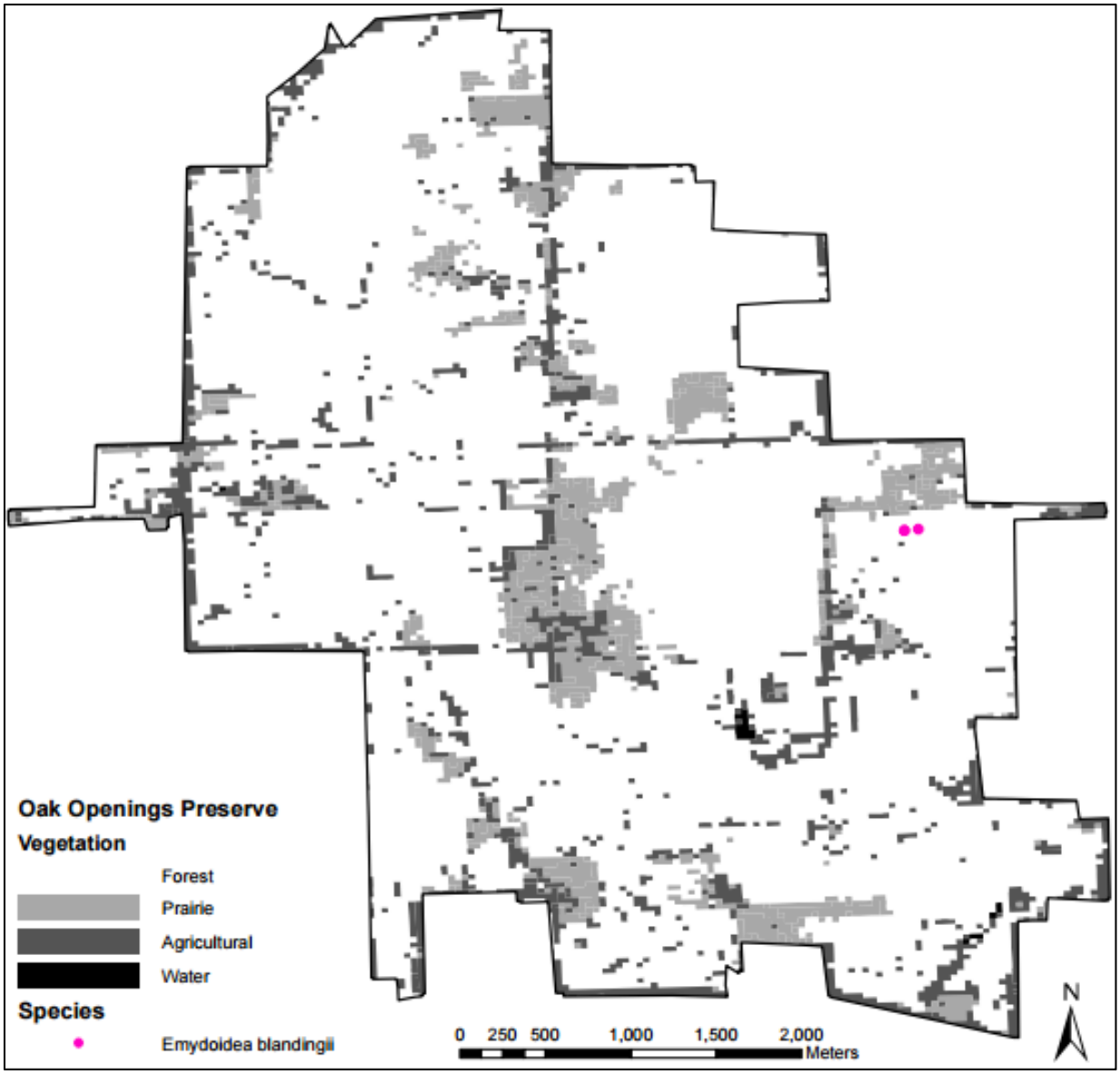



Figure A.25: Spatial locations of each *Emydoidea blandingii* individual sampled within the Oak Openings Preserve with land cover categorized into four types: forest, prairie, agricultural and water.

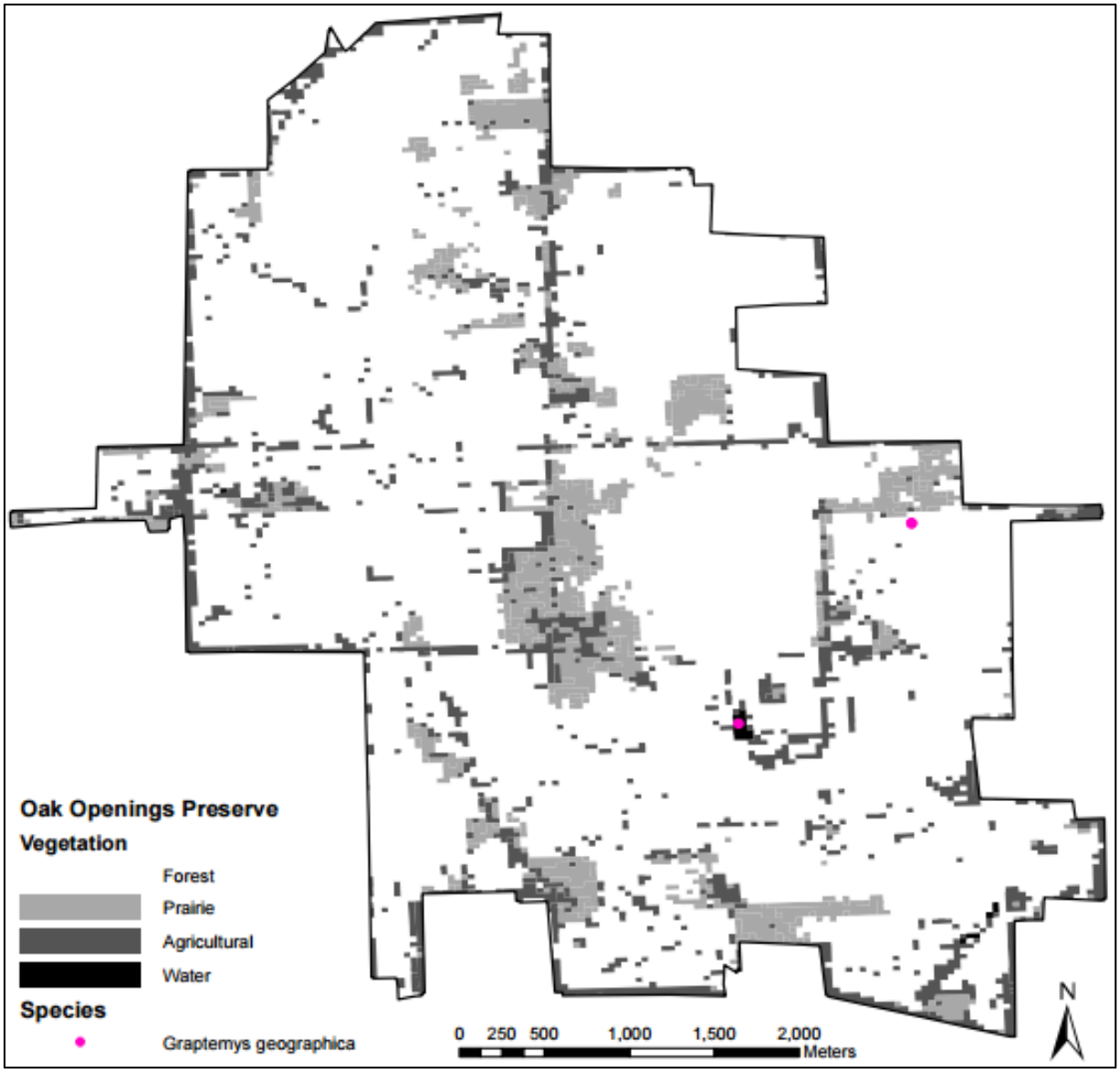



Figure A.26: Spatial locations of each *Graptemys geographica* individual sampled within the Oak Openings Preserve with land cover categorized into four types: forest, prairie, agricultural and water.

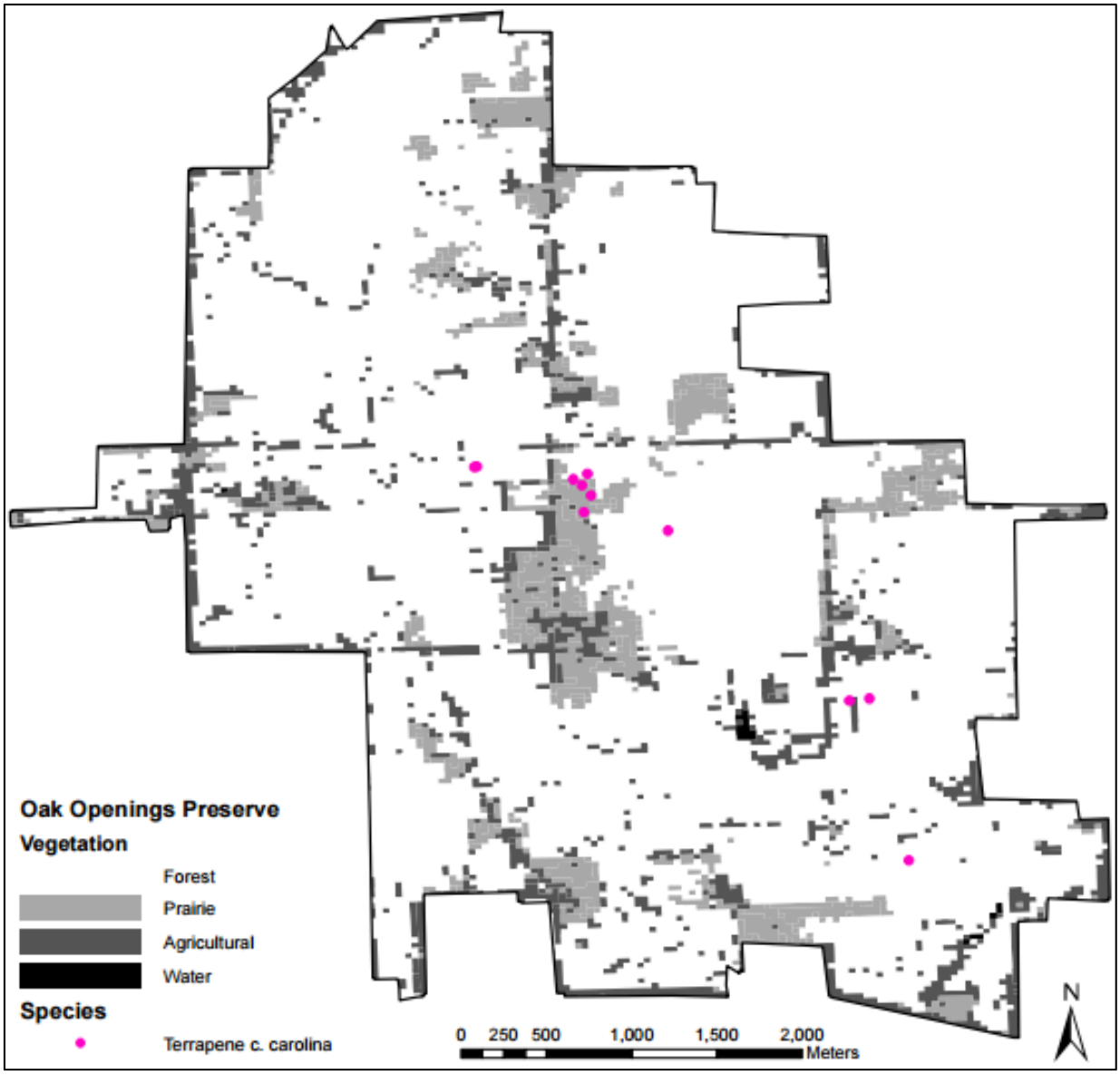
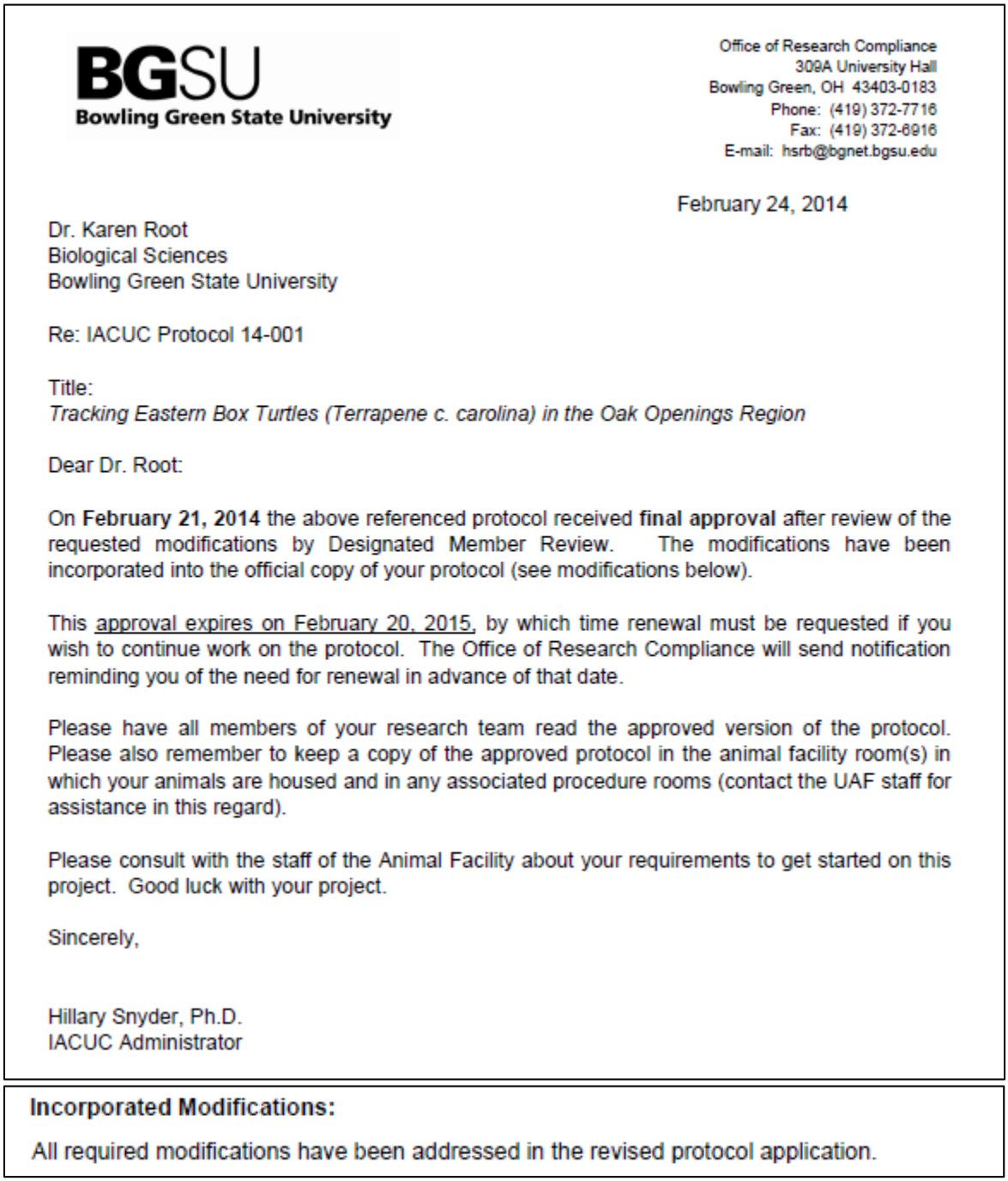



Figure A.27: Spatial locations of each *Terrapene c. carolina* individual sampled within the Oak Openings Preserve with land cover categorized into four types: forest, prairie, agricultural and water.

# APPENDIX B: IACUC APPROVAL LETTER



BGSU
Bowling Green State University

Office of Research Compliance
309A University Hall
Bowling Green, OH 43403-0183
Phone: (419) 372-7716
Fax: (419) 372-6916
E-mail: hsrb@bgnet.bgsu.edu

February 24, 2014

Dr. Karen Root
Biological Sciences
Bowling Green State University

Re: IACUC Protocol 14-001

Title:
*Tracking Eastern Box Turtles (Terrapene c. carolina) in the Oak Openings Region*

Dear Dr. Root:

On **February 21, 2014** the above referenced protocol received **final approval** after review of the requested modifications by Designated Member Review. The modifications have been incorporated into the official copy of your protocol (see modifications below).

This approval expires on February 20, 2015, by which time renewal must be requested if you wish to continue work on the protocol. The Office of Research Compliance will send notification reminding you of the need for renewal in advance of that date.

Please have all members of your research team read the approved version of the protocol. Please also remember to keep a copy of the approved protocol in the animal facility room(s) in which your animals are housed and in any associated procedure rooms (contact the UAF staff for assistance in this regard).

Please consult with the staff of the Animal Facility about your requirements to get started on this project. Good luck with your project.

Sincerely,

Hillary Snyder, Ph.D.
IACUC Administrator

**Incorporated Modifications:**

All required modifications have been addressed in the revised protocol application.

Figure B.1: IACUC approval for Tracking Eastern Box Turtles (*Terrapene c. carolina*) in the Oak Openings Region project.

# APPENDIX C: INDIVIDUAL TURTLE TRACKING DATA

## *Turtle 2*

We tracked turtle 2 for one day using thread trailing before the turtle was lost. He traveled at least 9 m before the thread trailer fell off; we know that he traveled further than recorded (13 m) because the fluorescent powder trail led us straight to him. In total, there were 3 flag markers, all of which were found in forested areas. Turtle 2's trail was found in forested areas 100% of the time (Figure C.1 A).

We tracked turtle 2 for two days using fluorescent powder before the turtle was lost. On average, he traveled 54 m with a range of 22 m to 87 m. He traveled 22 m on day one and traveled up to 87 m on day two. In total there were 36 flag markers; flag markers 1-11, 28-36 were found in forested areas and 12-27 were in agricultural areas. Turtle 2's trail was found in forested areas 43% of the time and found in agricultural areas 57% of the time (Figure C.1 B).

## *Turtle 3*

We tracked turtle 3 for one day using thread trailing before the turtle was lost. She traveled at least 34 m before the thread broke, which suggests that she traveled even further than recorded. In total, there were 11 flag markers; flag markers 1-11 were found in forested areas. Turtle 3's trail was found in forested areas 100% of the time (Figure C.2 A).

We tracked turtle 3 for one day using fluorescent powder before the turtle was lost. She traveled at least 39 m before the trail could not be tracked any further, which suggests that she traveled even further. In total, there were 13 flag markers, of which all were found in forested areas. Turtle 3's trail was found in forested areas 100% of the time (Figure C.2 B).

***Turtle 4***

We tracked turtle 4 for 10 days using fluorescent powder before the turtle was lost. On average, he traveled 41 m with a range of 11 m to 87 m (Table 4.2). In total, there were 134 flag markers. Flag markers 1-73, 83-99, 119-121 were found in forested areas, and 74-82, 100-118, 122-134 were in prairie areas. Turtle 4's trail was found in forested areas 71% of the time and in prairie areas 29% of the time (Figure C.3 A).

We tracked turtle 4 for 13 days using radio telemetry before the radio transmitter fell off and the turtle was lost. We identified the straight-line distance between two points collected from radio telemetry and on average; he traveled 18 m with a range of 3 m to 47 m (Table 4.2). In total, there were 23 detections. Detections 1-12, 15-16, 19, 21 were found in forested areas and 13-14, 17-18, 20, 22-23 were in prairie areas. Turtle 4's straight-line distance trail was found in forested areas 70% of the time and in prairie areas 30% of the time (Figure C.3 B).

***Turtle 5***

We tracked turtle 5 for three days using thread trailing before the thread trailer ran out of string and the turtle was lost. On average, he traveled 42 m with a range of 24 m to 57 m. He traveled 24 m on day one, 57 m on day 2 and 45 m on day three. In total, there were 42 flag markers. All flag markers were found in forested areas. Turtle 5's trail was found in forested areas 100% of the time (Figure C.4 A).

We tracked turtle 5 for four days using fluorescent powder before the turtle was lost. On average, he traveled 50 m with a range of 15 m to 67 m; see Table 4.2 for daily movements. In total, there were 66 flag markers. All flag markers were found in forested areas. Turtle 5's trail was found in forested areas 100% of the time (Figure C.4 B).

***Turtle 6***

We tracked turtle 6 for one day before the turtle was lost. He traveled at least 29 m before the thread broke, which suggests that he traveled further than recorded. In total, there were 9 flag markers and all flag markers were found in forested areas. Turtle 6's trail was found in forested areas 100% of the time (Figure C.5).

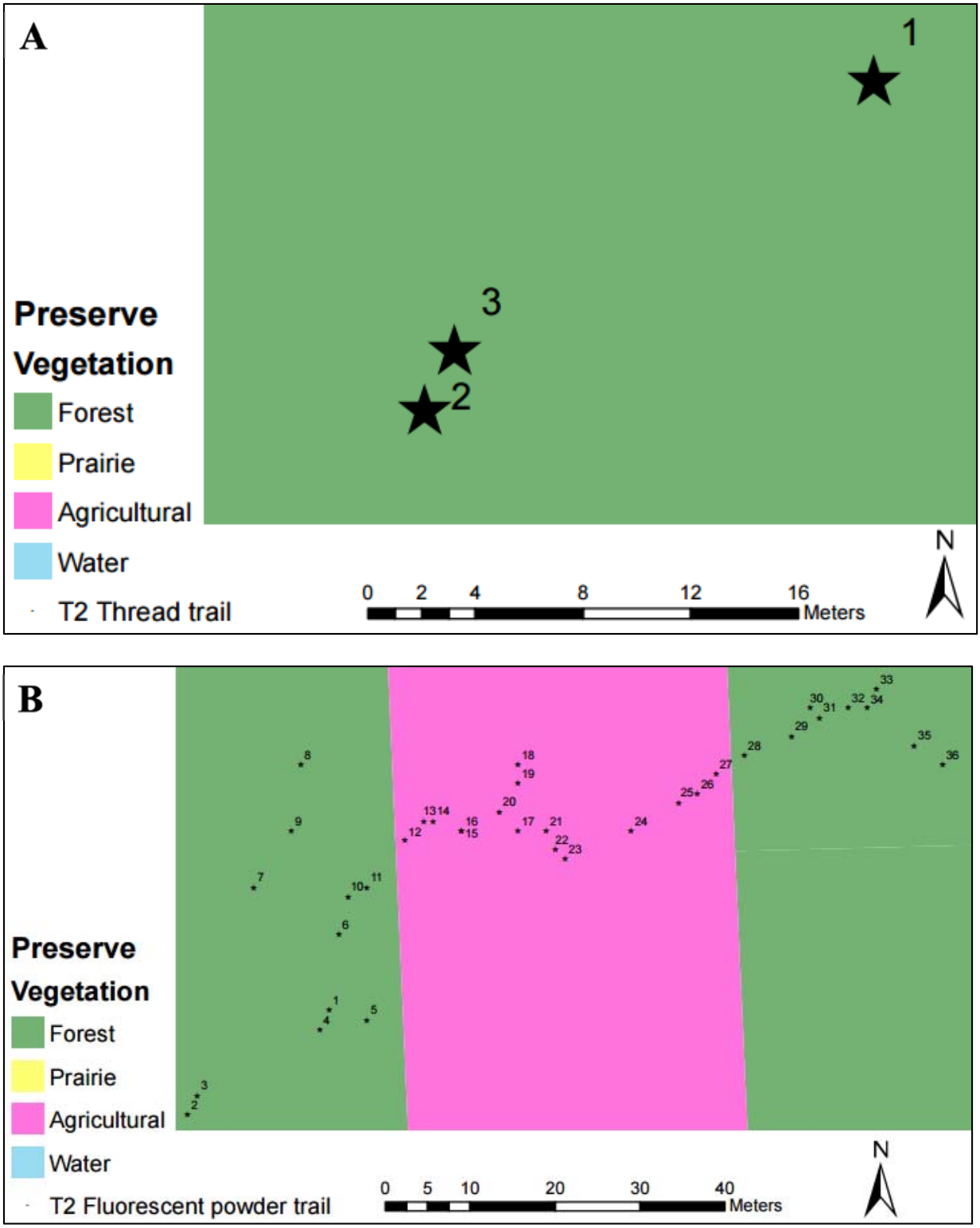



Figure C.1: Turtle 2's thread trail with label 1 as the starting point and 3 as the last point (A) and fluorescent powder trail with label 1 as the starting point and 36 as the last recorded point (B) with land cover.

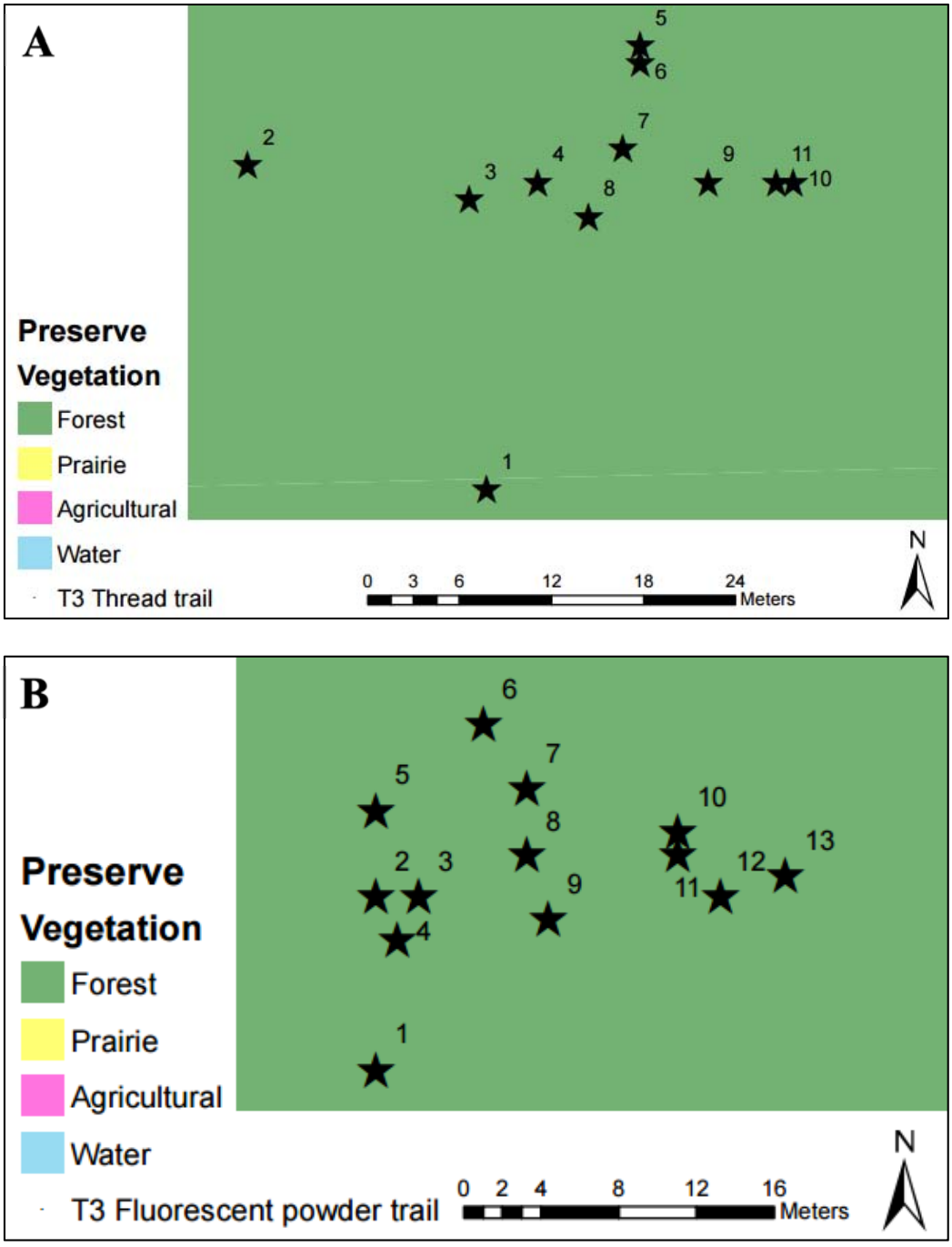



Figure C.2: Turtle 3's thread trail (A) with label 1 as the starting point and 11 as the last recorded point and fluorescent powder trail (B) with 1 as the starting point and 13 as the last recorded point with land cover.

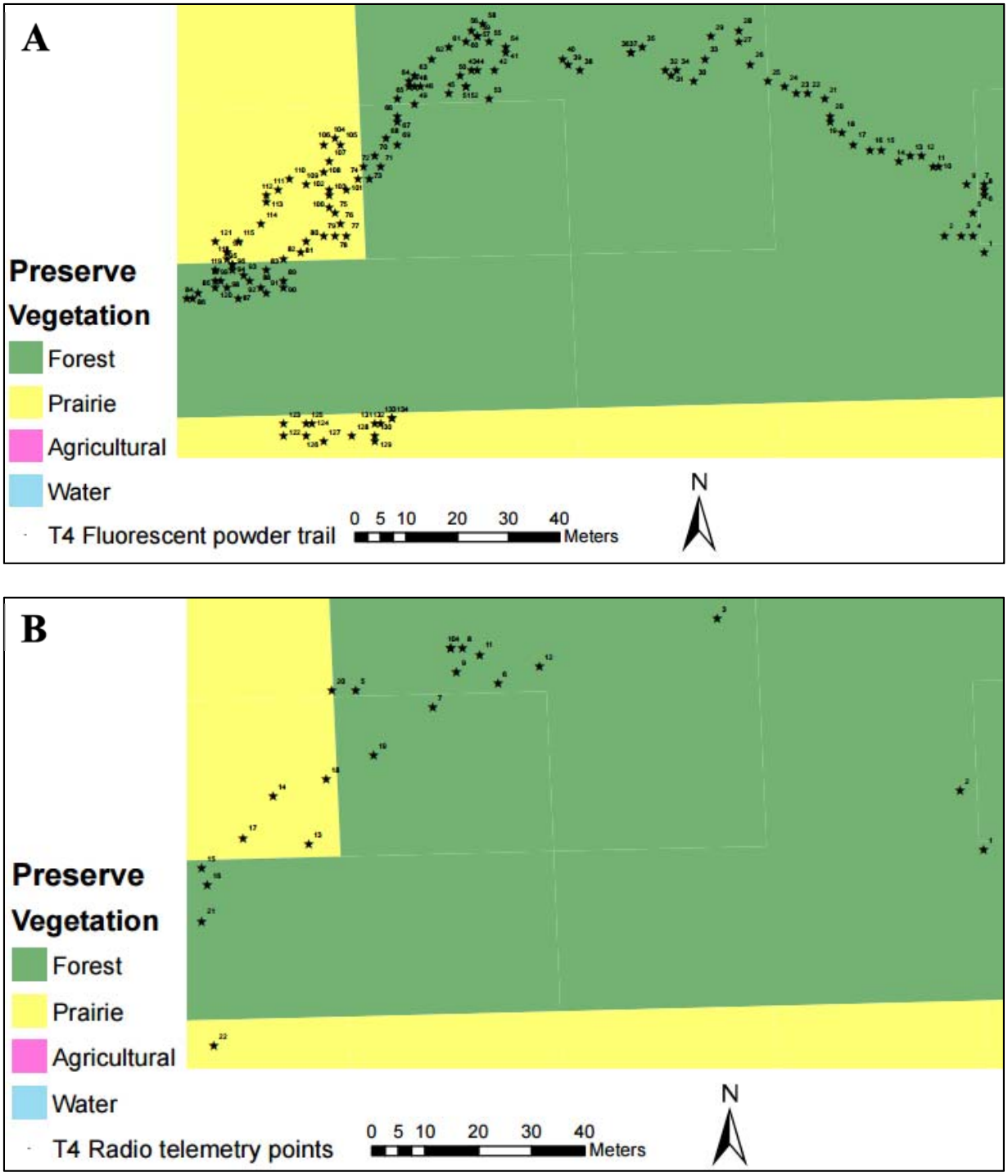



Figure C.3: Turtle 4's fluorescent powder trail (A) with label 1 as the starting point and 134 as the last recorded point and radio telemetry relocation points (B) with label 1 as the first location and 22 as the last relocation with land cover.

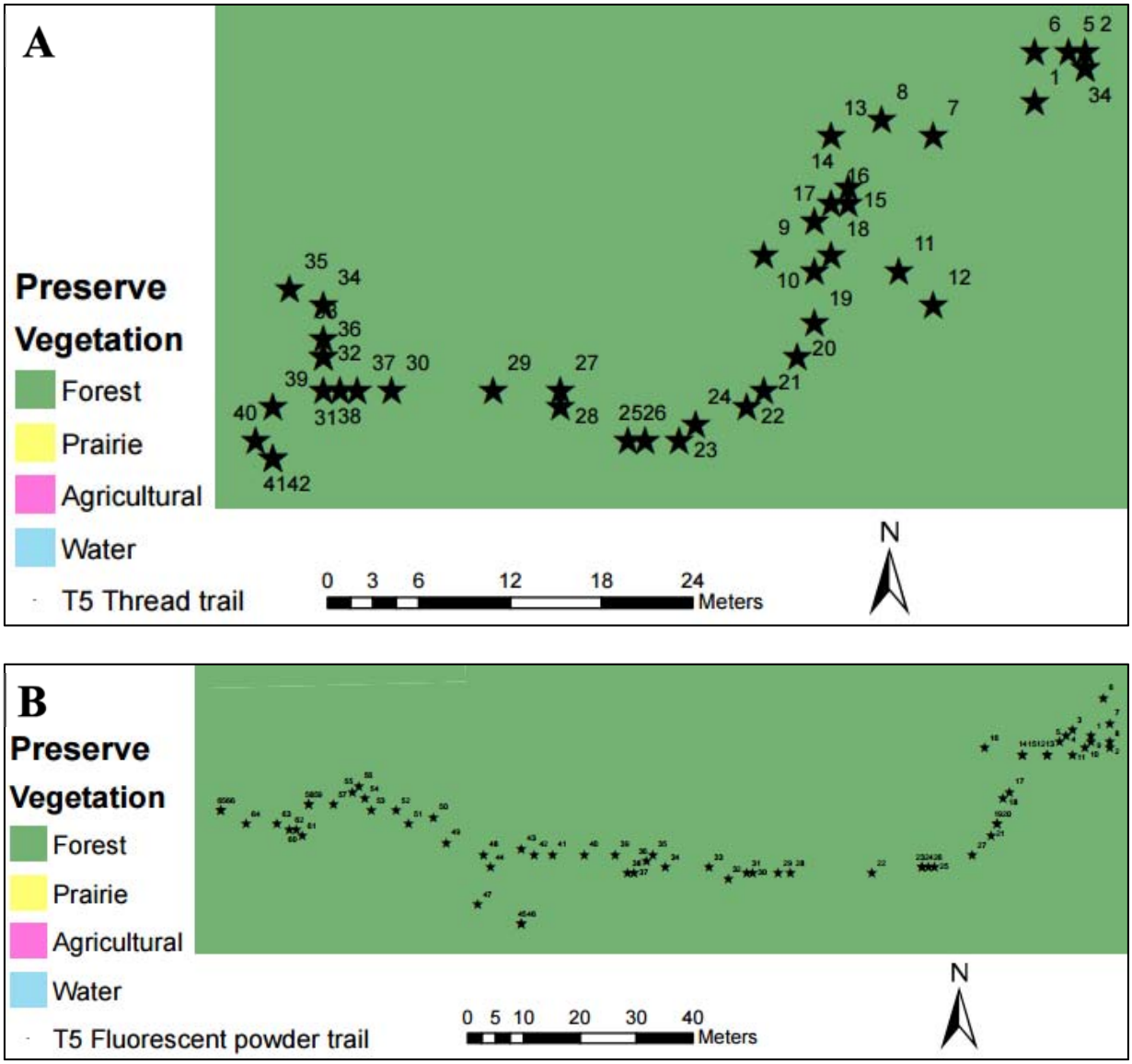



Figure C.4: Turtle 5's thread trail (A) with 1 as the starting point and 41 as the last recorded point and fluorescent powder trail (B) with 1 as the starting point and 66 as the last recorded point with land cover.

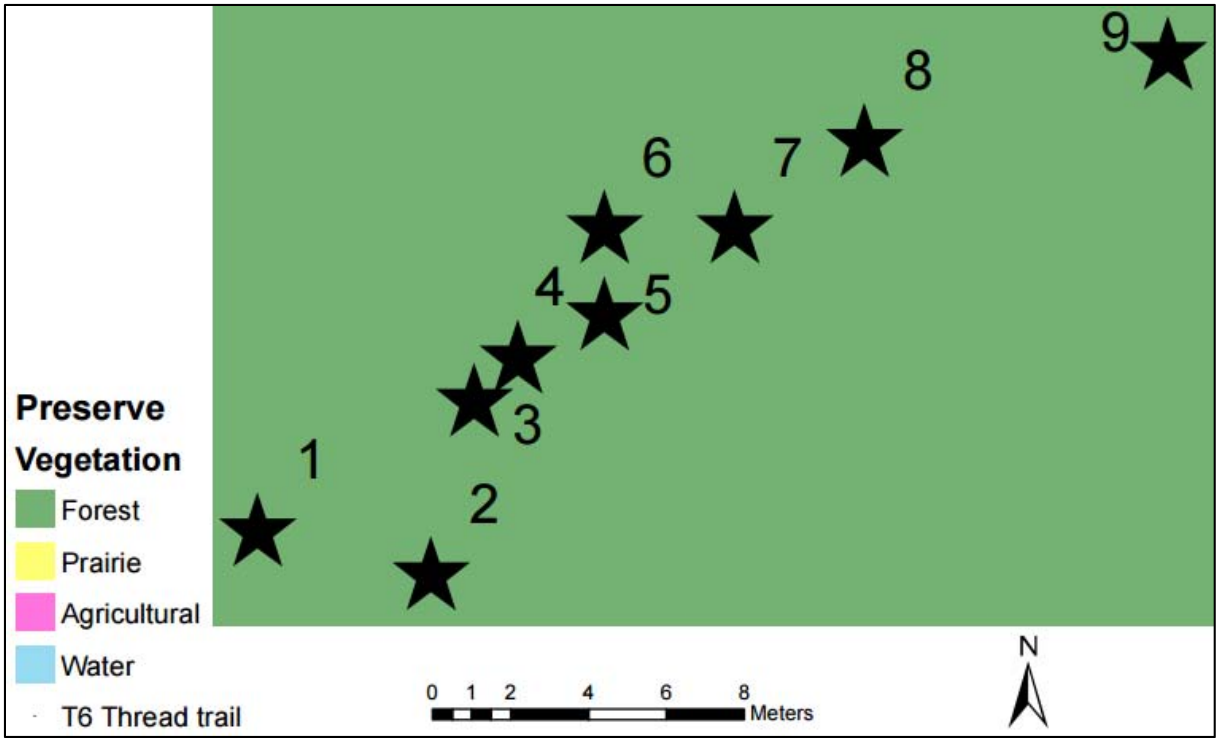



Figure C.5: Turtle 6's thread trail with 1 as the starting point and 9 as the last recorded point with land cover.