ANALYZING VERTEBRATE MOVEMENT IN AND AROUND NATURAL AREAS THROUGH ROAD SURVEYS

Victoria Freter

A Thesis

Submitted to the Graduate College of Bowling Green
State University in partial fulfillment of
the requirements for the degree of

MASTER OF SCIENCE

August 2020

Committee:

Karen V. Root, Advisor

Enrique Gomezdelcampo

Shannon Pelini

ABSTRACT

Karen V. Root, Advisor

With the expansion of the human population, new roads are continually being built, leading to an increase in fragmentation or loss of natural habitats. These roads can act as corridors for vertebrates connecting natural habitats or aiding in migration, but they can also act as barriers or boundaries leading to fragmentation, isolation, and/or mortality on roadways. Research has focused mainly on larger highways, outside of the U.S., and away from larger urban areas. The Oak Openings Region, a biodiversity hot spot in northwestern Ohio, is a matrix of human dominated land use and remnant natural patches. This research focused on identifying vertebrate mortality hot spots and the spatial and temporal variables associated with vertebrate road mortality. From mid-April to the end of September 2019, we surveyed 38 road transects within and around three protected (natural) areas to record diversity, abundance, and distribution of roadkilled vertebrates and to identify influencing factors (e.g., land use, canopy cover, environmental data, road characteristics). We found an uneven spread of roadkill, with 45% of roadkill found on nine out of the 38 (24%) surveyed transects, highlighting potential areas to prioritize for mitigation. During the spring months (April 14 - June 20), nine of the transects had no roadkill, but in the summer months (June 21 - September 18), only one transect had no roadkill. This suggests that seasonality influenced the abundance and distribution of roadkill. As transects moved northward, there was a positive trend of more roadkill with increased developed land use. We found a total of 297 roadkill on or around the roads, with all vertebrate taxa included. Mammals made up 49.8% of the roadkill found. Mammal roadkill showed a significant positive trend with average canopy cover ($p < 0.0001$). Amphibians made up the second largest roadkill group, but showed a seasonal peak in August. These results can help land managers predict where animal dispersal is occurring, which roads negatively impact vertebrates, and identify where better connectivity of the landscape could improve conditions for vertebrate populations. This study suggests that human dominated land use increases mortality and seasonality influences roadkill abundance and distribution.

ACKNOWLEDGMENTS

First and foremost, I would like to acknowledge and thank my advisor, Dr. Karen Root, for all the help, guidance, and support she has given me over the past two years. Secondly, I would like to thank my committee, Dr. Enrique Gomezdelcampo and Dr. Shannon Pelini, for helping make this project a success. A huge thank you to the Root Lab: Rachel Kappler, Amanda Martin, Sara Rair, Kelly Russo-Petrick, Jacob Schoen, Kelsey Stoneberg, and Kathryn Ware, for helping me grow, helping me edit, and dealing with my sarcasm. Finally, a thank you to all of my family and friends for encouraging me and pushing me to pursue my dreams.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Table Page

GENERAL INTRODUCTION

As the human population continues to grow, roads are being built and the landscape is changed, leading to conflict with other organisms. Habitat loss caused by anthropogenic change is one of the major threats to flora and fauna alike (Kautz et al., 2006; Rincón-Aranguri et al., 2019). Loss of habitat can lead to death or force mobile organisms to find new resources. As these organisms move across different landscapes, the chances of human-wildlife conflict increase. According to Sutherland et al. (2013), understanding the role that landscape structures play in the distribution and abundance of organisms is one of the 100 fundamental ecological questions. When looking at fragmentation and animal movement, roads are not always considered. By using road surveys to study animal mortality patterns, this knowledge gap can be reduced.

Vertebrates are the main focus for this study, as invertebrate carcass counts are generally unreliable due to the large number that stick onto vehicles or are unrecognizable on roads. Each taxon (amphibia, aves, mammalia, and reptilia) uses and responds to the roads differently and within each taxon, individual species can react differently. Amphibians use roadside ditches when they flood and will cross roads during seasonal migrations (Gibbs, 1998; Langen et al., 2009). Birds are greatly affected by noise, pollution, and visual stimuli potentially causing them to avoid roadways (Reijnen et al., 1997). Mammals use roads as corridors or to access resources, such as food, shelter, or mates (Oxley et al., 1974). Roads can attract reptiles to help with thermoregulation (Rincón-Aranguri et al., 2019; Tanner & Perry, 2007). Roads play an important ecological role to help these organisms access a variety of resources.

This study took place mainly within the Oak Openings Region, with 31 out of the 38 transects completely inside of the boundary of the region. The Oak Openings Region is a 476

km^2 hot spot of biodiversity in northwestern Ohio and southeastern Michigan (Brewer & Vankat, 2004). According to The Nature Conservancy, this region is considered “One of America’s Last Great Places” (Thieme, 2016). Within the Oak Openings Region, there are six natural plant communities, five of which are considered globally rare. These communities are: Black Oak/Lupine Barren, Mesic Sand Tallgrass Prairie, Midwest Sand Barren, Oak/Blueberry Forest, Great Lakes Pin Oak/Swamp White Oak Flatwoods, and Twigrush Wet Prairie (Gardner, 2016). These communities support a wide variety of endangered or rare plants, invertebrates, and vertebrates. Roughly a third of the rare plants in Ohio, can be found within the Oak Openings Region (Walters, 2016). All seven species of butterfly in the region are protected by Ohio and Michigan. In Ohio, of the seven butterfly species, four are only found within the region and nowhere else within the state (Parshall & Bradley, 2016). Because of the variety of ecosystems, plant communities, and insects, multiple bird species such as the lark sparrow, summer tanager, and the blue grosbeak, regularly spend summers in the Oak Openings Region, which is far from their normal distributions (Tramer, 2016). Many of the extirpated mammals (e.g. beaver, river otter, black bear, and bobcat) have started to return to the Oak Openings Region (Jacksy, 2016).

This region is a mosaic of human dominated land use and remnant natural patches. Roads fragment these natural areas and create hard edges between agriculture, residential, and urban environments. There is a gradient in land use types that shifts from agriculture in the southwest towards more residential and urban in the northeast with remnant natural land use types interspersed throughout. According to Schetter and Root (2011), approximately 25% of the Oak Openings Region has been converted to agriculture and another 45% is comprised of urban and residential development. Fragmented landscapes are ideal to research and analyze organisms’ movement responses and to identify areas that are more dangerous for them to move through.

The Oak Openings Region is a good model to analyze animal movement as human dominated landscapes are not unique to this area and natural habitats across the world are continually being fragmented by roads.

Within the Oak Openings Region, we focused our research on three surrounding protected (natural) areas: Kitty Todd Nature Preserve, Maumee State Forest, and Oak Openings Preserve Metropark. Kitty Todd Nature Preserve is near Irwin Prairie State Nature Preserve, Secor Metropark, and Wiregrass Lake Metropark and these natural areas were included with Kitty Todd Nature Preserve. Kitty Todd Nature Preserve, Maumee State Forest, and Oak Openings Preserve Metropark represent the larger natural locations left in a human dominated landscape, which can be used by native species for food, shelter, and reproduction. We assumed that these natural areas will have higher biodiversity than the surrounding human-modified areas, therefore acting as a source of road mortality. Since these natural areas provide food, shelter, and mating opportunities, organisms will be more likely to move between these natural areas, creating hot spots in roadkill.

This thesis is broken up into two standalone chapters, with a common focus on vertebrate mortality patterns throughout a human-dominated landscape. We addressed the spatial and temporal factors that affect where vertebrates are crossing the roads, affect animal mortality on roads, and whether there are roadkill hot spots within the study site. In addition to increasing our knowledge of the spatial ecology of native species, by understanding these patterns land managers would be able to locate where there are connectivity issues across the landscape and work towards mitigating road mortality.

Chapter 1. Chapter one focuses on vertebrate mortality hot spot locations across the study area and the influence of landscape features such as, land use type, road characteristics, and environmental variables. By examining roadkill patterns and their relationship to the surrounding environment, we analyze the spatial dynamics of native mammals. This chapter also investigates the changes in hot spots between vertebrate taxa and natural areas.

Chapter 2. Chapter two focuses on the temporal changes in vertebrate mortality hot spots throughout the study area and the influence of how landscape features such as, canopy cover, environmental variables, and ephemeral variables may play a role in the hot spots. This chapter considers the monthly changes in hot spots as well as seasonal changes in hot spots for the vertebrate taxa. By examining roadkill patterns and their patterns over different time intervals, we analyze the temporal dynamics of native mammals.

Literature Cited

Brewer, L. G., & Vankat, J. L. (2004). Description of vegetation of the Oak Openings of northwestern Ohio at the time of Euro-American settlement. *The Ohio Journal of Science, 104*(4), 76-85.

Gardner, R. (2016). Plant communities of the Oak Openings. In J. Thieme (Ed.), *Living in the Oak Openings: A guide to one of the world's last great places* (3rd ed., pp. 10-15). Retrieved from http://whitehouseoh.gov/Forms/Public-Service/Oak-Openings-Guide-3rd-Edition-2016.pdf

Gibbs, J. P. (1998). Amphibian movements in response to forest edges, roads, and streambeds in southern New England. *The Journal of Wildlife Management*, *62*(2), 584-589. https://doi.org/10.2307/3802333

Jacksy, B. (2016). Mammals of the Oak Openings. In J. Thieme (Ed.), *Living in the Oak Openings: A guide to one of the world's last great places* (3rd ed., pp. 28-33). Retrieved from http://whitehouseoh.gov/Forms/Public-Service/Oak-Openings-Guide-3rd-Edition-2016.pdf

Kautz, R., Kawula, R., Hoctor, T., Comiskey, J., Jansen, D., Jennings, D., Kasbohm, J., Mazzotti, F., McBride, R., Richardson, L., & Root, K. (2006). How much is enough? Landscape-scale conservation for the Florida panther. *Biological Conservation*, *130*(1), 118-133. https://doi.org/10.1016/j.biocon.2005.12.007

Langen, T. A., Ogden, K. M., & Schwarting, L. L. (2009). Predicting hot spots of herpetofauna road mortality along highway networks. *Journal of Wildlife Management*, *73*(1), 104-114. https://doi.org/10.2193/2008-017

Oxley, D. J., Fenton, M. B., & Carmody, G. R. (1974). The effects of roads on populations of small mammals. *Journal of Applied Ecology*, *11*(1), 51-59. https://doi.org/10.2307/2402004

Parshall, D. K., & Bradley, R. (2016). Insects & spiders of the Oak Openings. In J. Thieme (Ed.), *Living in the Oak Openings: A guide to one of the world's last great places* (3rd ed., pp. 40-45). Retrieved from http://whitehouseoh.gov/Forms/Public-Service/Oak-Openings-Guide-3rd-Edition-2016.pdf

Reijnen, R., Foppen, R., & Veenbaas, G. (1997). Disturbance by traffic of breeding birds: Evaluation of the effect and considerations in planning and managing road corridors. *Biodiversity & Conservation*, *6*, 567-581. https://doi.org/10.1023/A:1018385312751

Rincón-Aranguri, M., Urbina-Cardona, N., Galeano, S. P., Bock, B. C., & Páez, V. P. (2019). Road kill of snakes on a highway in an Orinoco ecosystem: Landscape factors and species traits related to their mortality. *Tropical Conservation Science, 12,* 1-18. https://doi.org/10.1177%2F1940082919830832

Schetter, T. A., & Root, K. V. (2011). Assessing an imperiled oak savanna landscape in northwestern Ohio using landsat data. *Natural Areas Journal*, *31*(2), 118-130. https://doi.org/10.3375/043.031.0204

Sutherland, W. J., Freckleton, R. P., Godfray, H. C. J., Beissinger, S. R., Benton, T., Cameron, D. D., Carmel, Y., Coomes, D. A., Coulson, T., Emmerson, M. C., Hails, R. S., Hays, G. C., Hodgson, D. J., Hutchings, M. J., Johnson, D., Jones, J. P. G., Keeling, M. J., Kokko, H., Kunin, W. E., . . . Wiegand, T. (2013). Identification of 100 fundamental ecological questions. *Journal of Ecology*, *101*(1), 58-67. https://doi.org/10.1111/1365-2745.12025

Tanner, D., & Perry, J. (2007). Road effects on abundance and fitness of Galápagos lava lizards (Microlophus albemarlensis). *Journal of Environmental Management*, *85*(2), 270-278. https://doi.org/10.1016/j.jenvman.2006.08.022

Thieme, J. (2016). Combining forces to protect the Oak Openings: Where to begin saving the Oak Openings. In J. Thieme (Ed.), *Living in the Oak Openings: A guide to one of the world's last great places* (3rd ed., pp. 56-59). Retrieved from http://whitehouseoh.gov/Forms/Public-Service/Oak-Openings-Guide-3rd-Edition-2016.pdf

Tramer, E. J. (2016). Birds of the Oak Openings. In J. Thieme (Ed.), *Living in the Oak Openings: A guide to one of the world's last great places* (3rd ed., pp. 22-27). Retrieved from http://whitehouseoh.gov/Forms/Public-Service/Oak-Openings-Guide-3rd-Edition-2016.pdf

Walters, T. L. (2016). Rare plants of the Oak Openings. In J. Thieme (Ed.), *Living in the Oak Openings: A guide to one of the world's last great places* (3rd ed., pp. 16-21). Retrieved from http://whitehouseoh.gov/Forms/Public-Service/Oak-Openings-Guide-3rd-Edition-2016.pdf

CHAPTER 1. SPATIAL HOT SPOTS: WHERE ARE VERTEBRATES MEETING THEIR DEMISE ON THE ROADS?

Introduction

Roads (paved and unpaved) are unavoidable across the globe. According to the United States Department of Transportation, as of 2016, there were 6.6 million kilometers (4.1 million miles) of roads in the United States. In 2016, there were 268.8 million registered vehicles, which traveled 3.2 trillion miles within the United States (Federal Highway Administration, 2019). These roads play an important role in human and wildlife movement alike. Roads affect roughly 22% of the contiguous United States (Forman, 2000). Many studies have found vertebrate mortality on the roads from human-wildlife collision, the leading cause of terrestrial vertebrate mortality worldwide (Canova & Balestrieri, 2019; Ferreras et al., 1992; Forman & Alexander, 1998; Trombulak & Frissel, 2000). This major human impact on wildlife leads to injury or death of individuals, affects population density, changes sex structure of populations, and can lead to low genetic diversity (Fahrig et al., 1995; Forman & Alexander, 1998; Gibbs & Steen, 2005; Laurance et al., 2009; Meza-Joya et al., 2019; Trombulak & Frissel, 2000; Valladares-Padua et al., 1995). Not only do vehicle collisions impact animals, but they can injure humans and cause damage to vehicles. The Federal Highway Administration estimated the cost of wildlife-vehicle collisions to be $8.4 billion annually in the United States (Creech et al., 2019; Federal Highway Administration, 2008). As more roads are created and humans continue to fragment the landscape, the amount of money spent yearly will most likely continue to increase and we need to understand how these roads affect the ecology of native organisms.

Road ecology is a fairly new field, beginning in the 1970s in Europe and gradually spreading to the United States. Richard T. T. Forman is often considered one of the fathers of road ecology. He along with 13 others wrote the textbook, *Road Ecology* (Tepper, 2011). Road ecology focuses on the effects on the environment, both biotic and abiotic, when humans alter the landscape with roads (Coffin, 2007). The main goal of road ecology is to reduce, prevent, or offset the negative effects of roads (van der Ree et al., 2011), such as acting as barriers or potentially leading to collisions with vehicles. However, roads are not always a threat to the organisms using them; roads can also benefit the organisms in various ways. Roads can act as corridors for movement (Oxley et al., 1974; Seabrook & Dettmann, 1996; Transportation Research Board and National Research Council, 2005). These corridors can function as connections between fragmented landscapes and create an easily traversed path for vertebrates. These corridors, if along migration routes, can help speed up migratory movements. Supplementary food can be found in the form of roadkill or access to agriculture areas. Temporary water can form if there are ditches next to the roads, which can act as a temporary source or facilitate amphibian movement (Frey & Conover, 2006; Garriga et al., 2012; Sillero et al., 2019).

Roads can reduce the amount of natural habitat and resources available for organisms by separating different land use types such as natural, residential, and agriculture. As roads spread throughout the landscape, habitats are fragmented, and edges are created. Road edges tend to have higher rates of predation, as both birds and mammals have been linked to using edges for prey (Forman & Alexander, 1998). Edges have also been shown to lead to a degradation of forest habitat (Marsh, 2010). With increased fragmentation, more mobile organisms, i.e., large mammals and birds, may cross more of these road edges looking for specific resources and find themselves as roadkill (Lidicker, 1999). Garriga et al. (2012) found that less mobile species, i.e., amphibians and reptiles, are more susceptible to habitat fragmentation. Increased number and density of roads will add more edges that can potentially lead to less biodiversity and degradation of the remaining habitats.

Although roads can help animal movement, they primarily act as a barrier for less mobile organisms and those that are more averse to human disturbance. Barriers can cause populations to crash by cutting off individuals from each other, i.e., reduce gene flow, and the loss of individuals to road mortality, leading to low genetic diversity (Fahrig et al., 1995; Forman et al., 2003; Meza-Joya et al., 2019; Riley et al., 2006). In addition to individual mortality, this disconnection and low genetic diversity could lead to the crash of isolated populations and eventually the community (Cortes & Steury, 2016; Huijser & Bergers, 2000; McGregor et al., 2008). Roads can increase human disturbance in an environment and negatively impact organisms. Predators that rely on auditory cues can be impacted by the noise pollution from roads, leading to less successful hunting (Siemers & Schaub, 2011). It is important to understand how animals use roads by examining the factors that influence road mortality, i.e., roadkill.

This chapter focuses on the spatial factors that potentially influence the diversity, abundance, and distribution of roadkill in the Oak Openings Region. Spatial factors include but are not limited to: road topography, presence or absence of road signs and other human structures, traffic volume and speed, surrounding land use, and local vegetation cover (Barthelmess, 2014). Clevenger et al., (2003), found that decreased distance to vegetation and increased distance from wildlife road-crossing structures increased the amount of roadkill. With so many variables affecting roadkill diversity, abundance, and distribution, it is important to understand the influences and to look at features that can be examined from fine to landscape scale to help understand why organisms are moving across the landscape. Our research will help locate road mortality hot spots and see how spatial factors may play a role in the movement of organisms in a human dominated landscape, leading to land management actions that can help reduce human-wildlife conflicts.

Our goal was to identify the factors that: (1) affect where vertebrates are crossing the roads, (2) affect where animal mortality happens on roads, and (3) determine whether there are roadkill hot spots within the study area. This study will also look at how roadkill hot spots vary between taxon and natural areas. There are also more specific issues that we try to address, such as the influence of land use on movement, the impact of proximity to natural areas and water on movement, the critical structural and environmental factors that increase roadkill abundance, and the variation in the relationships within and among taxa for these characteristics. We hypothesize that there will be a greater abundance of roadkill in areas where there was more land use heterogeneity, as organisms need to move more to find fragmented resources. We predict that ephemeral water resources will attract organisms and increase road mortality. We also predict that nearby natural areas (e.g. forests and prairies), will act as a source for organisms and their resources, so roadkill would be higher closer to natural areas and lower farther away.

Methods

Study Location

Our research was conducted in and around Irwin Prairie State Nature Preserve, Kitty Todd Nature Preserve, Oak Openings Preserve Metropark, Maumee State Forest, Secor Metropark, and Wiregrass Lake Metropark in northwestern Ohio. These protected (natural) areas are all located within the Oak Openings Region (Figure 1.1). We focused on Kitty Todd Nature Preserve, Maumee State Forest, and Oak Openings Preserve, as they are the largest protected areas in the region. Irwin Prairie State Nature Preserve, Secor Metropark, and Wiregrass Lake Metropark were included with Kitty Todd Nature Preserve. Kitty Todd Nature Preserve is 566.6

hectares and managed by The Nature Conservancy. Maumee State Forest is just over 1,250.0 hectares and managed by the Ohio Department of Natural Resources Division of Forestry. Oak Openings Preserve is the largest natural area at roughly 2,023.4 hectares and is managed by Metroparks Toledo. Table 1.1 lists the key characteristics for the entirety of each protected natural area.

The Oak Openings Region has been categorized by 15 land cover types (turf/pasture, wet prairie, residential/mixed, perennial ponds, upland savannah, wet shrubland, swamp forest, upland coniferous forest, upland deciduous forest, floodplain forest, sand barrens, Eurasian meadow, upland prairie, dense urban, and cropland) (Root & Martin 2018). These land cover types were simplified into four land use types (agriculture, developed, natural, and mosaic) for this study. Natural was recorded when land use consisted of natural ecosystems (i.e., wet prairie, perennial ponds, upland savanna, wet shrubland, swamp forest, upland coniferous forest, upland deciduous forest, floodplain forest, sand barrens, Eurasian meadow, or upland prairie) on each side of the road. Agriculture was recorded when land use was inactive agriculture, active agriculture, fallow agriculture, or farm on each side of the road. Inactive agriculture was defined as fields that were left undisturbed throughout the spring and summer. Active agriculture was defined as fields that were plowed, seeded, had plant growth, and harvested throughout the spring and summer. Fallow agriculture was defined as fields that were plowed, but no plant growth or harvest was recorded throughout the spring and summer. Farm was defined as land with a barn present and/or fenced in pastures with livestock and/or poultry. Developed was recorded when land use was made up of suburban and/or urban development (housing, businesses, etc.). Mosaic was recorded when land use categories were different on each side of the road, i.e., natural and developed, natural and agriculture, or developed and agriculture.

Road Surveys

We surveyed roads in and around Kitty Todd Nature Preserve, Maumee State Forest, and Oak Openings Preserve (see Figure 1.2) for all dead vertebrates except for deer. Deer were not included as carcasses are more likely to be taken by humans, which would bias our results. A total of 38 roads were surveyed covering 59.26 kilometers (km), Figure 1.2. Road lengths ranged from 1.17 km - 1.82 km. Roads selected were in a grid pattern radiating outward from the natural areas varying from 0.00 km - 5.63 km. Roads within 6.00 km were chosen to reduce the total mileage between transects and roads with more than 5,000 cars in annual average daily traffic (AADT) were eliminated for safety of the surveyors. All roads were two lane, paved roads, with speed limits that varied between 32.2 km/h (20.0 mph) and 88.5 km/h (55.0 mph) (Table 1.2). Many of the country roads did not have speed limits marked; these roads are recognized to have a speed limit of 88.5 km/h (55.0 mph) in Ohio. The percentage of land use types did vary between the natural areas (Figure 1.3).

For Kitty Todd Nature Preserve, we surveyed a total of 12 roads, covering 17.98 km, Table 1.2. Transects ranged from 1.17 km to 1.73 km. Roads were in all cardinal directions except for south, as shown in Figure 1.4, with three roads inside the natural areas. Surveys were not done in the southern direction to avoid the Toledo Express Airport. Mosaic was the prominent land use type around roads for Kitty Todd Nature Preserve, but there was variation across the transects (Figure 1.5). For Maumee State Forest, we surveyed a total of 13 roads, covering 20.76 km, Table 1.2. Transects ranged from 1.54 km to 1.63 km. Roads were in all four cardinal directions moving outward from Maumee State Forest, as shown in Figure 1.6, with one road inside the natural area. Maumee State Forest had the most even distribution of land use types across the entire area, however, land use type per transect was not as evenly distributed compared to the other natural areas (Figure 1.7). For Oak Openings Preserve, we surveyed a total of 13 roads, covering 20.52 km, Table 1.2. Transects ranged from 1.20 km to 1.82 km. Roads were in all four cardinal directions moving outward from Oak Openings Preserve, as shown in Figure 1.8, with one road inside the park. Oak Openings Preserve was predominantly made up of mosaic and developed land use type around the roads, but once again there was variation across transects (Figure 1.9).

All dead terrestrial vertebrates (e.g. amphibians, birds, mammals, and reptiles) were counted during road surveys. We created a protocol based on the Collinson et al. (2014) standardized protocol for counting flattened fauna. All transects were surveyed every other week for a total of two to three times a month from April to October 2019. To be consistent, we started all surveys within an hour or two of sunrise. Following Garrah et al. (2015) and Smith and Dodd (2003), surveys were performed at slower speeds on bike when possible, riding 20.1 - 24.9 km/h (12.5 - 15.5 mph). The only exception was for two weeks in April and two weeks in May when surveys were done by vehicle, driving 40.2 - 48.3 km/h (25.0 - 30.0 mph), when the weather was too cold to ride a bike.

We defined the road as one edge of the pavement to the other edge. Based on previous work in the Oak Openings Region (Jonaitis, 2017), the verge was defined as 6.50 meters (m) from the edge of the road (Figure 1.10). For each roadkill found we recorded the location with a Garmin Etrex GPS, identified to the species (or taxonomic class, if species identification was not possible), took a photograph, and recorded a variety of environmental and spatial variables, which included land use type, presence of human structures, presence of ditches, presence of ephemeral variables, canopy cover percentage, presence of understory, and height of roadside vegetation (Table 1.3). Due to specimen quality, amphibians were only identified to the category of ‘frog’ or ‘toad’, as species identification was not usually possible. Animals on the verges were counted as roadkill, as we assumed that they may have been injured by a vehicle and moved off the road before dying. We did not remove animal carcasses, but we noted if they still remained on the road during the next survey. Carcasses that persisted on the roads were identified by GPS location, prior data, and photographs to prevent duplicate entries.

Environmental Characteristics of Roads

For all the roads, we obtained environmental variables: season (i.e., spring, summer), temperature (C°), humidity (%), precipitation (cm), and moon illumination (%). Spring was considered from April 14, 2019 (start of the surveys) to June 20, 2019. Summer was considered June 21, 2019 to September 22, 2019 (end of the surveys). Both daily precipitation and daily temperature were obtained from the NOAA weather data (https://ncdc.noaa.gov/). Moon illumination was obtained from timeanddate.com (https://www.timeanddate.com/moon/). In addition, humidity, temperature, and weather variables were recorded at the start of all surveys using a Brunton Atmosphere Pro handheld weather station.

Spatial and Structural Features of Roads

For all roads, we measured spatial and structural features including; canopy cover, vegetation cover, presence of understory, distance from natural area, land use type, traffic volume, road speed limit, and other road features. We measured canopy cover at fixed sampling points, every 400 m on both sides of the road and at roadkill points. On average, each transect had four fixed sampling points. At each point, four canopy cover measurements were taken and averaged together. Canopy cover measurements were taken once a month using HabitApp Version 1 (Scrufster, 2014), approximately in the middle of the month. At each roadkill point, three canopy cover measurements were taken and averaged together. Vegetation cover type (e.g., grasses, shrubs, berry bushes, etc.) and height of vegetation at each sampling point were also recorded in centimeters for the verges on both sides of the road and for the verges on both sides of the roadkill points. Vegetation height measurements were taken monthly, at the same time that canopy cover measurements were taken. Understory was considered the layer of plant growth between the forest floor and the forest canopy (Brookshire, 2018). Road distance from the protected areas was measured in Google Maps for each transect.

Roads were categorized based on the type of land use at the fixed points and at roadkill points. The land use types were simplified into four categories: natural, agriculture, developed, and mosaic. Land use types were originally identified using the Oak Openings Region land cover map (Root & Martin, 2018), then confirmed in Google Maps, and finally verified in the field.

Road features measured included traffic volume (AADT), road speed limit (km/h), length of road (km), width of road (m), road quality (newly paved, few cracks, tarred and chipped, many cracks and holes), road topography (raised, buried, level, mixed), presence of ditches, and presence of human structures (e.g., signs, telephone poles, fences, mailboxes, etc.). Traffic volume, annual average daily traffic (AADT), was obtained through the Ohio Department of Transportation (https://odot.ms2soft.com/tcds/tsearch.asp?loc=Odot&mod=). Road speed limits, in km/h, were recorded for each road during the study. Length of road, in km, was measured in Google Maps and confirmed with a cycling computer, Garmin Edge 25. Width of road, in m, was measured in Google Maps and confirmed with a Meter Man, Komelon Series 45 surveyor's wheel. Road quality or the degree of road deterioration was noted at the beginning of the survey and updated with any road construction changes (e.g., roads repaved, cracks sealed, holes patches, lines repainted, etc.).

At the beginning of the study, road topography was categorized at each fixed point based on one of six categories that refer to the cross section of the road (Clevenger et al., 2003) (Figure 1.11). Buried, raised, and level referred to when both sides of the roads were the same topography. Mixed was recorded when both sides of the road were not the same category (buried-raised, part-buried, part-raised). Buried-raised is defined, when both sides of the road are opposite of each other, but neither are level (i.e., one side buried and the other raised). Part-buried and part-raised, referred to when half of the road is level and the other half is either buried or raised, respectively. Presence of roadside ditches and human structures (e.g. signs, telephone poles, bridges, guardrails, etc.) were noted at both the fixed points and the roadkill points. We noted if any construction or repairs happened on or near roads that could change the road features.

Ephemeral Variables

During all surveys, we recorded the presence of ephemeral variables at roadkill points and for each road, including water presence (in ditches or fields), invertebrate presence (alive or dead), temporary vegetation presence (flowers or berries), presence of recycling or trash cans, and presence of living vertebrates. These were measured as they may all act as temporary resources for organisms.

<u>Analysis</u>

Totals, averages, minimums, maximums, and roadkill per km were all highly correlated, therefore, we created 200 m buffers around all the fixed sampling points to run statistical analysis using total roadkill per buffered sampling point. More information about the 200 m buffered fixed sampling points can be found in the analysis section under “Road Surveys”. We also checked for association between variables and each individual taxon, except for reptiles, for which there was insufficient sample size. The Bonferroni correction was applied to the significance values to account for repeated statistical analyses.

Road Surveys

We performed hot spot analysis using Getis-Ord Gi* in ArcGIS version 10.2 (Environmental Systems Research Institute, 2014). We chose Getis-Ord Gi* instead of Moran's I because not only did we want to know if our roadkill points were clustered, but more specifically we wanted to know whether or not there were clusters of high/low values. Following the methodology in Jonaitis (2017), we created 200 m buffers around each of the fixed sampling points for all transects; 200 m represented the midpoint between each of the fixed survey points. We assumed that the 200 m buffers accurately represented the variation in variables on and around the roads. The total number of roadkill found within each buffered point was summed across all surveys. The total number of roadkill from each taxon was also summed individually across all surveys. We did not perform a hot spot analysis for reptiles because of low sample size. We used the spatial join tool in ArcGIS to sum the roadkill points within each buffer. This hot spot analysis (Getis-Ord Gi* statistic) identified where significantly high or low clusters of roadkill were found on the roads.

The test is defined as:

$$\Sigma\, G_i{*}(d) = \Sigma\, W_{ij}\,(d)\, X_j / X_j$$

The Gi* statistic gives a z-score for each feature signifying the presence of a hot or cold spot and its level of significance. For a 90% confidence level or p-value of < 0.10 the z-score value is ± 1.65 away from zero. For a 95% confidence level or p-value of < 0.05 the z-score value is ± 1.96 away from zero. For a 99% confidence level or p-value of < 0.01 the z-score value is ± 2.58 away from zero. A high positive z-score value indicates high clusters of roadkill or hot spots and a low negative z-score value indicates low clusters of roadkill or cold spots (Mitchell, 2020). We also analyzed hot spots using the road and vegetation data from the fixed sampling points to see if there were similar features present at hot spots between natural areas and taxa specific hot spots.

To compare if there were any significant differences between natural areas in total roadkill abundance by taxa, we ran a Wilcoxon signed-rank test.

Spatial and Structural Features of Roads

We used the nonparametric correlation, Spearman's ρ, in JMP version 15.0 (SAS Institute Inc., 2019) to look at trends and significance between canopy cover, vegetation cover, understory presence, and total roadkill. Roadkill was summed for each 200 m buffer point for the entirety of the survey period. Total roadkill at the 200 m buffer points for amphibians, birds, and mammals were also examined to see if a relationship between individual taxon and canopy cover, vegetation cover, and understory presence existed.

To look at significant relationships between total roadkill per 200 m buffer point, land use type, distance from natural area, and human structures, we performed a nonparametric correlation test. We ran separate tests for each taxon to explore the relationship between total roadkill per 200 m buffer point for the entire study, land use type, and human structures, for each taxon.

We also analyzed the road features (traffic volume, width, topography, and presence of ditches) to look at the relationships between the road features and total roadkill per 200 m buffer points. We did this for total amphibians, birds, and mammals as well.

In addition, we also explored the relationships between all spatial and structural features. We eliminated one of each pair of variables that were highly correlated with one another

(Spearman $\rho \geq 0.700$). After removing the highly correlated variables, we utilized stepwise regression in JMP to check for significant relationships between multiple spatial and structural features and total roadkill per 200 m buffer points. We also ran a stepwise regression between all the variables and the individual taxon (except reptiles). The best model was based on the lowest Akaike's Information Criterion (AIC_c). The final model was checked for normality using the Shapiro-Wilks test.

Ephemeral Variables

We used a nonparametric correlation analysis to look at trends between the ephemeral variables (water presence, invertebrate presence, temporary vegetation presence, or presence of recycling/trash cans), and total roadkill, as well as, total roadkill for each individual taxon per 200 m buffer points. Ephemeral variables were also included in the stepwise regression models in JMP after highly correlated variables (Spearman $\rho \geq 0.700$) were removed. The best model was based on the lowest AIC_c. The final model was checked for normality using the Shapiro-Wilks test.

Results

Road Surveys

We surveyed a total of 706 km over 100 hours between April 14, 2019 - September 18, 2019. A total of 297 roadkill vertebrates were found on the 38 transects within and around the three major natural areas. Roadkill from all four terrestrial vertebrate taxa (amphibia, aves, mammalia, and reptilia) were found and they were split across 24 identifiable species (Table 1.4). Mammals had the highest number of roadkill at 148 (49.8%), followed by amphibians at 84 (28.3%), birds at 58 (19.5%), and reptiles at 7 (2.4%) (Figure 1.12). On average there were five dead animals per kilometer, however, roadkill was not evenly spread across the transects as seen by our hot spot analysis (Figure 1.13). We found that 135 (45.4%) out of the 297 roadkill were found on nine out of 38 the transects: with three roads in and around Kitty Todd Nature Preserve, one road in and around Maumee State Forest, and five roads in and around Oak Openings Preserve. Roadkill was absent on only one of the transects, West Tupelo Way.

Kitty Todd Nature Preserve had 102 (34.3%) roadkill over the 12 transects. This translates to 5.7 roadkill per kilometer; 48% of the roadkill found in and around Kitty Todd Nature Preserve were found on three roads: Bancroft Street, Dorr Street (west), and Frankfort Road (west) (Table 1.5). Our hot spot analysis revealed eight significant clusters (Figure 1.14). Three of the hot spots were significant at the 99% confidence level ($z > 2.58$); these were located on Bancroft Street and two on Frankfort Road (west). One was at the 95% confidence level ($z > 1.96$) and the other at the 90% confidence level ($z > 1.65$). These hot spots represent locations of frequent terrestrial vertebrate road mortality. The remaining three clusters were cold spots with a 90% confidence level ($z < -1.65$), suggesting that there are low levels of terrestrial vertebrate road mortality on West Tupelo Way. The significant factors that influenced hot spots in and around Kitty Todd Nature Preserve were the lack of presence of natural land use ($p = 0.0004$), presence of ditches ($p = 0.0047$), increased traffic volume ($p = 0.0023$), and wider roads ($p = 0.0004$). Roadkill counts in Kitty Todd Nature Preserve were significantly greater than roadkill counts in Maumee State Forest (Wilcoxon signed-rank, $p < 0.05$).

Across all 13 transects in and around Maumee State Forest, we found 71 (23.9%) roadkill. Maumee State Forest had the lowest rate of roadkill with 3.4 roadkill per kilometer of road. We found 35.2% of the roadkill in and around Maumee State Forest on two roads: Township Road EF and County Road C (Table 1.6). Hot spot analysis revealed seven significant clusters (Figure 1.15). Three of these hot spots were located on Township Road EF: two of which have a 99% confidence level ($z > 2.58$) and one at the 90% confidence level ($z > 1.65$). Two of the other hot spots were located on County Road C: one with a 95% confidence level ($z > 1.96$) and the other with a 90% confidence level ($z > 1.65$). The final two significant clusters were found on County Road 5 with a 90% confidence level ($z > 1.65$). These areas had significantly higher roadkill mortality rates than the other transects. The significant factors that influenced hot spots in and around Maumee State Forest were the lack of presence of agriculture land use ($p = 0.0155$), higher percentage of canopy cover ($p = 0.0266$), understory presence ($p = 0.004$), and increased traffic volume ($p = 0.0061$).

The 13 transects in and around Oak Openings Preserve had a total of 124 (41.8%) roadkill. Oak Openings Preserve had the highest rate of roadkill with six roadkill per kilometer of road. We found 38.7% of the roadkill in and around Oak Openings Preserve on three roads: County Road 3, Waterville-Swanton Road, and Archbold-Whitehouse Road (Table 1.7). Hot spot analysis revealed six significant hot spots (Figure 1.16). Three were located on Archbold-Whitehouse Road: two at the 99% confidence level ($z > 2.58$) and one at the 95% confidence level ($z > 1.96$). Two hot spots were located on County Road 3: one at the 99% confidence level ($z > 2.58$) and one at the 90% confidence level ($z > 1.65$). The last significant hot spot was located on Old State Line Road with a 90% confidence level ($z > 1.65$). There were no significant factors that influenced hot spots in and around Oak Openings Preserve. The three factors that had the lowest p-value and highest Spearman ρ were the presence of mosaic land use (Spearman $\rho = 0.2417$, $p = 0.0783$), wider roads (Spearman $\rho = 0.2319$, $p = 0.0916$), and higher percentage of canopy cover (Spearman $\rho = 0.2121$, $p = 0.1312$). Roadkill counts in Oak Openings Preserve were significantly greater than roadkill counts in Maumee State Forest (Wilcoxon signed-rank, $p < 0.0005$).

Roadkill was not evenly spread across the study area and hot spots changed location when looking at each taxon separately. Out of the 84 dead amphibians found, 52.4% were found in and around Kitty Todd Nature Preserve, 26.2% were found in and around Maumee State Forest, and 21.4% were found in and around Oak Openings Preserve. We found 11 hot spots for amphibians; six were at a 99% confidence level ($z > 2.58$) and five at a 95% confidence level ($z > 1.96$). The 99% confidence level hot spots were all located in and around Kitty Todd Nature Preserve: Bancroft Street, Dorr Street (west), and South Raab Road (Figure 1.17). The significant factor that influenced amphibian roadkill hot spots was presence of ditches ($p = 0.0103$). Both Maumee State Forest and Oak Openings Preserve had significantly fewer dead amphibians than Kitty Todd Nature Preserve (Wilcoxon signed-rank, $p < 0.005$ and $p < 0.01$, respectively).

Out of the 58 dead birds, 46.5% were found in and around Oak Openings, 27.6% were found in and around Kitty Todd Nature Preserve, and 25.9% were found in and around Maumee State Forest. We found 11 hot spots for birds; five hot spots at the 99% confidence level ($z > 2.58$) and six at the 95% confidence level ($z > 1.96$). The 99% confidence level hot spots were in and around Oak Openings Preserve (Old State Line Road and County Highway 1-2) and Kitty Todd Nature Preserve (Bancroft Street) (Figure 1.18). The significant factors that influenced bird roadkill hot spots were the lack of ephemeral water presence ($p = 0.0257$), wider roads ($p = 0.0137$), increased distance from the natural areas ($p = 0.0299$), and increased traffic volume ($p = 0.0144$).

Out of the 148 dead mammals found, 51.3% were found in and around Oak Openings Preserve, 25.7% were found in and around Kitty Todd Nature Preserve, and 23% were found in and around Maumee State Forest. We found 19 hot spots for mammals; six hot spots at a 99%

confidence level ($z > 2.58$), six at a 95% confidence level ($z > 1.96$), and seven at a 90% confidence level ($z > 1.65$). The 99% confidence level hot spots were in and around Oak Openings Preserve (County Road 3, Waterville-Swanton Road, and Archbold-Whitehouse Road) and Maumee State Forest (County Road EF) (Figure 1.19). There were no significant factors that influenced mammal roadkill hot spots. Oak Openings Preserve had significantly more mammalian roadkill than Kitty Todd Nature Preserve or Maumee State Forest (Wilcoxon signed-rank, $p < 0.05$ and $p < 0.001$, respectively). The comparison between hot spots by roadkill taxon can be found in Appendix A Figure 1.S1.

Out of the seven reptilian roadkill found, 57% were found in and around Kitty Todd Nature Preserve and 43% were found in and around Oak Openings Preserve. No reptiles were found in or around Maumee State Forest. Kitty Todd Nature Preserve had significantly higher reptile counts than Maumee State Forest (Wilcoxon signed-rank, $p < 0.05$). Due to low sample size, reptiles were not included in any other statistical analysis.

Spatial and Structural Features of Roads

Mammals made up the largest portion of all the roadkill, therefore similar trends were seen for mammalian roadkill and total roadkill across the fixed sampling points. As a result of the high correlation (Spearman $\rho \geq 0.700$) and the strong explanatory power mammalian roadkill had on total roadkill trends, we looked at individual roadkill taxon separately and not all roadkill together.

The total number of mammal roadkill per fixed sampling point showed a significant positive trend with average canopy cover ($p < 0.0001$). Amphibian and bird roadkill per sampling point also had a positive relationship with canopy cover, but was not significant. Amphibian roadkill and average canopy cover was not significant after Bonferroni correction.

Average vegetation height did not have a significant impact on total roadkill per sampling point for any taxa. We found that all taxa roadkill increased with increasing understory presence (amphibian $p < 0.0005$, mammal roadkill $p < 0.0001$, bird roadkill $p < 0.005$).

We found that land use type and human structures were highly correlated (Spearman $\rho \geq 0.700$), therefore, human structures were removed from multivariate analysis. Amphibian roadkill across the sampling points had a significant positive relationship with agriculture and mosaic land use, but developed and natural land use type were not significantly related. The strongest relationship between dead amphibians and land use type was with mosaic land use (Spearman $\rho = 0.3456$, $p < 0.0001$) and the weakest significant relationship was with agriculture land use (Spearman $\rho = 0.2419$, $p < 0.005$) (Appendix A Table 1.S1). Bird roadkill only had a significant positive relationship with mosaic land use (Spearman $\rho = 0.3637$, $p < 0.0001$) (Appendix A Table 1.S2). Agriculture, developed, and natural land use types were not significantly related to bird roadkill. Mammal roadkill had a significant positive relationship with natural, developed, and mosaic. The strongest relationship between dead mammals and land use type was with mosaic land use (Spearman $\rho = 0.4756$, $p < 0.0001$) and the weakest significant relationship was with developed land use (Spearman $\rho = 0.3223$, $p < 0.0001$) (Appendix A Table 1.S3). There were no significant associations between roadkill taxa and distance from natural area. Amphibian roadkill had a negative association with distance from natural area, whereas bird roadkill had a positive association and mammal roadkill had a weak positive association. Transects further away from the protected natural areas had a decrease in amphibian roadkill, but an increase in bird and mammal roadkill. Bird and mammal roadkill had a positive relation to presence of human structures, but only mammal roadkill was significantly related ($p < 0.0001$).

Amphibian roadkill had a nonsignificant weak negative relationship with the presence of human structures.

Traffic volume and road width were highly correlated (Spearman $\rho \geq 0.700$), therefore, road width was removed from the multivariate analysis. Traffic volume was positively related to all taxa roadkill across the sampling points and this relationship was significant for mammals ($p < 0.0001$). Amphibian and bird roadkill were not significantly related to traffic volume. All roadkill taxa had a positive relationship with road width, but it was only significant for dead mammals ($p < 0.0001$). The relationship between dead amphibians, dead birds, and road width was not significant. As traffic volume and road width increased, all taxa roadkill count increased and as traffic volume and road width decreased, so did roadkill counts. There were no significant associations between any taxa roadkill totals and road topography type. Amphibian and bird roadkill increased with raised and mixed topography, but decreased with buried and level topography. Mammal roadkill decreased with raised and buried, but increased with level and mixed topography. Amphibian and mammal roadkill significantly increased with the presence of ditches ($p < 0.0001$ and $p < 0.0001$, respectively). Bird roadkill had a positive relationship with the presence of ditches, but it was not significant.

Ephemeral Variables

As a result of a combination of low occurrence rate and high correlation (Spearman $\rho \geq 0.700$), statistical analysis was not performed on invertebrate presence, temporary vegetation presence, or presence of recycling/trash cans. We found that all three taxa had a significant positive association with water presence (amphibian $p < 0.005$, bird $p < 0.05$, mammal $p < 0.0001$) at the fixed sampling points.

The multivariate analysis in JMP did not result in models with high explanatory power and all models had a significant lack of fit.

Discussion

Road Surveys

Roadkill surveys help determine the abundance, diversity, and distribution of terrestrial vertebrates across the landscape and study potential areas of high mortality concern. By including environmental characteristics, spatial and structural features, and ephemeral variables, we are better able to understand their effects on vertebrate mortality patterns. Understanding these patterns can help better evaluate where animals are moving in highly fragmented landscapes, i.e., the effect of composition and configuration of the landscape on vertebrate mortality. Mesopredators (e.g. raccoons and opossums) were the most abundant mammals, making up 57.4% of mammals found and 28.6% of total roadkill. These findings are similar to another roadkill survey within the Oak Openings Region (Jonaitis, 2017). The mesopredators found in this study are all generalist species. As a result of the heterogeneous landscape in this study, mesopredators may take advantage to the plethora of resources available to them, increasing their chances of being hit on the road. Though we simplified the 15 land cover types (Root & Martin, 2018) into four land use types, 79% of the roads we surveyed had two or more land use types highlighting how heterogeneous the landscape is. Of the land use types, mosaic had the highest rate of occurrence, making up just under 40% of all land use on the surveyed transects.

The main difference between land use type around transects within the natural areas and transects outside of the natural areas, was that transects within protected areas had a higher occurrence rate of natural land use type, whereas transects outside of these protected areas had a higher occurrence of agriculture land use type. According to Prange et al. (2004), mesopredators tend to have relatively stable populations within suburban and urban environments due to the availability of artificial food resources. This too, could increase their chances of being hit by a car. Carceres (2011) showed that the more mobile a mammal was, the higher potential there was for it being hit while crossing a road.

Birds and amphibians were found in greater abundances than a prior study done by Jonaitis (2017), but both studies reported a low number of reptiles. For birds, we found that 46.6% of roadkill was made up of robins or a type of sparrow, both generalist species. Similar to the generalist mesopredators, these birds were able to take advantage of more resources across the landscape, as they have the potential to move more between habitats. Birds have been shown to change their behavior in response to daytime road noises (Fuller et al., 2007). This could explain why birds were found in lower abundances than mammals. Frogs made up 66.7% of the amphibian roadkill. Mazerolle et al. (2005), found that amphibians reacted to cars by becoming immobile and extending their time on the road. Reptiles only made up 2.4% of all roadkill. Road avoidance behavior has been seen in both snakes and turtles (Colino-Rabanal & Lizana, 2012; Paterson et al., 2019).

Our roadkill numbers are most likely underestimates of total roadkill due to carcass permanency and methodology. Although we did not track how long all roadkill remained on the roads, we did note if carcasses remained for multiple road surveys. Out of the 19 carcasses that were noted, all but two were larger mammals. For the roadkill that remained on the roads, permanency ranged from six days to 51 days, with a median of 13 days. According to Garrah et al. (2015), frogs, birds, and juvenile turtles lasted less than 24 hours on roads, whereas mammals, snakes, and adult turtles lasted for multiple days. Permanency can be impacted by predation and scavengers, traffic volume, and weather (Ratton et al., 2014). Though we used a standardized method (Collinson et al., 2014) and we traveled the roads slower on bikes, it is still possible that some roadkill was missed. To understand carcass permanency across the taxa, further studies are needed, which could help get a better estimate of mortality on the roadways.

As we predicted, all taxa roadkill had a positive relationship with mosaic land use, which suggests that there may be more movement between fragmented resources. The higher rate of mortality suggests a greater need for connectivity or mitigation in these areas. Distance from protected (natural) areas did not seem to play a major role in location of roadkill hot spots, but mammal roadkill was higher closer to natural areas, as we predicted. As mentioned above, many of the species found were generalist and/or are suited to living in more urban environments. Therefore, natural areas may act as a food, water, and shelter source, but they are not the only source available for these animals and it is advisable to survey roads throughout a region and not only those immediately adjacent to parks to better understand the impact on vertebrate mortality from landscape characteristics.

Hot spot analysis was helpful in analyzing significant clusters of roadkill across the landscape, within each natural area, and by taxa. These locations can help highlight areas of interest for varied goals such as management, restoration, and mitigation. Hot spots across the landscape help larger initiatives like Ohio's Green Ribbon Initiative (GRI) ("Green Ribbon Initiative", n.d.). The goal of the GRI is to conserve biodiversity and restore critical natural areas and connections among them (Woods, 2016). Hot spots could help pinpoint critical areas that need protected or restored to facilitate this functionality. Many of the natural areas in the study site were managed by different organizations. Looking at hot spots within each natural area, provides each of these management groups focal points that they can work on specifically. Finally, by looking at taxa specific hot spots, we are able to see how each individual taxon is affected, where their areas of concern are, and identify if there are hot spots for species of concern.

Spatial and Structural Features of Roads

Mammals were the only taxa to have a significant relationship between roadkill and canopy cover. All roadkill taxa had a significant positive trend with understory presence, but none of the taxa had a significant relationship with vegetation height. This contrasts with a prior study done within the Oak Openings Region; Jonaitis (2017) found that roadkill was associated with low to intermediate levels of vegetation cover. In our study, more vegetation and higher canopy cover levels were related to increases in mammal roadkill, which may suggest that more vegetation may obscure the road more, making it harder to see all oncoming traffic. This difference could be a result of the differences in our study sites or methodology. For example, Jonaitis (2017) conducted road surveys on roads with speeds varying between 80.0 km/h (50.0 mph) - 88.5 km/h (55.0 mph), whereas, this study had greater speed variation. There were a few roads as slow as 32.2 km/h (20.0 mph), going all the way up to roads marked as 88.5 km/h (55.0 mph). Jonaitis (2017) also surveyed fewer roads all within or next to Oak Openings Preserve and Maumee State Forest. Both canopy cover and vegetation height were measured and reported as a score zero to five based on density, whereas our measurements were reported as the raw percentage or height measured (Jonaitis, 2017). This could suggest the earlier study had less variation in their variables, than our study. Baigas et al. (2017) found that Canada lynx crossed roads closer to areas of greater vegetation cover. Canal et al. (2019) reported an increase in bird and mammal roadkill with the increase in vegetation height to about five meters and then a decreasing amount of roadkill with taller vegetation. Based on the inverted U curve, Canal et al. (2019) suggests that lower vegetation provides more visibility and therefore more reaction time for both the animals and humans, medium height vegetation may act to reduce visibility for both animals and humans, and for birds taller vegetation would encourage higher flight patterns when crossing the roads.

Once again, all taxa had significant relationships with land use type. Amphibian, bird, and mammal roadkill all had at least one nonsignificant relation with land use type. Significant trends did show minor variations based on taxa, recommending taxa specific research. As we predicted, there was an increase in roadkill for all taxa with mosaic land use. With highly fragmented landscapes, animals may be forced to cross more roads to find specific resources. Because of different types and amounts of land use types in other studies and different methodologies, it is hard to compare land use types across studies. Which provides, another reason that region specific studies should be done and used to inform management practices. Though nonsignificant, amphibian roadkill response provides evidence for another prediction that there would be more roadkill closer to the natural areas. This did not hold true for birds and mammals, however, again recommending a multi-taxa approach.

Traffic volume and road width were associated with increased roadkill across all taxa. Higher traffic increases the chance of organisms getting hit by vehicles and wider roads increase the time it takes an animal to cross. A few studies have suggested that roads with higher traffic volumes actually create more disturbance, therefore, higher road avoidance leading to less roadkill (Trombulak & Frissel, 2000). In this study, traffic on roads had great variability. One road had as few as 52 cars daily, with the busiest road having 3,105 cars daily. Jacobson et al. (2016) reviewed road barrier effects to wildlife from different traffic volumes based on animal behavior. Animals were grouped into four categories: nonresponders, pausers, speeders, and avoiders; nonresponders (i.e., some frogs, snakes, turtles, and owls) crossed roads regardless of traffic volume, pausers (i.e., skunks, cryptic snakes, and some amphibians and turtles) and speeders (i.e., ungulates) had less success crossing roads as traffic increased, and avoiders (i.e., pumas, bear, and some bats) rarely crossed roads (Jacobson et al., 2016). Taking species specific behavior into account can help identify where animals are more likely to cross roads.

Ephemeral Variables

There was only a significant trend for water presence in relation to roadkill. This follows our prediction that ephemeral water would attract organisms and increase road mortality. Ephemeral occurrences were low enough that statistical analysis was not possible for most variables. Within the literature, an extremely low number of studies include ephemeral variables in their research. The most common ephemeral variable accounted for is water presence, but many studies focus on water bodies and distance to water. We found all taxa roadkill numbers to be significantly higher with water presence, which is similar to other studies (Santos et al., 2007; Seo et al., 2015). Studies focusing on permanent water presence also recorded the trend of increased amphibian roadkill near water (D'Amico et al., 2015; Langen et al., 2009; Orlowski, 2007). This is most likely from amphibians' reliance on water for habitat, food, and breeding.

The stepwise regressions for each individual roadkill taxa all resulted in a significant lack of fit and all had low explanatory power. This suggests that the story for what spatial variables are affecting roadkill counts is complex and there are variables that potentially were not captured during this study. Also, as a result of the high variation within each vegetation fixed sampling point and transect, a long-term detailed analysis of hot spots areas may be needed to tease apart the details of this complexity and its influence.

Conclusion

This study shows that road surveys are a valid method to help understand where animals are dying on the roadways and what spatial factors may impact roadkill numbers. Based on our results, roadkill does not occur evenly across the region and does not occur evenly across taxa. Hot spots also change slightly based on the scale you are analyzing. These results suggest that to better understand animal movement and mortality, a multi-taxa and multi-scale approach is recommended.

There is a plethora of variables that impact roadkill numbers and their impact changes based on taxa response and location. There is not one variable that solely drives roadkill, but the interaction of many variables. As we predicted, land use heterogeneity and water presence both showed an increase in roadkill for all taxa. Continued fragmentation of the landscape is inevitable and that means smaller habitats, more roads, and a higher probability of animals getting hit by vehicles. Many spatial factors, such as canopy cover, understory presence, and ditches, do influence the amount of roadkill and where roadkill is found. These variables along with traffic volume and road width impact roadkill, but can be altered by land managers.

Species of concern and areas of concern can be identified using hot spot analysis. This can be useful for protecting local fauna and locating areas where mitigation (e.g., wildlife crossing structures, signs, and culverts) or connectivity needs improvement. This approach is helpful in identifying spatial and structural features that can be managed to reduce the amount of animal mortality on the roads. In addition, it is applicable across all landscapes that are fragmented by roadways.

Literature Cited

Baigas, P. E., Squires, J. R., Olson, L. E., Ivan, J. S., & Roberts, E. K. (2017). Using environmental features to model highway crossing behavior of Canada lynx in the Southern Rocky Mountains. *Landscape and Urban Planning, 157*, 200-213. https://doi.org/10.1016/j.landurbplan.2016.06.007

Barthelmess, E. L. (2014). Spatial distribution of road-kills and factors influencing road mortality for mammals in northern New York state. *Biodiversity Conservation*, *23*, 2491-2514. https://doi.org/10.1007/s10531-014-0734-2

Brookshire, B. (2018, October 22). *Scientists say: Understory*. Retrieved May 15, 2020, from https://www.sciencenewsforstudents.org/article/scientists-say-understory

Canal, D., Camacho, C., Martin, B., de Lucas, M., & Ferrer, M. (2019). Fine-scale determinants of vertebrate roadkills across a biodiversity hotspot in southern Spain. *Biodiversity and Conservation*, *28*, 3239-3256. https://doi.org/10.1007/s10531-019-01817-5

Canova, L., & Balestrieri, A. (2019). Long-term monitoring by roadkill counts of mammal populations living in intensively cultivated landscapes. *Biodiversity and Conservation, 28*(1), 97-113. https://doi.org/10.1007/s10531-018-1638-3

Carceres, N. C. (2011). Biological characteristics influence mammal road kill in an Atlantic Forest-Cerrado interface in south-western Brazil. *Italian Journal of Zoology*, *78*(3), 379-389. https://doi.org/10.1080/11250003.2011.566226

Clevenger, A. P., Chruszcz, B., & Gunson, K. E. (2003). Spatial patterns and factors influencing small vertebrate fauna road-kill aggregations. *Biological Conservation*, *109*(1), 15-26. https://doi.org/10.1016/S0006-3207(02)00127-1

Climate data online. (n.d.). Retrieved October 1, 2019, from https://www.ncdc.noaa.gov/cdo-web/

Coffin, A. W. (2007). From roadkill to road ecology: A review of the ecological effects of roads. *Journal of Transport Geography*, *15*, 396-406. https://doi.org/10.1016/j.jtrangeo.2006.11.006

Colino-Rabanal, V. J., & Lizana, M. (2012). Herpetofauna and roads: A review. *Basic and Applied Herpetology*, *26*, 5-31. https://doi.org/10.11160/bah.12008

Collinson, W. J., Parker, D. M., Bernard, R. T. F., Reilly, B. K., & Davies-Mostert, H. T. (2014). Wildlife road traffic accidents: A standardized protocol for counting flattened fauna. *Ecology and Evolution*, *4*(15), 3060-3071. https://doi.org/10.1002/ece3.1097

Cortes, F. E., & Steury, T. D. (2016). Factors that influence the risk of carnivore road mortality in central Alabama. *Journal of the Southeastern Association of Fish and Wildlife Agencies*, 190-194. Retrieved from http://webhome.auburn.edu/~tds0009/PDFs/Factors%20that%20Influence%20the%20Risk%20of%20Carnivore%20Road%20Mortality%20in%20Central%20Alabama-2016-03-01.pdf

Creech, T. G., Fairbank, E. R., Clevenger, A. P., Callahan, A. R., & Ament, R. J. (2019). Differences in spatiotemporal patterns of vehicle collisions with wildlife and livestock. *Environmental Management*, *64*(6), 736-745. https://doi.org/10.1007/s00267-019-01221-3

D'Amico, M., Roman, J., de los Reyes, L., & Revilla, E. (2015). Vertebrate road-kill patterns in Mediterranean habitats: Who, when and where. *Biological Conservation*, *191*, 234-242. https://doi.org/10.1016/j.biocon.2015.06.010

Fahrig, L., Pedlar, J. H., Pope, S. E., Taylor, P. D., & Wegner, J. F. (1995). Effect of road traffic on amphibian density. *Biological Conservation*, *73*(3), 177-182. https://doi.org/10.1016/0006-3207(94)00102-V

Federal Highway Administration. (2008). *Wildlife-vehicle collision reduction study* (Report No. FHWA-HRT-08-034). U.S. Department of Transportation. https://www.fhwa.dot.gov/publications/research/safety/08034/03.cfm

Federal Highway Administration. (2019). *Highway statistics 2018*. U.S. Department of Transportation. https://www.fhwa.dot.gov/policyinformation/statistics/2018/

Ferreras, P., Aldama, J. J., Beltran, J. F., & Delibes, M. (1992). Rates and causes of mortality in a fragmented population of Iberian lynx Felis pardina Temminck, 1824. *Biological Conservation*, *61*(3), 197-202. https://doi.org/10.1016/0006-3207(92)91116-A

Forman, R. T. T. (2000). Estimate of the area affected ecologically by the road system in the United States. *Conservation Biology*, *14*(1), 31-35. Retrieved from https://www.jstor.org/stable/2641901

Forman, R. T. T., & Alexander, L. E. (1998). Roads and their major ecological effects. *Annual Review of Ecology and Systematics*, *29*, 207-231. Retrieved from https://www.jstor.org/stable/221707

Forman, R. T. T., Sperling, D., Bissonette, J. A., Clevenger, A. P., Cutshall, C. D., Dale, V. H., Fahrig, L., France, R., Goldman, C. R., Heanue, K., Jones, J. A., Swanson, F. J., Turrentine, T., & Winter, T. C. (2003). *Road ecology: Science and solutions*. Island Press.

Frey, S. N., & Conover, M. R. (2006). Habitat use by meso-predators in a corridor environment. *BioOne*, *70*(4), 1111-1118. https://doi.org/10.2193/0022-541X(2006)70[1111:HUBMIA]2.0.CO;2

Fuller, R. A., Warren, P. H., & Gaston, K. J. (2007). Daytime noise predicts nocturnal singing in urban robins. *Biology Letter*, *3*(4), 368-370. https://doi.org/10.1098/rsbl.2007.0134

Garrah, E., Danby, R. K., Eberhardt, E., Cunnington, G. M., & Mitchell, S. (2015). Hot spots and hot times: Wildlife road mortality in a regional conservation corridor. *Environmental Management*, *56*, 874-889. https://doi.org/10.1007/s00267-015-0566-1

Garriga, N., Santos, X., Montori, A., Richter-Boix, A., Franch, M., & Llorente, G. A. (2012). Are protected areas truly protected? The impact of road traffic on vertebrate fauna. *Biodiversity Conservation*, *21*, 2761-2774. https://doi.org/10.1007/s10531-012-0332-0

Gibbs, J. P., & Steen, D. A. (2005). Trends in sex ratios of turtles in the United States: Implications of road mortality. *Conservation Biology*, *19*(2), 552-556. https://doi.org/10.1111/j.1523-1739.2005.000155.x

The Green Ribbon Initiative. (n.d.). Retrieved April 14, 2020, from https://www.oakopenings.org/about/

Huijser, M. P., & Bergers, P. J. M. (2000). The effect of roads and traffic on hedgehog (Erinaceus europaeus) populations. *Biological Conservation*, *95*(1), 111-116. https://doi.org/10.1016/S0006-3207(00)00006-9

Jacobson, S. L., Bliss-Ketchum, L. L., de Rivera, C. E., & Smith, W. P. (2016). A behavior-based framework for assessing barrier effects to wildlife from vehicle traffic volume. *Ecosphere*, *7*(4). https://doi.org/10.1002/ecs2.1345

Jonaitis, L. (2017). *Using roadkill as a lens to understand animal movement and mortality.* (Electronic Thesis or Dissertation). Retrieved from https://etd.ohiolink.edu/

Langen, T. A., Ogden, K. M., & Schwarting, L. L. (2009). Predicting hot spots of herpetofauna road mortality along highway networks. *Journal of Wildlife Management*, *73*(1), 104-114. https://doi.org/10.2193/2008-017

Laurance, W. F., Goosem, M., & Laurance, S. G. W. (2009). Impacts of roads and linear clearings on tropical forests. *Trends in Ecology & Evolution*, *24*(12), 659-669. https://doi.org/10.1016/j.tree.2009.06.009

Lidicker, W. Z., Jr. (1999). Responses of mammals to habitat edges: An overview. *Landscape Ecology*, *14*, 333-343. https://doi.org/10.1023/A:1008056817939

Marsh, D. M. (2010). Edge effects of gated and ungated roads on terrestrial salamanders. *The Journal of Wildlife Management*, *71*(2), 389-394. https://doi.org/10.2193/2005-556

Mazerolle, M. J., Huot, M., & Gravel, M. (2005). Behavior of amphibians on the road in response to car traffic. *BioOne*, *61*(4), 380-388. https://doi.org/10.1655/04-79.1

McGregor, R. L., Bender, D. J., & Fahrig, L. (2008). Do small mammals avoid roads because of the traffic? *Journal of Applied Ecology*, *45*(1), 117-123. https://doi.org/10.1111/j.1365-2664.2007.01403.x

Meza-Joya, F. L., Ramos, E., & Cardona, D. (2019). Spatio-temporal patterns of mammal road mortality in Middle Magdalena Valley, Colombia. *Oecologia Australis, 23*(3), 575-588. https://doi.org/10.4257/oeco.2019.2303.15

Mitchell, A. (2020). *The Esri guide to GIS analysis: Geographic patterns and relationships, Volume 1* (2nd ed.).

[Moon illumination for Toledo, Ohio]. (n.d.). Retrieved April 20, 2020, from https://www.timeanddate.com/moon/usa/toledo?month=4&year=2019

Orlowski, G. (2007). Spatial distribution and seasonal pattern in road mortality of the common toad Bufo bufo in an agricultural landscape of south-western Poland. *Amphibia-Reptilia*, *28*(1), 25-31. https://doi.org/10.1163/156853807779799045

Oxley, D. J., Fenton, M. B., & Carmody, G. R. (1974). The effects of roads on populations of small mammals. *Journal of Applied Ecology*, *11*(1), 51-59. https://doi.org/10.2307/2402004

Paterson, J. E., Baxter-Gilbert, J., Beaudry, F., Carstairs, S., Chow-Fraser, P., Edge, C. B., Lentini, A. M., Litzgus, J. D., Markle, C. E., McKeown, K., Moore, J. A., Refsnider, J. M., Riley, J. L., Rouse, J. D., Seburn, D. C., Zimmerling, J. R., & Davy, C. M. (2019). Road avoidance and its energetic consequences for reptiles. *Ecology and Evolution*, *9*(17), 9794-9803. https://doi.org/10.1002/ece3.5515

Prange, S., Gehrt, S. D., & Wiggers, E. P. (2004). Influences of anthropogenic resources on raccoon (Procyon lotor) movements and spatial distribution. *Journal of Mammalogy*, *85*(3), 483-490. https://doi.org/10.1644/1383946

Ratton, P., Secco, H., & da Rosa, C. A. (2014). Carcass permanency time and its implications to the roadkill data. *European Journal of Wildlife Research*, *60*(3). https://doi.org/10.1007/s10344-014-0798-z

Riley, S. P., Pollinger, J. P., Sauvajot, R. M., York, E. C., Bromley, C., Fuller, T. K., & Wayne, R. K. (2006). A southern California freeway is a physical and social barrier to gene flow in carnivores. *Molecular Ecology*, *15*(7), 1733-1741. https://doi.org/10.1111/j.1365-294X.2006.02907.x

Root, K. V., & Martin, A. K. (2018, June). *Oak Openings Region biodiversity model final report (phase II)*. Retrieved from http://karenroot.net/documents/Oak_Openings_Model_Report_Phase_II.pdf

Santos, X., Llorente, G. A., Montori, A., Carretero, M. A., Franch, M., Garriga, N., & Richter-Boix, A. (2007). Evaluating factors affecting amphibian mortality on roads: The case of the common toad Bufo bufo, near a breeding place. *Animal Biodiversity and Conservation*, *30*(1), 97-104.

Seabrook, W. A., & Dettmann, E. B. (1996). Roads as activity corridors for cane toads in Australia. *The Journal of Wildlife Management, 62*(2), 363-368. https://doi.org/10.2307/3802236

Seo, C., Thorne, J. H., Choi, T., Kwon, H., & Park, C.-H. (2015). Disentangling roadkill: The influence of landscape and season on cumulative vertebrate mortality in South Korea. *Landscape Ecological Engineering*, *11*, 87-99. https://doi.org/10.1007/s11355-013-0239-2

Siemers, B. M., & Schaub, A. (2011). Hunting at the highway: Traffic noise reduces foraging efficiency in acoustic predators. *Proceedings of the Royal Society B: Biological Sciences*, *278*(1712), 1646-1652. https://doi.org/10.1098/rspb.2010.2262

Sillero, N., Poboljšaj, K., Lešnik, A., & Šalamun, A. (2019). Influence of landscape factors on amphibian roadkills at the national level. *Diversity*, *11*(1). https://doi.org/10.3390/d11010013

Smith, L. L., & Dodd, C. K., Jr. (2003). Wildlife mortality on U.S. highway 441 across Paynes Prairie, Alachua county, Florida. *Florida Academy of Sciences, Inc*, *66*(2), 128-140. Retrieved from https://www.jstor.org/stable/24321153

Tepper, L. (2011). Road ecology: Wildlife habitat and highway design. *Places Journal*. https://doi.org/10.22269/110922

Transportation data management system. (n.d.). Retrieved April 15, 2020, from https://odot.ms2soft.com/tcds/tsearch.asp?loc=Odot&mod=

Transportation Research Board and National Research Council. (2005). *Assessing and managing the ecological impacts of paved roads*. Washington, DC: The National Academies Press. https://doi.org/10.17226/11535

Trombulak, S. C., & Frissel, C. A. (2000). Review of ecological effects of roads on terrestrial and aquatic communities. *Conservation Biology*, *14*(1), 18-30. https://doi.org/10.1046/j.1523-1739.2000.99084.x

Valladares-Padua, C., Cullen, L., Jr, & Padua, S. (1995). A pole bridge to avoid the primate road kills. *Neotropical Primates*, *3*(1), 13-15.

van der Ree, R., Jaeger, J. A. G., van der Grift, E. A., & Clevenger, A. P. (2011). Effects on roads and traffic on wildlife populations and landscape function road ecology is moving toward larger scales. *Ecology and Society*, *16*(1). https://doi.org/10.5751/ES-03982-160148

Woods, S. (2016). Combining forces to protect the Oak Openings: The green ribbon initiative. In J. Thieme (Ed.), Living in the Oak Openings: A guide to one of the world's last great places (3rd ed., pp. 50-55). Retrieved from http://whitehouseoh.gov/Forms/Public-Service/Oak-Openings-Guide-3rd-Edition-2016.pdf

Figures

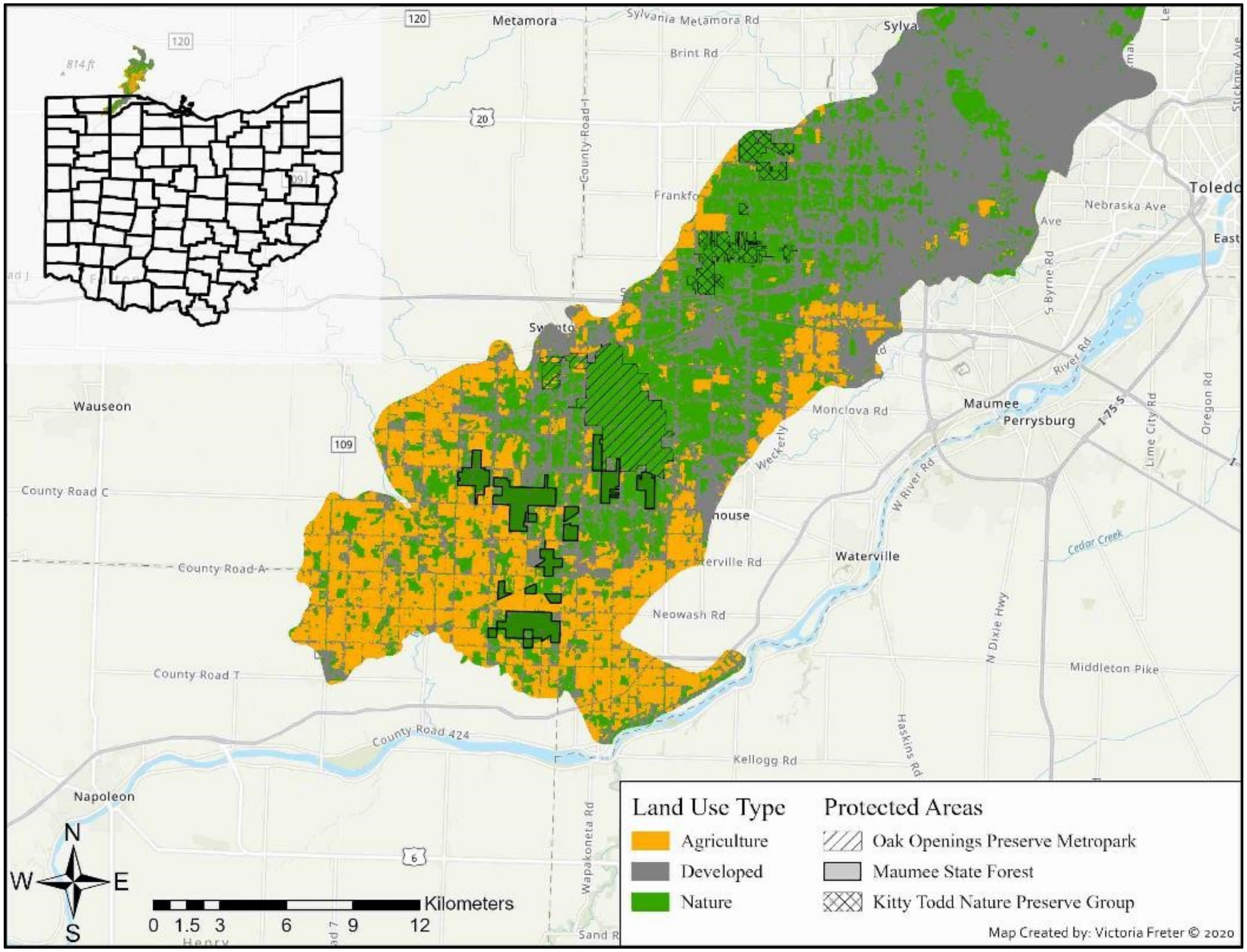

Figure 1.1. The Oak Openings Region in Northwest Ohio categorized by three land use types: agriculture (yellow), developed (gray), and nature (green) (Root & Martin, 2018). Oak Openings Preserve Metropark (diagonal), Maumee State Forest (black outline), and Kitty Todd Nature Preserve group (cross hatch) are highlighted.

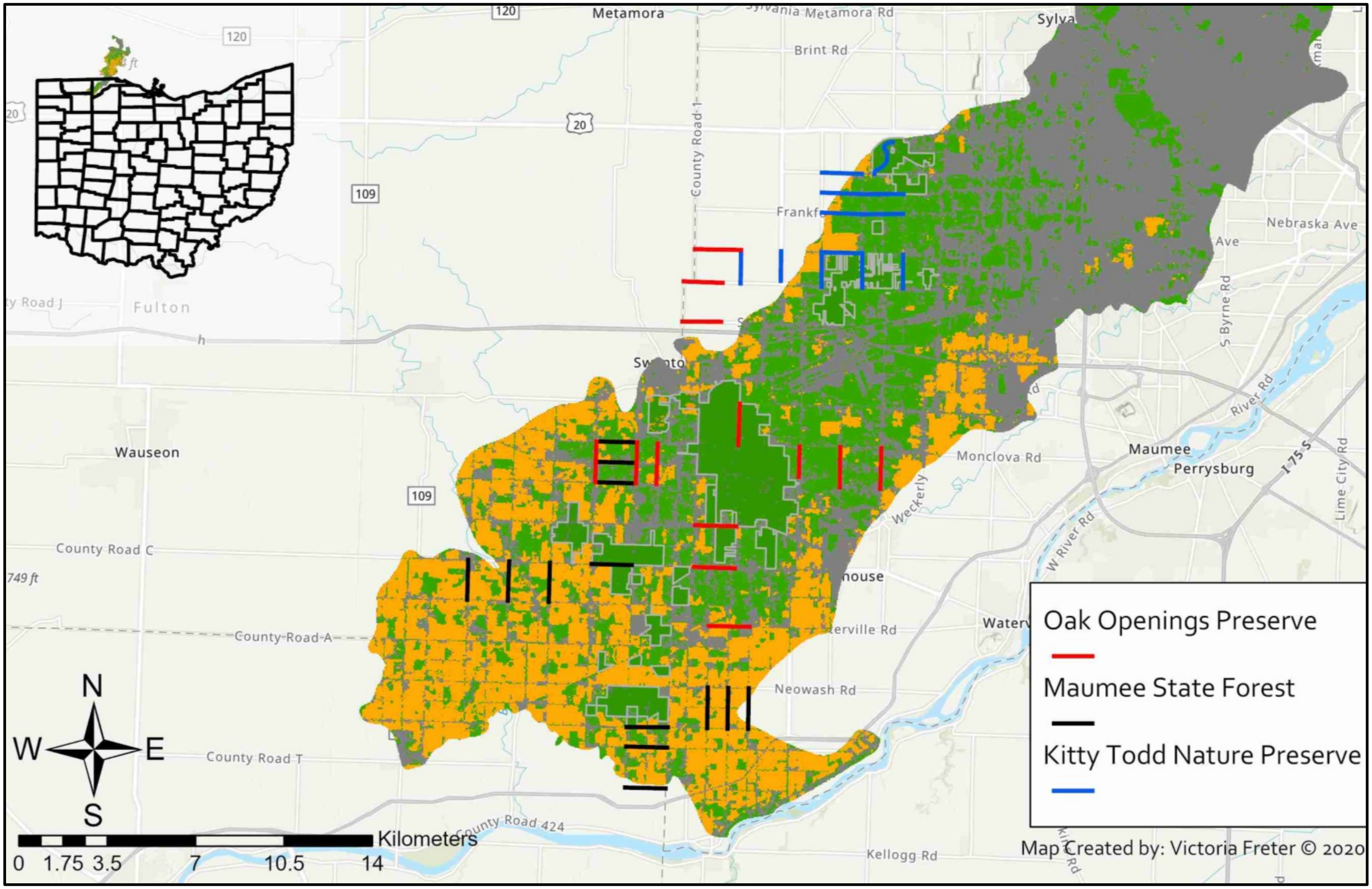

Figure 1.2. Map of surveyed road transects in and around Kitty Todd Nature Preserve (blue), Oak Openings Preserve (red), and Maumee State Forest (black) in the Oak Openings Region. Protected natural areas (gray outline) are highlighted (Root & Martin, 2018).

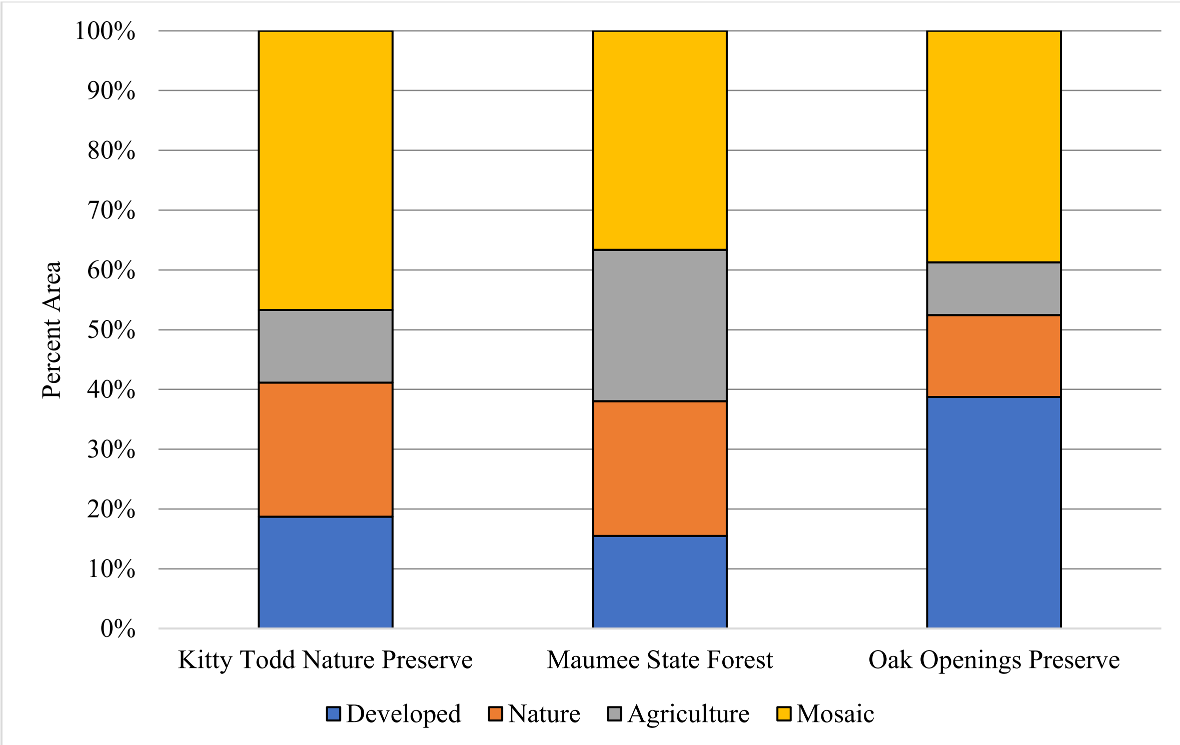

Figure 1.3. The percentage of land use type (agriculture, developed, mosaic, nature) for Kitty Todd Nature Preserve, Maumee State Forest, and Oak Openings Preserve.

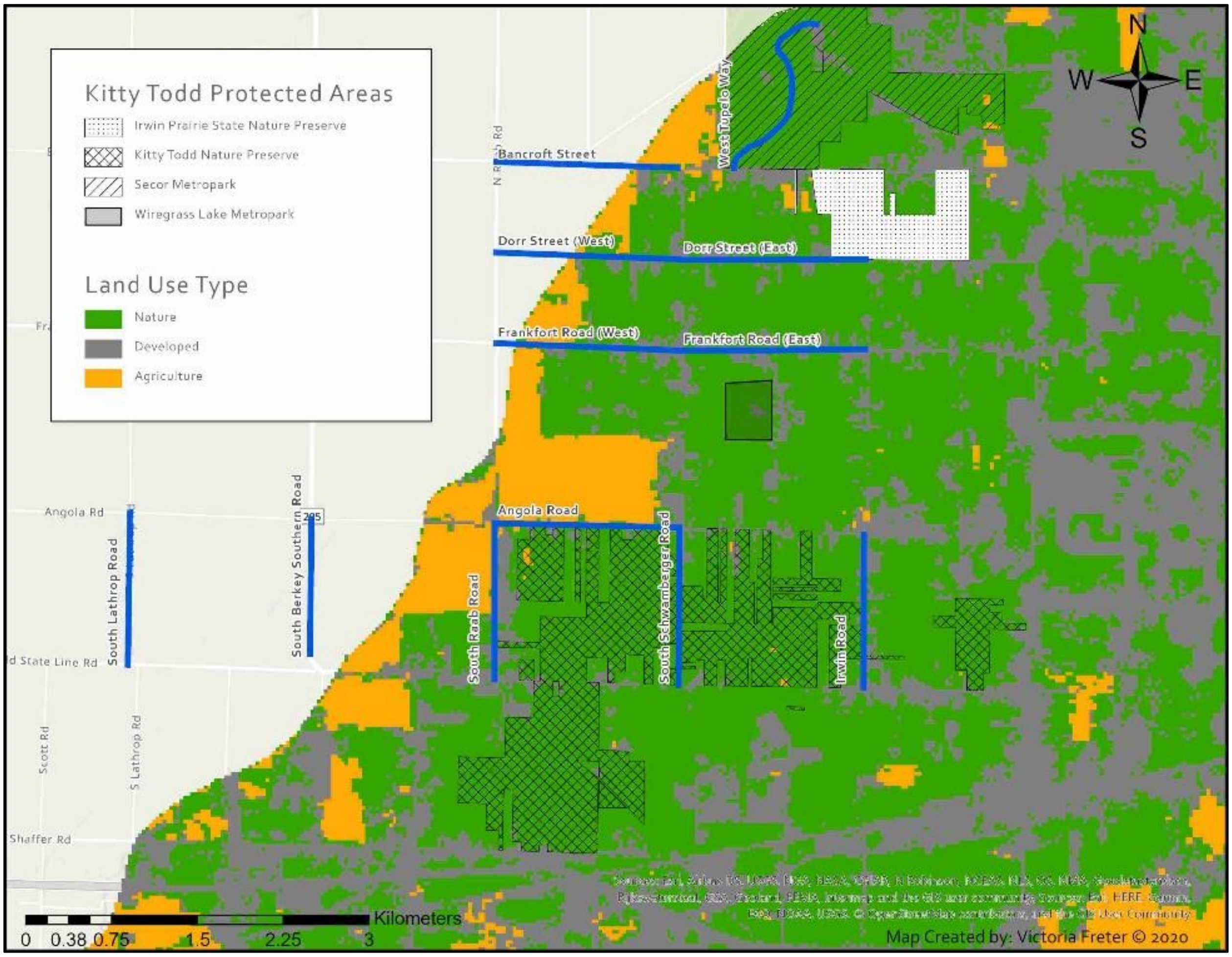

Figure 1.4. Map of surveyed road transects (blue) in and around Kitty Todd Nature Preserve. Irwin Prairie State Nature Preserve (dots), Kitty Todd Nature Preserve (cross hatch), Secor Metropark (diagonal), and Wiregrass Lake Metropark (black outline) are highlighted (Root & Martin, 2018).

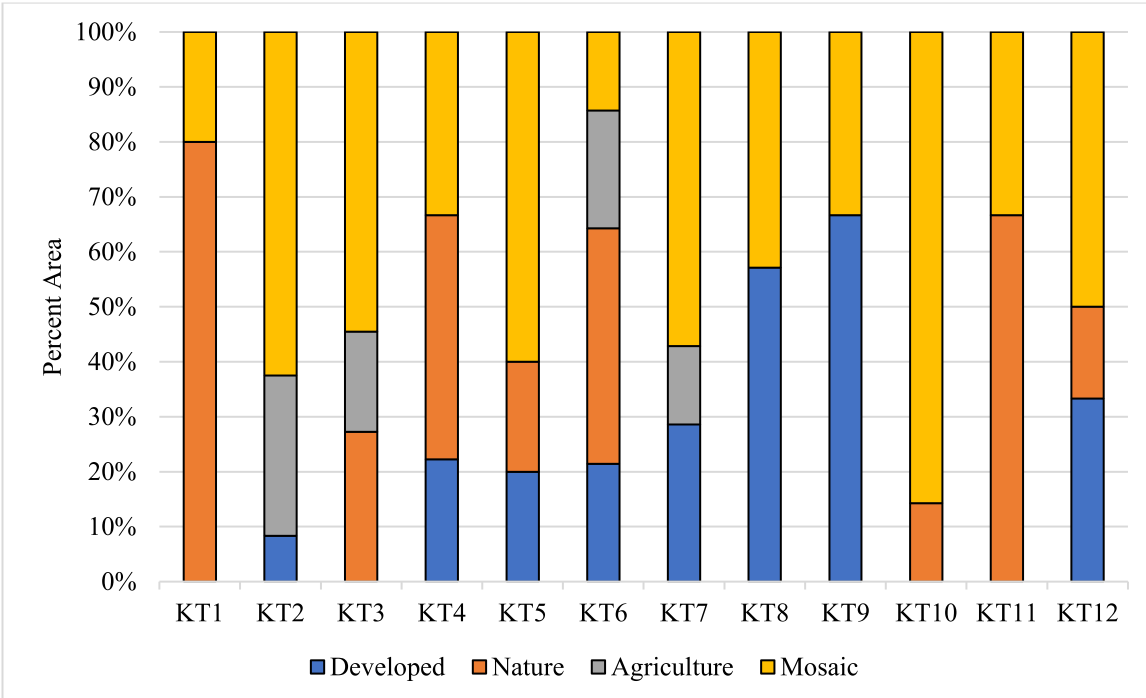

Figure 1.5. The percentage of Kitty Todd Nature Preserve's land use type (agriculture, developed, mosaic, nature) by road transect.

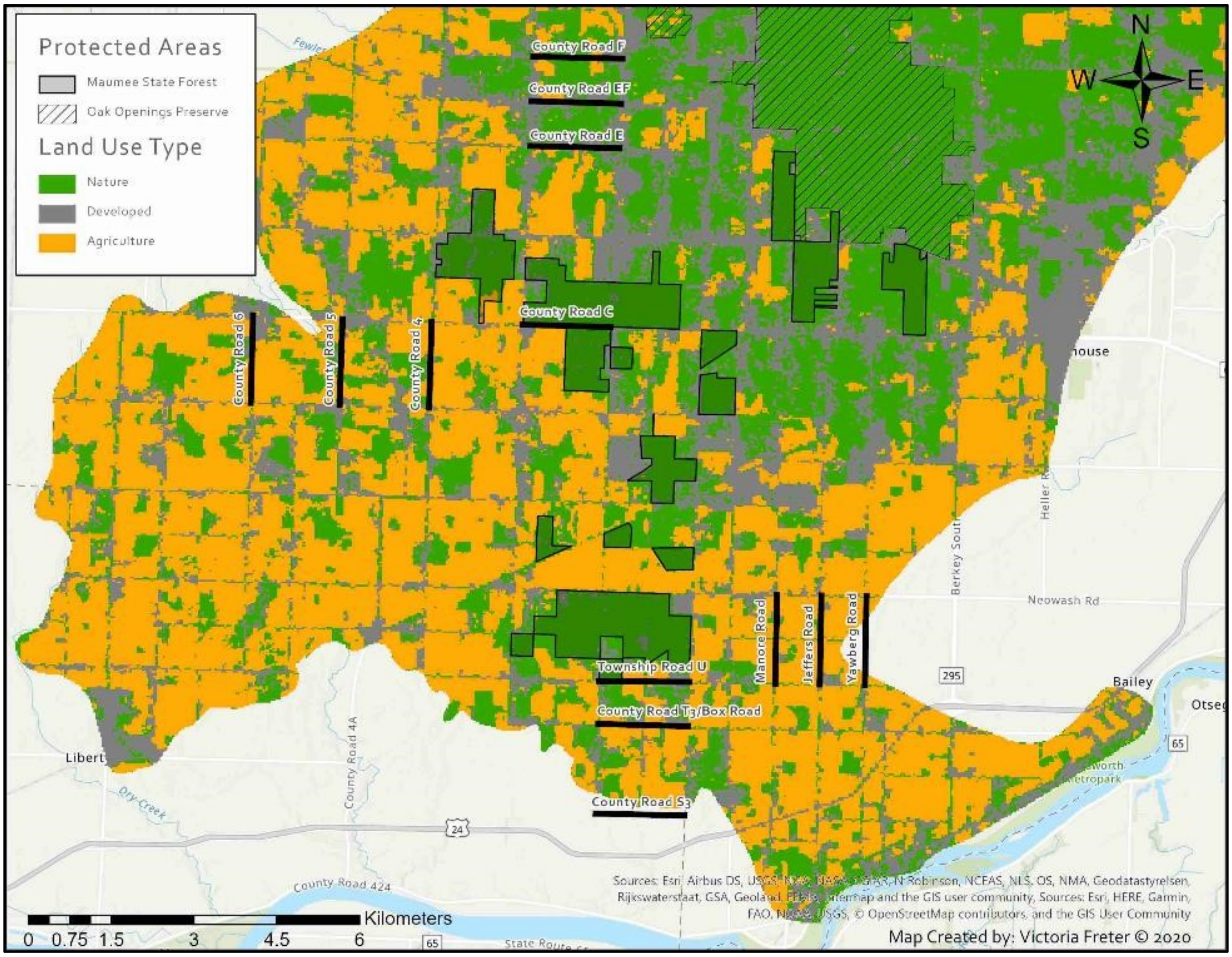

Figure 1.6. Map of surveyed road transects (black) in and around Maumee State Forest. Maumee State Forest (black outline) and Oak Openings Preserve (diagonal) are highlighted (Root & Martin, 2018).

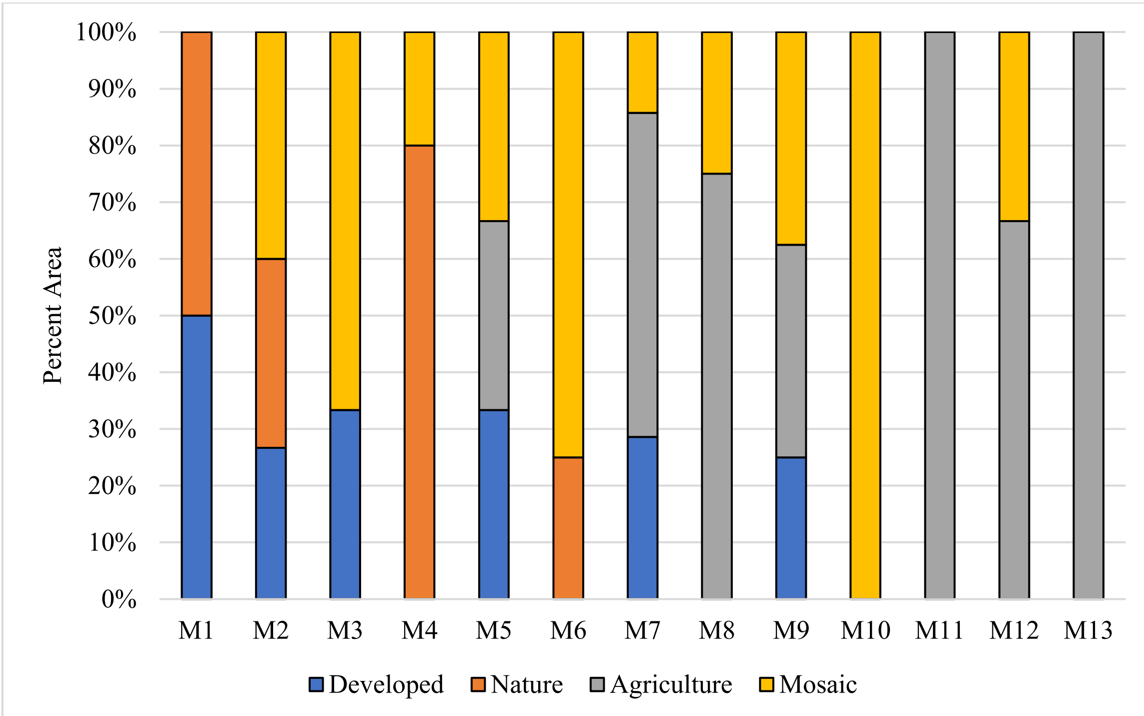

Figure 1.7. The percentage of Maumee State Forest's land use type (agriculture, developed, mosaic, nature) by road transect.

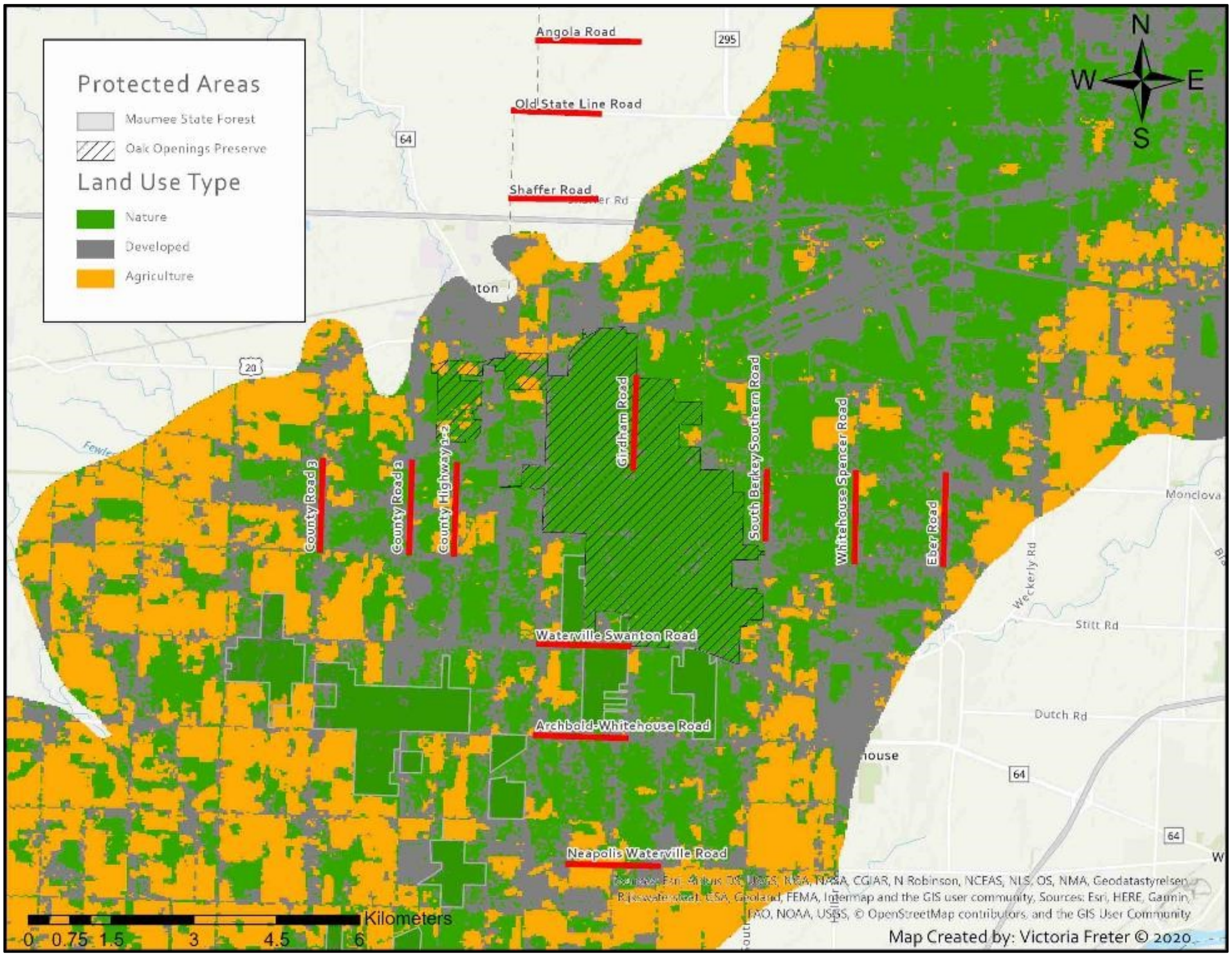

Figure 1.8. Map of surveyed road transects (red) in and around Oak Openings Preserve. Maumee State Forest (gray outline) and Oak Openings Preserve (diagonal) are highlighted (Root & Martin, 2018).

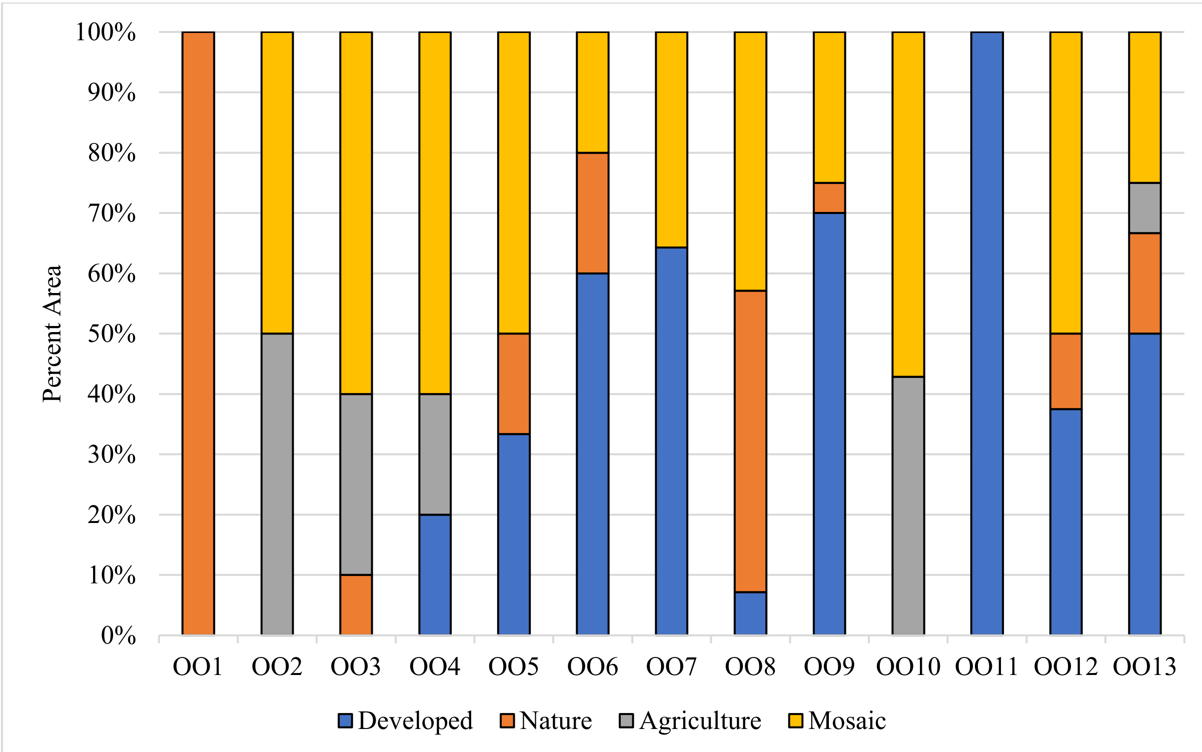

Figure 1.9. The percentage of Oak Openings Preserve's land use type (agriculture, developed, mosaic, nature) by road transect.

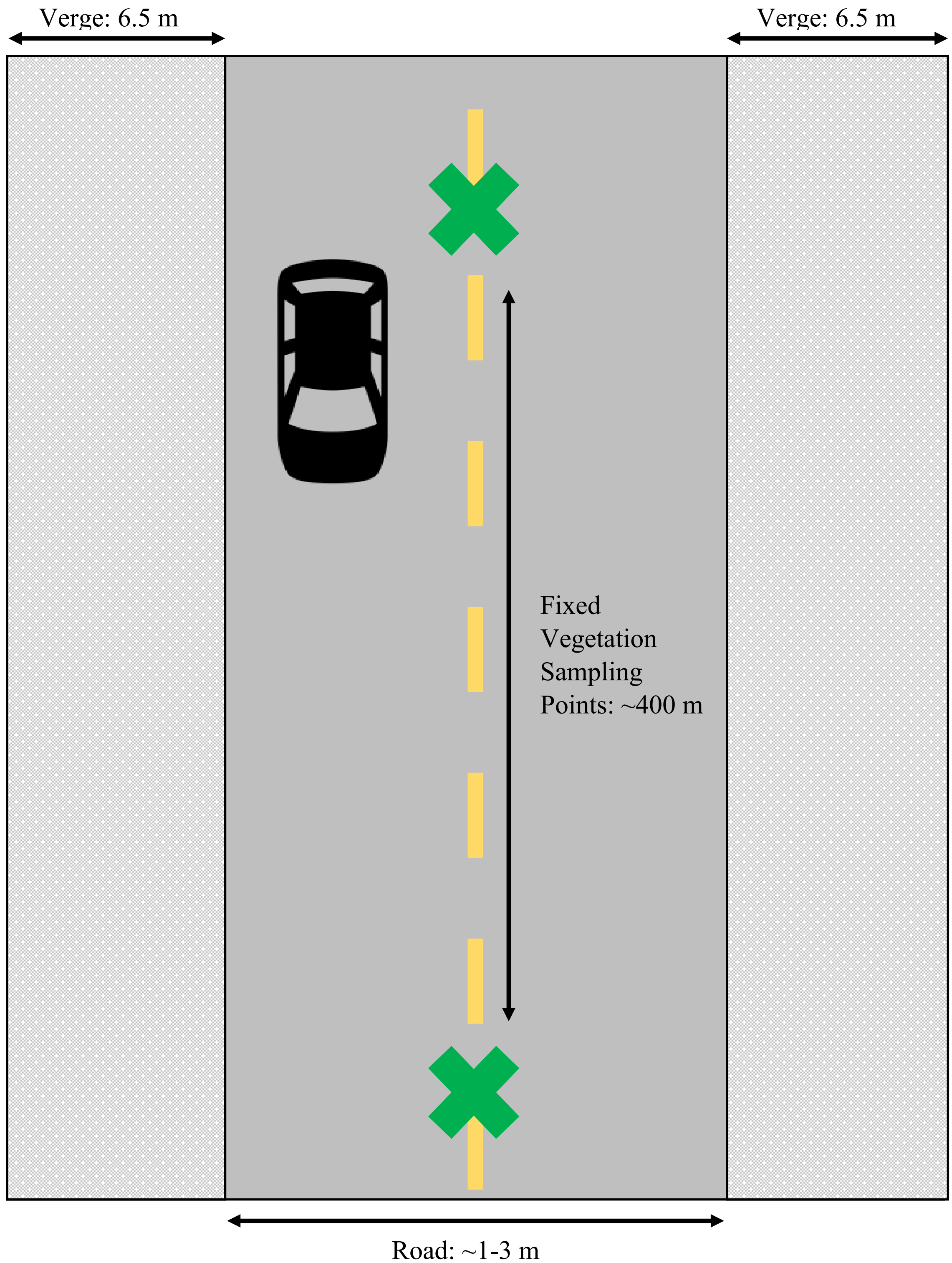

Figure 1.10. Road diagram showing approximate widths of roadways, verges, and distance between fixed vegetation points. Not to scale.

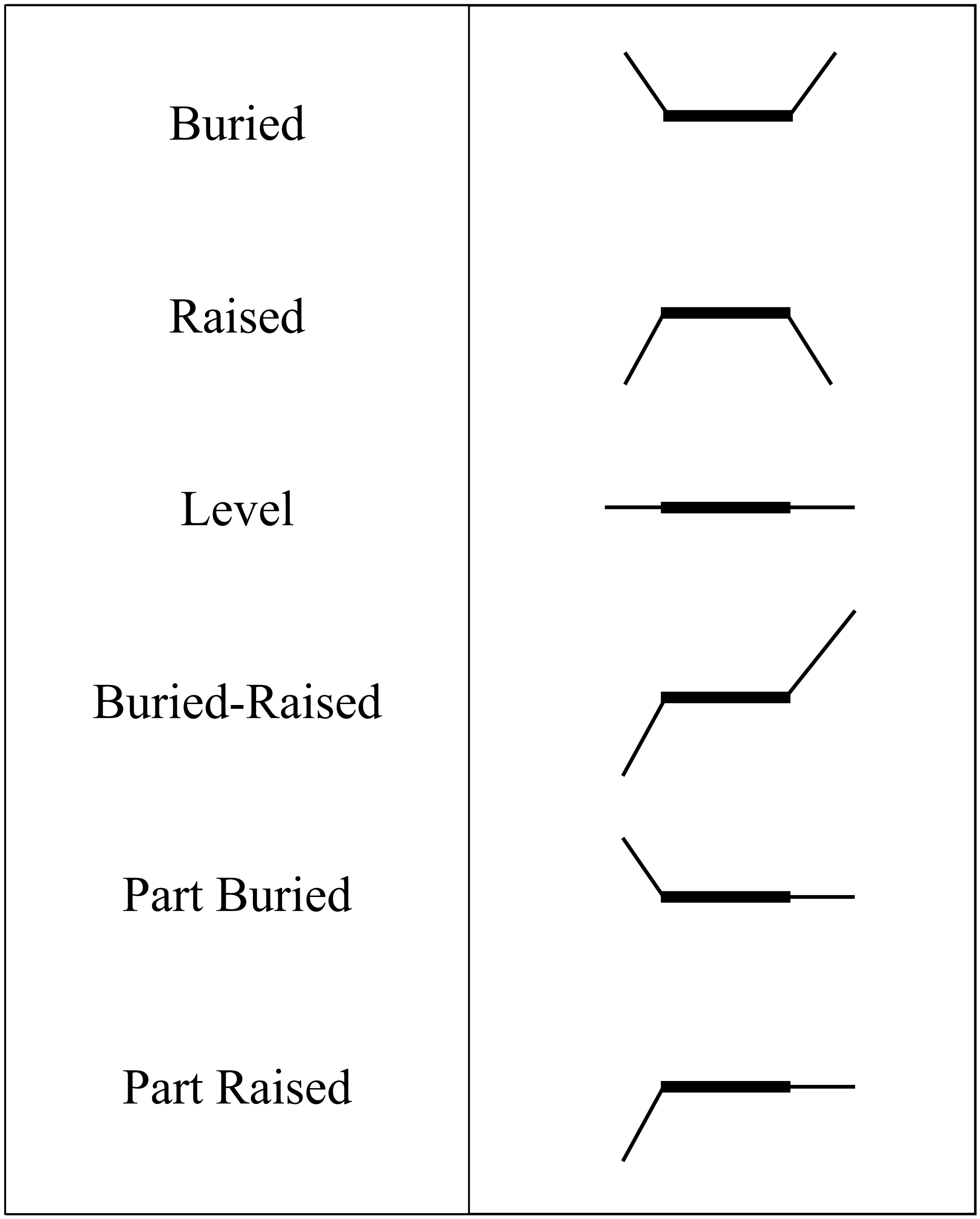

Figure 1.11. Categorization of road type based on road cross section. The thick black lines in the middle represents the road, the thin black lines on both sides represents the verges. Adapted from Clevenger et al. (2003).

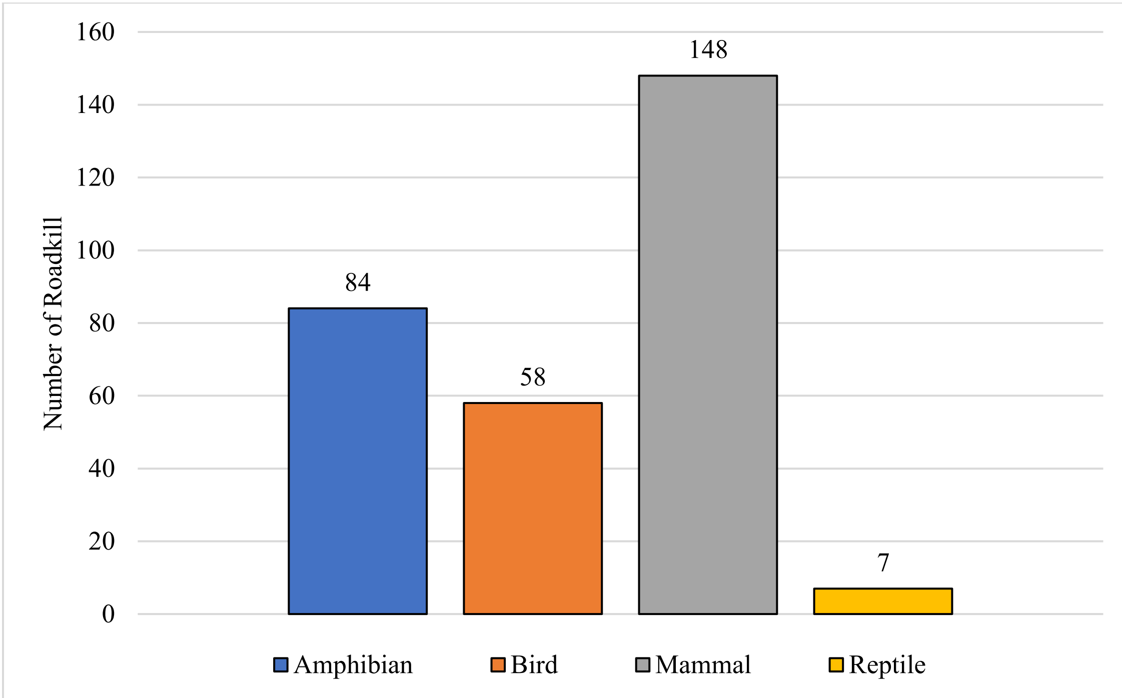

Figure 1.12. Number of roadkill per taxa that was found in or around the transects. Total number for each taxon listed above bar on graph. The blue bar represents amphibians found as roadkill. The orange bar represents birds found as roadkill. The gray bar represents mammals found as roadkill. The yellow bar represents reptiles found as roadkill.

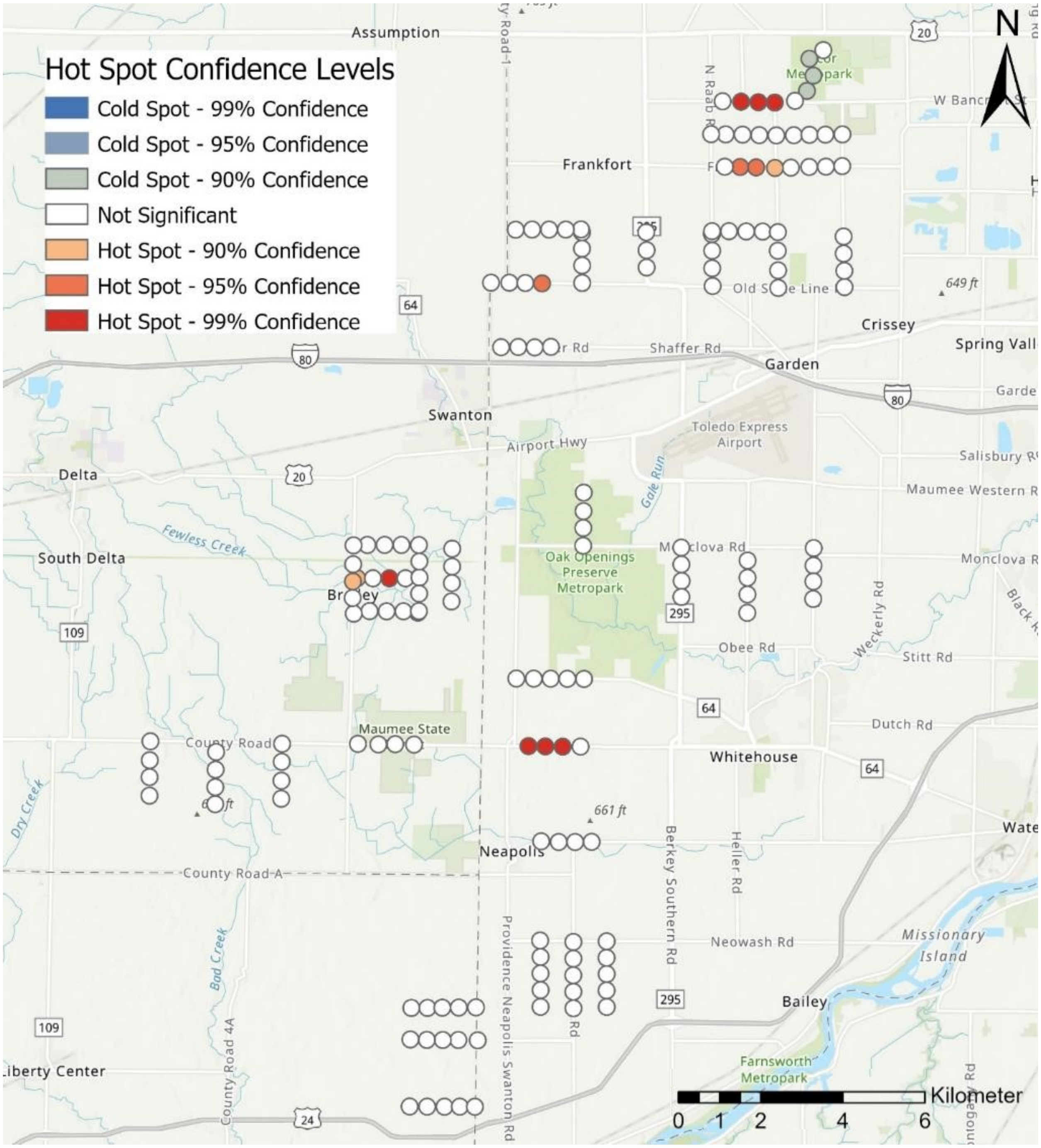

Figure 1.13. Getis-Ord Gi* hot spot analysis for all roadkill across the study area in Northwest Ohio. White circles show 200 m buffers that were not significant. Warm colored circles (orange-red) show buffers with 90-99% significance of being a hot spot. Cold colored circles (blue-green) show buffers with 90-99% significance of being a cold spot.

Figure 1.14. Getis-Ord Gi* hot spot analysis for all roadkill in and around Kitty Todd Nature Preserve. White circles show 200 m buffers that were not significant. Warm colored circles (orange-red) show buffers with 90-99% significance of being a hot spot. Cold colored circles (blue-green) show buffers with 90-99% significance of being a cold spot.

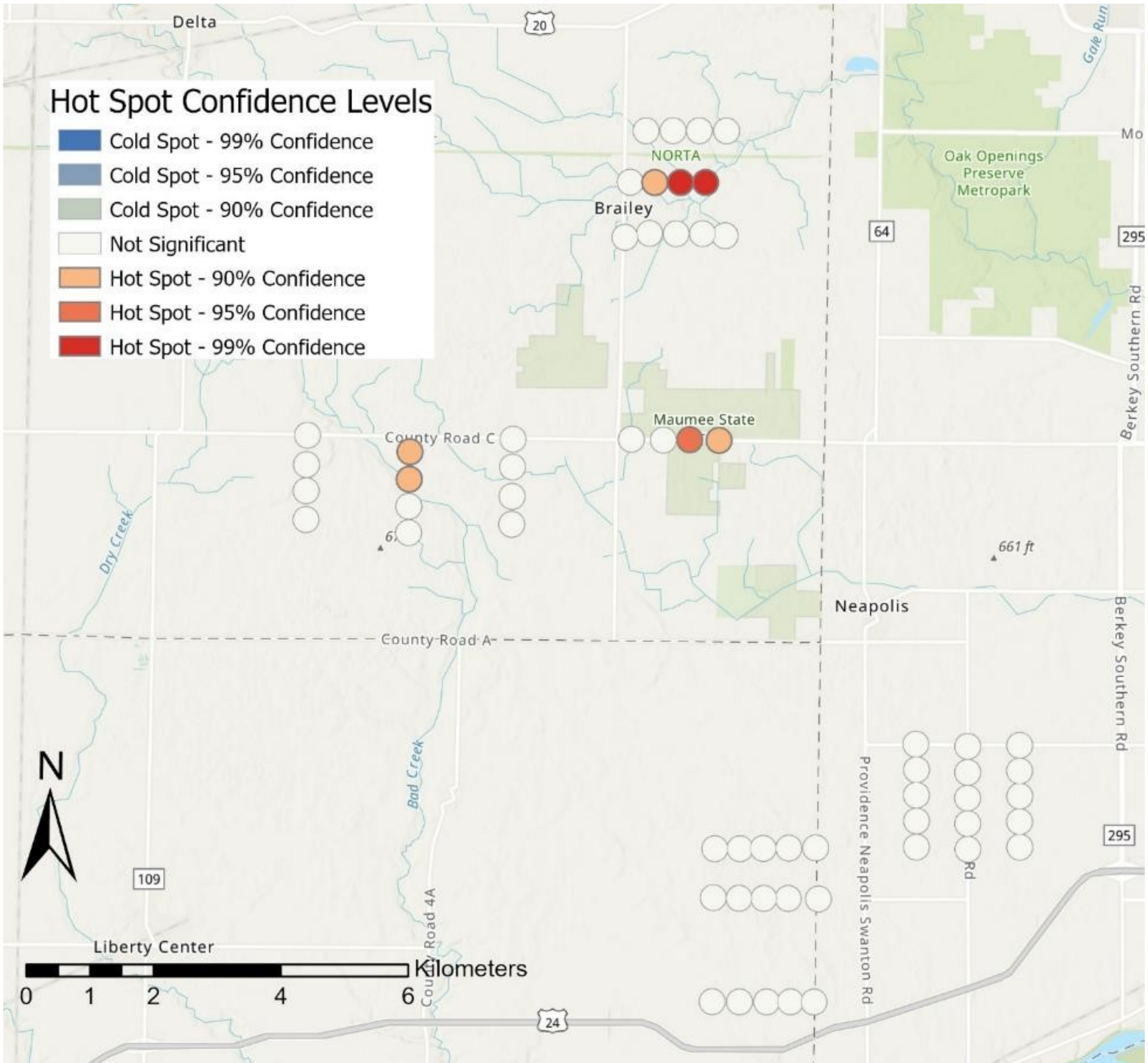

Figure 1.15. Getis-Ord Gi* hot spot analysis for all roadkill in and around Maumee State Forest. White circles show 200 m buffers that were not significant. Warm colored circles (orange-red) show buffers with 90-99% significance of being a hot spot. Cold colored circles (blue-green) show buffers with 90-99% significance of being a cold spot.

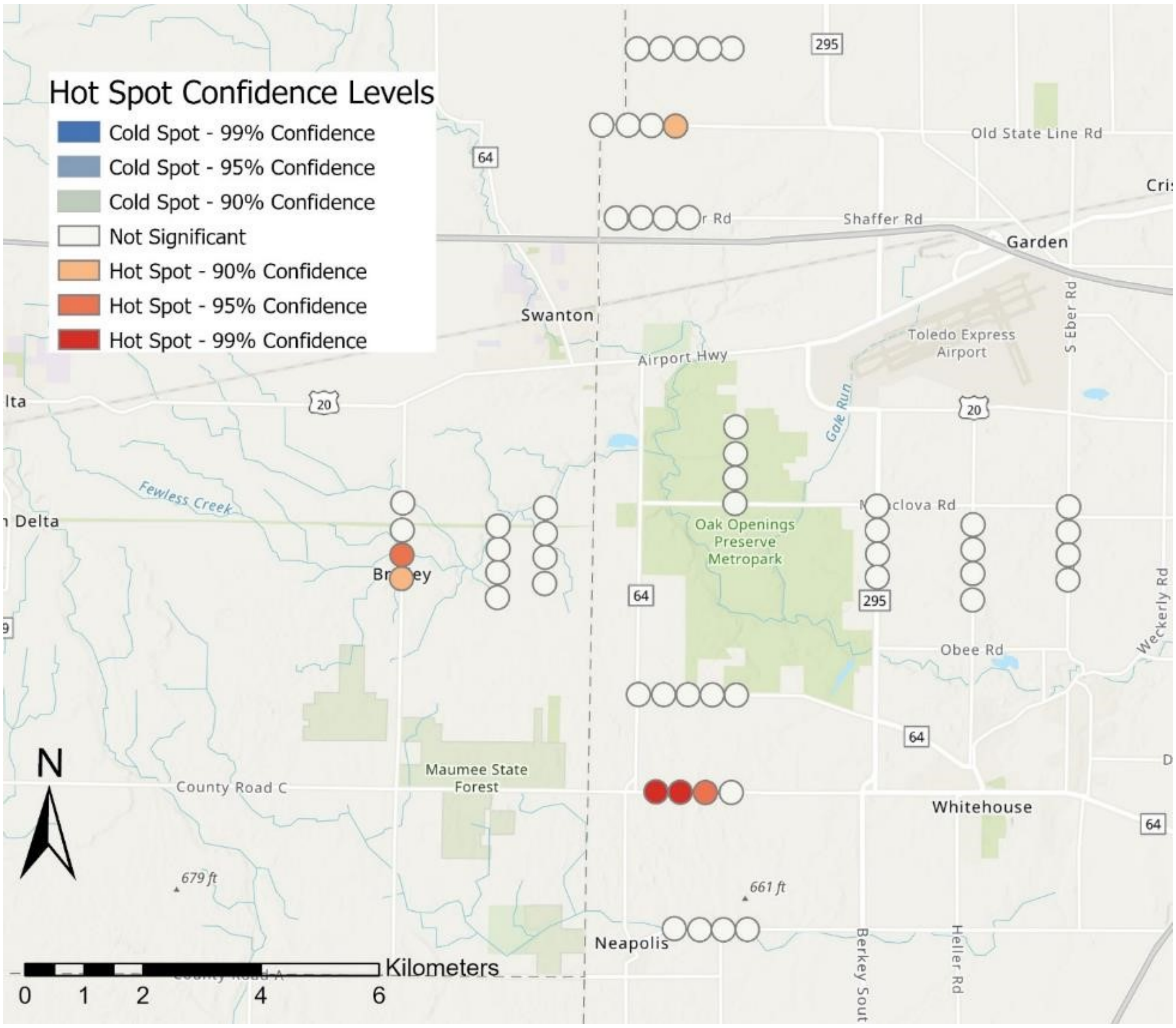

Figure 1.16. Getis-Ord Gi* hot spot analysis for all roadkill in and around Oak Openings Preserve. White circles show 200 m buffers that were not significant. Warm colored circles (orange-red) show buffers with 90-99% significance of being a hot spot. Cold colored circles (blue-green) show buffers with 90-99% significance of being a cold spot.

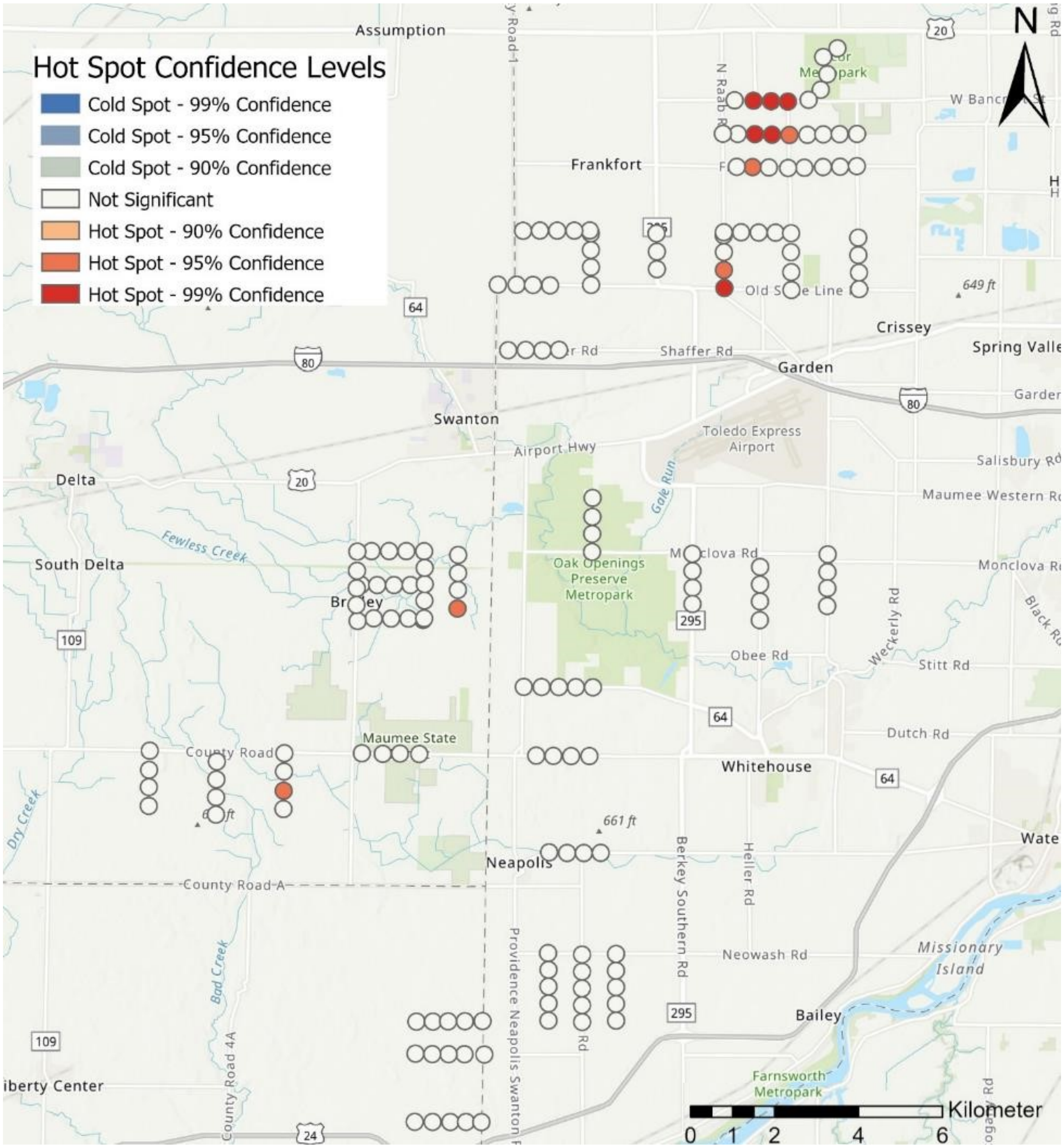

Figure 1.17. Getis-Ord Gi* hot spot analysis for all amphibian roadkill across the study area in Northwest Ohio. White circles show 200 m buffers that were not significant. Warm colored circles (orange-red) show buffers with 90-99% significance of being a hot spot. Cold colored circles (blue-green) show buffers with 90-99% significance of being a cold spot.

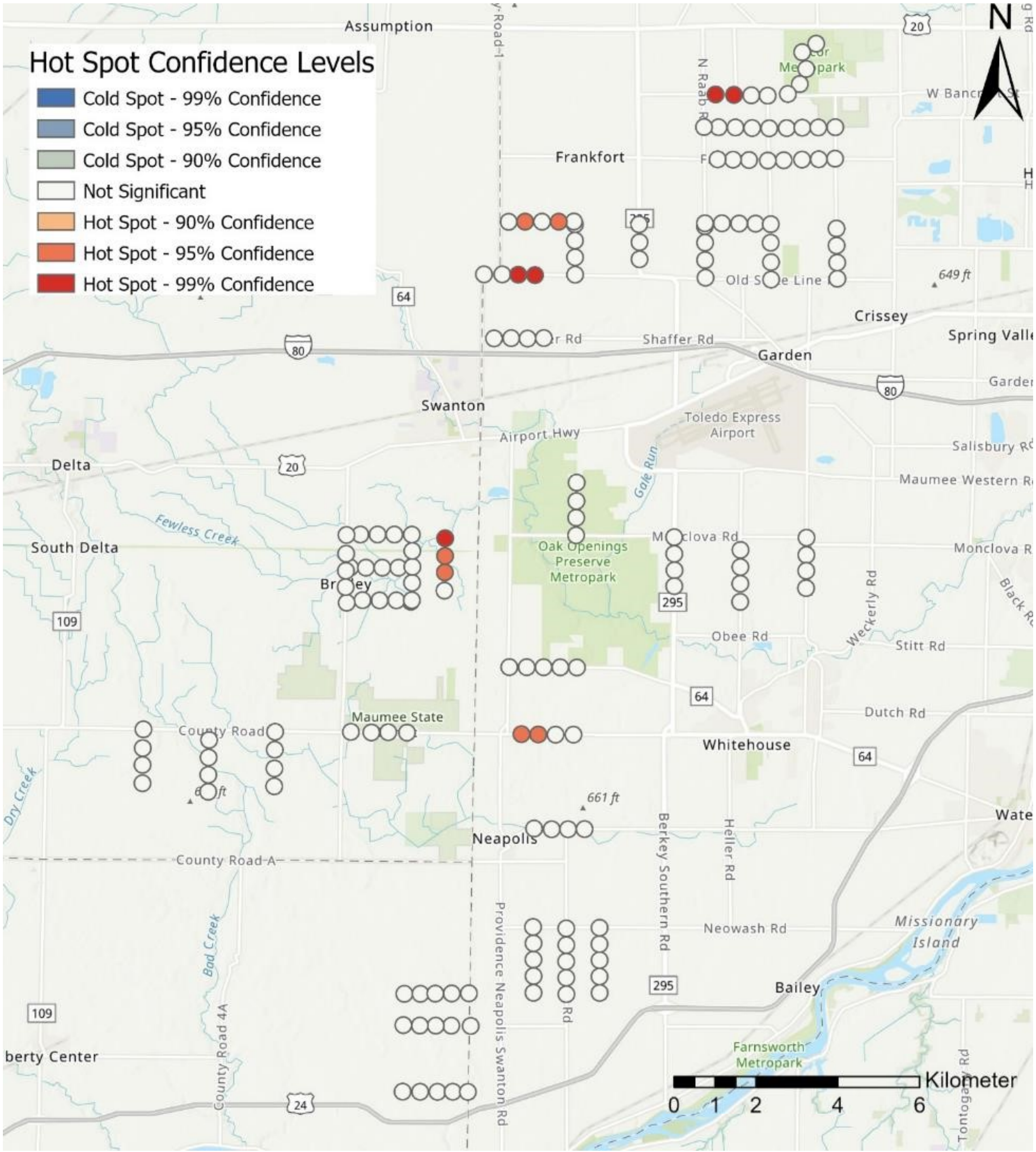

Figure 1.18. Getis-Ord Gi* hot spot analysis for all bird roadkill across the study area in Northwest Ohio. White circles show 200 m buffers that were not significant. Warm colored circles (orange-red) show buffers with 90-99% significance of being a hot spot. Cold colored circles (blue-green) show buffers with 90-99% significance of being a cold spot.

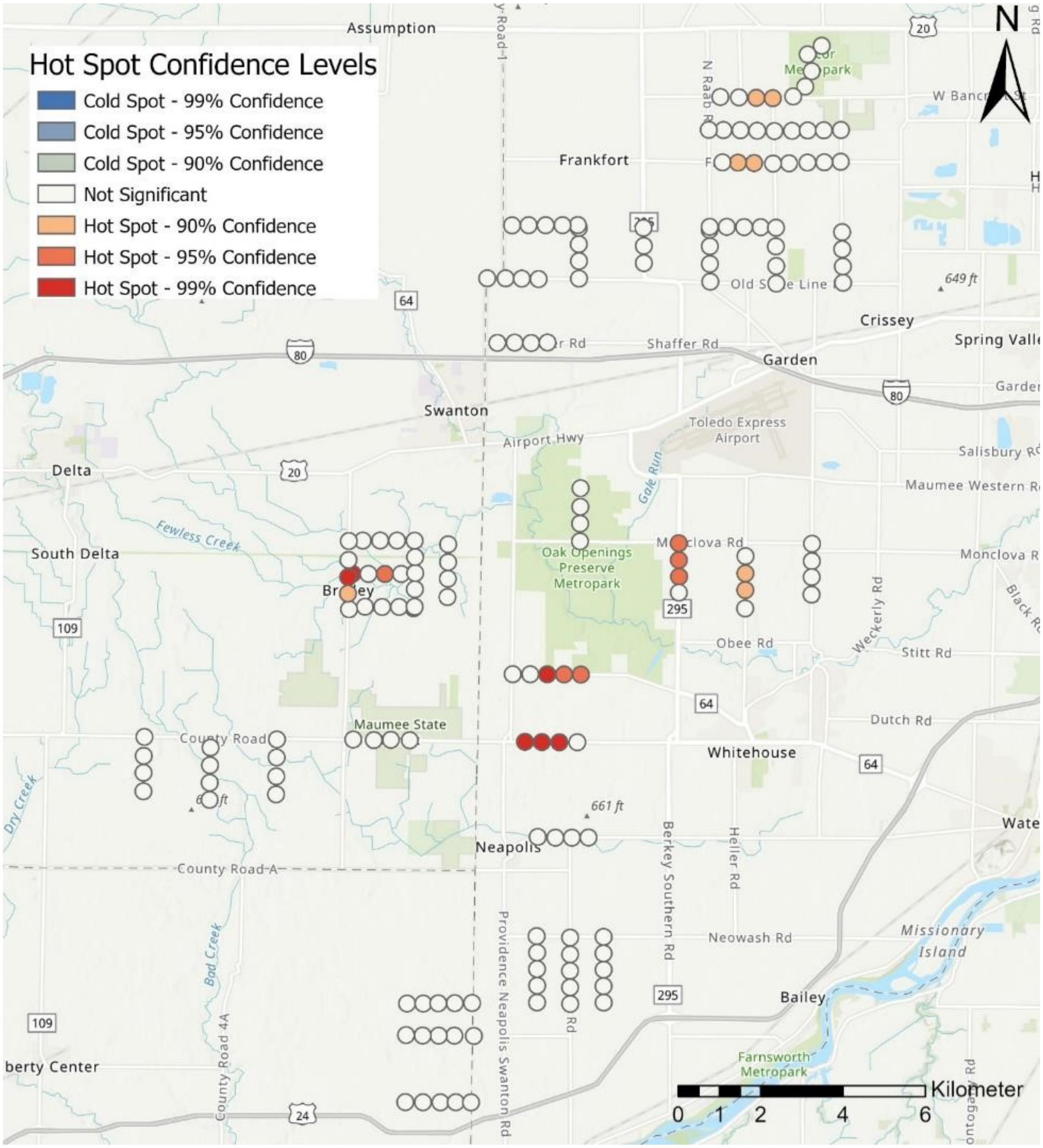

Figure 1.19. Getis-Ord Gi* hot spot analysis for all mammal roadkill across the study area in Northwest Ohio. White circles show 200 m buffers that were not significant. Warm colored circles (orange-red) show buffers with 90-99% significance of being a hot spot. Cold colored circles (blue-green) show buffers with 90-99% significance of being a cold spot.

<u>Tables</u>

Table 1.1. The natural areas within the Oak Openings Region that were surveyed with key characteristics. Shown is the area in hectares and land manager for each protected area.

Natural Area	**Managed by**	**Size (hectares)**
Irwin Prairie State Nature Preserve	Ohio Division of Natural Areas and Preserve	83.8
Kitty Todd Nature Preserve	The Nature Conservancy	566.6
Maumee State Forest	Ohio Department of Natural Resources Division of Forestry	>1,254.5
Oak Openings Preserve Metropark	Metroparks Toledo	~2,023.4
Secor Metropark	Metroparks Toledo	237.1
Wiregrass Lake Metropark	Metroparks Toledo	~5

Table 1.2. All surveyed road transects broken down by natural area. Shown are the key characteristics for the roads: road name, transect number, length, width, traffic volume, speed limit, and distance from natural area.

Natural Area	Road Name	Transect Number	Length (km)	Width (m)	Traffic (AADT)	Speed Limit (km/h, mph)	Distance from Natural Area (km)
Kitty Todd Nature Preserve	West Tupelo Way	KT1	1.73	6.00	-	32.2, 20.0	0.00
	Bancroft Street	KT2	1.57	9.00	1132	88.5, 55.0*	3.15
	Dorr Street (west)	KT3	1.58	6.50	277	88.5, 55.0	2.35
	Dorr Street (east)	KT4	1.63	6.50	497	88.5, 55.0	2.35
	Frankfort Road (east)	KT5	1.62	6.50	869	88.5, 55.0*	1.55
	Frankfort Road (west)	KT6	1.59	6.50	734	88.5, 55.0*	1.55
	South Lathrop Road	KT7	1.32	6.00	209	88.5, 55.0	3.18
	South Berkey Southern Road	KT8	1.17	8.00	1124	88.5, 55.0	1.60
	South Raab Road	KT9	1.36	6.50	277	88.5, 55.0*	0.00
	Angola Road	KT10	1.61	6.00	300	88.5, 55.0*	0.00
	South Schwamberger Road	KT11	1.37	6.00	243	88.5, 55.0*	0.00
	Irwin Road	KT12	1.34	6.00	435	72.4, 45.0	0.00
Maumee State Forest	County Road F	M1	1.60	5.50	154	88.5, 55.0*	3.25
	Township Road EF	M2	1.61	6.00	412	88.5, 55.0*	2.44
	County Road E	M3	1.61	6.00	469	88.5, 55.0*	1.62
	County Road C	M4	1.59	6.50	958	88.5, 55.0*	0.00
	County Road 6	M5	1.58	6.50	245	88.5, 55.0*	3.63
	County Road 5	M6	1.55	6.00	320	88.5, 55.0*	1.62

	County Road 4	M7	1.54	5.50	199	88.5, 55.0*	0.00
	County Road S3	M8	1.60	6.00	100	88.5, 55.0*	2.40
	County Road T3/Box Road	M9	1.62	6.00	264	88.5, 55.0*	0.78
	Township Road U	M10	1.63	3.50	296	88.5, 55.0*	0.00
	Manore Road	M11	1.61	5.50	88	88.5, 55.0*	1.51
	Jeffers Road	M12	1.61	7.00	677	88.5, 55.0*	2.32
	Yawberg Road	M13	1.61	6.00	52	88.5, 55.0*	3.14
Oak Openings Preserve	Girdham Road	OO1	1.62	5.00	387	88.5, 55.0*	0.00
	Shaffer Road	OO2	1.52	6.50	850	88.5, 55.0*	2.36
	Old State Line Road	OO3	1.53	9.50	1659	88.5, 55.0*	3.90
	Angola Road	OO4	1.82	6.50	309	88.5, 55.0	5.22
	County Highway 1-2	OO5	1.55	6.00	1291	88.5, 55.0	1.57
	County Road 2	OO6	1.62	6.50	1451	88.5, 55.0*	2.40
	County Road 3	OO7	1.61	7.00	2410	56.3, 35.0	4.00
	Waterville Swanton Road	OO8	1.61	7.50	3105	88.5, 55.0	0.00
	Archbold-Whitehouse Road	OO9	1.61	8.50	1660	88.5, 55.0	1.67
	Neapolis Waterville Road	OO10	1.61	9.00	1500	88.5, 55.0	3.97
	South Eber Road	OO11	1.61	10.00	3057	88.5, 55.0*	3.24
	Whitehouse Road	OO12	1.61	6.50	532	88.5, 55.0*	1.62
	South Berkey Southern Road	OO13	1.20	8.00	2949	88.5, 55.0*	0.00

* Speed limit not posted, assumed 88.5 km/h (55.0 mph).

Table 1.3. Example of variables measured at each roadkill spot. In bold are the variables measured.

Roadkill 104:	Racoon
Transect:	M4 (County Road C)
GPS:	41.515844, -83.915660
Land Use Left/Right:	Inactive Ag/Forest
Structure Presence:	Telephone Lines, Sign
Ditch Presence:	Ditch Present
Ephemeral Presence:	None
Canopy Cover:	12%
Ground Vegetation Type/Height:	Grass/5.0 cm

Table 1.4. Frequency of vertebrate species found across the roadways in Northwest Ohio from April - September 2019. Species of concern in Ohio indicated by *.

	Common Name	Scientific Name	N	% of Taxa	% of Total Roadkill
Amphibian	Frog	-	56	66.7	18.9
	Toad	-	20	23.8	6.7
	Unidentified Amphibian	-	8	9.5	2.7
Bird	American Goldfinch	*Spinus tristis*	1	1.7	0.3
	Cardinal	*Cardinalis cardinalis*	3	5.2	1.0
	Chicken	*Gallus gallus domesticus*	3	5.2	1.0
	Common Grackle	*Quiscalus quiscula*	1	1.7	0.3
	Horned Lark	*Eremophila alpestris*	1	1.7	0.3
	Mourning Dove	*Zenaida macroura*	1	1.7	0.3
	Robin	*Turdus migratorius*	12	20.7	4.0
	Sparrow	*Passeridae sp.*	15	25.9	5.1
	Yellow Warbler	*Setophaga petechia*	1	1.7	0.3
	Unidentified Bird	-	20	34.5	6.7
Mammal	American Red Squirrel	*Tamiasciurus hudsonicus*	2	1.4	0.7
	Eastern Chipmunk	*Tamias striatus*	7	4.7	2.4
	Eastern Fox Squirrel	*Sciurus niger*	11	7.4	3.7
	Eastern Mole	*Scalopus aquaticus*	2	1.4	0.7
	Groundhog	*Marmota monax*	8	5.4	2.7
	House Cat	*Felis catus*	1	0.7	0.3
	Mouse	*Peromyscus spp.*	8	5.4	2.7
	Northern Short Tailed Shrew	*Blarina brevicauda*	14	9.5	4.7
	Opossum	*Didelphis virginiana*	38	25.7	12.8
	Racoon	*Procyon lotor*	47	31.8	15.8
	Unidentified Mammal	-	10	6.8	3.4
Reptile	Eastern Box Turtle*	*Terrapene carolina carolina*	1	14.3	0.3
	Common Snapping Turtle	*Chelydra serpentina*	1	14.3	0.3
	Garter Snake	*Thamnophis spp.*	3	42.9	1.0
	Unidentified Reptile	-	2	28.6	0.7
	Total Roadkill		297		
	Total Species		24		

Table 1.5. Frequency of vertebrate taxa on the roadways in and around Kitty Todd Nature Preserve from April - September 2019.

Road Name	**Transect Number**	**Amphibian**	**Bird**	**Mammal**	**Reptile**	**Total Roadkill**	**% of Roadkill**
West Tupelo Way	KT1	0	0	0	0	0	0.0
Bancroft Street	KT2	11	5	7	1	24	23.5
Dorr Street (west)	KT3	8	0	3	0	11	10.8
Dorr Street (east)	KT4	2	3	3	1	9	8.8
Frankfort Road (east)	KT5	0	1	3	1	5	4.9
Frankfort Road (west)	KT6	6	1	7	0	14	13.7
South Lathrop Road	KT7	4	1	2	0	7	6.9
South Berkey Southern Road	KT8	2	2	2	0	6	5.9
South Raab Road	KT9	5	0	1	0	6	5.9
Angola Road	KT10	2	2	3	0	7	6.9
South Schwamberger Road	KT11	2	1	2	1	6	5.9
Irwin Road	KT12	2	0	5	0	7	6.9
Total		44	16	38	4	102	

Table 1.6. Frequency of vertebrate taxa on the roadways in and around Maumee State Forest from April - September 2019.

Road Name	Transect Number	Amphibian	Bird	Mammal	Reptile	Total Roadkill	% of Roadkill
County Road F	M1	0	0	2	0	2	2.8
Township Road EF	M2	4	3	8	0	15	21.1
County Road E	M3	1	1	1	0	3	4.2
County Road C	M4	4	1	5	0	10	14.1
County Road 6	M5	0	1	2	0	3	4.2
County Road 5	M6	2	2	4	0	8	11.3
County Road 4	M7	5	1	1	0	7	9.9
County Road S3	M8	0	0	4	0	4	5.6
County Road T3/Box Road	M9	4	1	3	0	8	11.3
Township Road U	M10	0	2	0	0	2	2.8
Manore Road	M11	1	0	0	0	1	1.4
Jeffers Road	M12	0	2	4	0	6	8.5
Yawberg Road	M13	1	1	0	0	2	2.8
Total		22	15	34	0	71	

Table 1.7. Frequency of vertebrate taxa on the roadways in and around Oak Openings Preserve Metropark from April - September 2019.

Road Name	Transect Number	Amphibian	Bird	Mammal	Reptile	Total Roadkill	% of Roadkill
Girdham Road	OO1	0	0	2	1	3	2.4
Shaffer Road	OO2	1	1	2	0	4	3.2
Old State Line Road	OO3	0	8	1	1	10	8.1
Angola Road	OO4	4	5	1	0	10	8.1
County Highway 1-2	OO5	4	5	3	0	12	9.7
County Road 2	OO6	2	0	3	0	5	4.0
County Road 3	OO7	1	2	11	0	14	11.3
Waterville Swanton Road	OO8	2	0	12	0	14	11.3
Archbold-Whitehouse Road	OO9	3	4	13	0	20	16.1
Neapolis Waterville Road	OO10	0	1	6	0	7	5.6
South Eber Road	OO11	1	0	5	0	6	4.8
Whitehouse Road	OO12	0	0	7	1	8	6.5
South Berkey Southern Road	OO13	0	1	10	0	11	8.9
Total		18	27	76	3	124	

CHAPTER 2. TEMPORAL HOT SPOTS: HOW VERTEBRATE ROAD MORTALITY CHANGES OVER TIME

Introduction

Just like humans, animals use roadways throughout the year to move from one location to another, to find resources, and to find mates. These movements may change throughout the year based on abiotic factors (e.g. temperature, precipitation, and weather) (Winkler et al., 2014). They may also change based on animal dispersal patterns. Two common types of long-term movement are seasonal migration and dispersal (Breed & Moore, 2016). Shorter term movements are made up of smaller, daily movements, such as movements to find food/water, shelter, or mates. Movement based on environmental factors and regular dispersal movement can vary based on taxa and potentially vary based on species. These movements may impact the type and abundance of roadkill found throughout the year. Anthropogenic barriers (e.g. roads) are considered one of the most serious threats to animal movement (Croteau, 2010; Meza-Joya et al., 2019).

Migration is simplistically described as a long, seasonal population movement from one habitat to another. Though there is variation when migrations occur, starting in spring one can find caribou, monarch butterflies, northern elephant seals, ruby-throated humming birds, and others starting their trek across different parts of North America away from their winter locations (Handwerk, 2019). This movement is generally roundtrip in vertebrates and links up habitats that are favorable during different seasons. In some cases, this could be a warmer climate for the winter or a safe place for breeding (Dingle & Drake, 2007). If roads fall along migration routes, they may be used for quicker migration. Regardless of route, roads will need to be crossed during migrations. This movement may increase an organism's time on or crossing roads and increase the chance of unsuccessful crossing. Therefore, temporal hot spots in road mortality may occur with these seasonal migration movements. Temporary road closures may occur to reduce the number of organisms killed on roadways during major movements. The National Park Service annually closes a road within the Delaware Water Gap National Recreational Area for evenings with precipitation and mild temperatures from March to mid-April to help protect amphibians who are moving for the breeding season (Sandt, 2018).

Unlike migrations, dispersals tend to be a permanent movement of individuals. This movement generally benefits the organism by increasing mate choice, locating new resources, or reducing competition (Breed & Moore, 2016; Songer, 2019). These movements vary based on sex, life stage, and taxa. Dispersal based on sex is common throughout vertebrate taxa. This is where one sex leaves and one sex stays. In mammals, it is generally the males that disperse, but in birds the opposite is true, with the females generally dispersing (Lawson Handley & Perrin, 2007; Prugnolle & de Meeus, 2002). Dispersal difference can change which sex is found on the roadways in greater numbers. In a study done by Steen et al. (2006), female freshwater turtles were found more likely to be on roadways than males. This could increase their chance of road mortality and greatly skew the sex ratio for that population. Juvenile dispersal or juveniles leaving their birth site has also been linked to increased roadkill (Grilo et al., 2009; Kowalczyk et al., 2009; Lin et al., 2019). This suggests that with the increase in population, there may be more movement to access resources. In addition, juveniles are less experienced with roads and may have a higher chance of being hit (Schwartz et al., 2020). While migrations tend to be based around seasonal changes, dispersals vary based on the type of dispersal and species. For example, juvenile dispersal will vary based on breeding season, length of gestation, and amount of parental care. Groundhogs mate in spring and drive off their young by July or August, whereas striped skunks mate in late winter and the babies leave in the fall. Raccoons mate around the same time as skunks, but young racoons can stay with their mother through winter (Ohio Division of Wildlife, 2018).

Daily animal movements happen more frequently than seasonal migrations or dispersals and arguably are less predictable. These movements can vary in length, from a short walk to find nearby water to a longer trek to mark territory. Throughout these movements, organisms may be required to cross roads to reach resources or change their path/find different resources to avoid roadways. Animals are active at different times throughout the day, some like humans are awake with the sun and sleep at night (diurnal) and some are awake during the night and sleep during the day (nocturnal). Crepuscular organisms are generally awake during twilight and cathemeral organisms are awake whenever they need when they need food (Bennie et al., 2014). When organisms move throughout the day can alter their interaction with humans. Nocturnal movement potentially allows for crossing of roadways when less traffic is present. However, crossing roadways when visibility is lower may shorten driver reaction time. Animals may also be blinded or stunned by vehicle headlights, increasing chances of being hit (Chyn et al., 2019). All types of movement, long or short, increase the likelihood that organisms will cross roads, interact with humans, and meet their demise on the roadways.

This chapter focuses on the temporal factors that potentially influence the diversity, abundance, and distribution of roadkill. Temporal factors include but are not limited to: season, temperature, weather variables, and canopy cover. Many studies have found that the amount of roadkill peaked in different months based on taxa (Ashley & Robinson, 1996; Canal et al., 2018; Canova & Balestrieri, 2018; Clevenger et al., 2003; Seo et al., 2015). Some taxa even had multiple peaks depending on dispersal behavior. With a variety of variables affecting when animals move resulting in a change in roadkill diversity, abundance, and distribution, it is important to look at how those changes vary between taxa. Understanding this would help land managers make decisions on temporary road closures, leading to the reduction of human-wildlife conflicts. This research will locate monthly mortality hot spots and see how temporal factors may play a role in the movement of organisms across a fragmented landscape.

Our goal was to identify the factors that: (1) affect when vertebrates are crossing the roads, (2) affect when mortality happens on roads, and (3) whether there are temporal hot spots in roadkill within the study site. We hypothesize that there will be some variation between taxa, but a general roadkill peak in June. With this research we examine if changes in climate, weather variables, and vegetation affect animal movement and the hot spots. We analyze how peaks and hot spots vary between taxa as well. We predict that amphibian roadkill will have an early peak in the spring, following normal dispersal patterns. Mammal and bird roadkill will potentially have later peaks for juvenile dispersal.

Methods

Study Location

Our research was conducted in and around Irwin Prairie State Nature Preserve, Kitty Todd Nature Preserve, Oak Openings Preserve Metropark, Maumee State Forest, Secor Metropark, and Wiregrass Lake Metropark in northwestern Ohio. These protected (natural) areas are all located within the Oak Openings Region (Figure 2.1). We focused on Kitty Todd Nature Preserve, Maumee State Forest, and Oak Openings Preserve, as they are the largest protected areas in the region. Irwin Prairie State Nature Preserve, Secor Metropark, and Wiregrass Lake Metropark were included with Kitty Todd Nature Preserve. Kitty Todd Nature Preserve is 566.6 hectares and managed by The Nature Conservancy. Maumee State Forest is just over 1,250.0

hectares and managed by the Ohio Department of Natural Resources Division of Forestry. Oak Openings Preserve is the largest natural area at roughly 2,023.4 hectares and is managed by Metroparks Toledo. Table 2.1 lists each protected natural area and its key characteristics.

Road Surveys

We surveyed roads in and around Kitty Todd Nature Preserve, Maumee State Forest, and Oak Openings Preserve, see Figure 2.2, for all dead vertebrates except for deer. Deer were not included as carcasses are more likely to be taken by humans, which would bias our results. A total of 38 roads were surveyed covering 59.26 kilometers (km), Figure 2.2. Road lengths ranged from 1.17 km - 1.82 km. Roads selected were in a grid pattern radiating outward from the natural areas varying from 0.00 km - 5.63 km. All roads were two lane, paved roads, with speed limits that varied between 32.2 km/h (20.0 mph) and 88.5 km/h (55.0 mph) (Table 2.2). Many of the country roads did not have speed limits marked; these roads are recognized to have a speed limit of 88.5 km/h (55.0 mph) in Ohio.

For Kitty Todd Nature Preserve, we surveyed a total of 12 roads, covering 17.98 km, Table 2.2. Transects ranged from 1.17 km to 1.73 km. Roads were in all cardinal directions except for south, as shown in Figure 2.3, with three roads inside the natural areas. Surveys were not done in the southern direction to avoid the Toledo Express Airport. For Maumee State Forest, we surveyed a total of 13 roads, covering 20.76 km, Table 2.2. Transects ranged from 1.54 km to 1.63 km. Roads were in all four cardinal directions moving outward from Maumee State Forest, as shown in Figure 2.4, with one road inside the natural area. For Oak Openings Preserve, we surveyed a total of 13 roads, covering 20.52 km, Table 2.2. Transects ranged from 1.20 km to 1.82 km. Roads were in all four cardinal directions moving outward from Oak Openings Preserve, as shown in Figure 2.5, with one road inside the park.

All dead terrestrial vertebrates (e.g. amphibians, birds, mammals, and reptiles) were counted during road surveys. We created a protocol based on the Collinson et al. (2014) standardized protocol for counting flattened fauna. All transects were surveyed every other week for a total of two to three times a month from April to October 2019. To be consistent, we started all surveys within an hour or two of sunrise. Following Garrah et al. (2015) and Smith and Dodd (2003), surveys were performed at slower speeds on bike when possible, riding 20.1 - 24.9 km/h (12.5 - 15.5 mph). The only exception was for two weeks in April and two weeks in May when surveys were done by vehicle, driving 40.2 - 48.3 km/h (25.0 - 30.0 mph), when the weather was too cold to ride a bike.

We defined the road as one edge of the pavement to the other edge. Based on previous work in the Oak Openings Region (Jonaitis, 2017), the verge was defined as 6.5 meters (m) from the edge of the road (Figure 2.6). For each roadkill found we recorded the location with a Garmin Etrex GPS, identified to the species (or taxonomic class, if species identification was not possible), took a photograph, and recorded a variety of temporal and spatial variables, which included land use type, presence of human structures, presence of ditches, presence of ephemeral variables, canopy cover percentage, presence of understory, and type of roadside vegetation (Table 2.3). Due to specimen quality, amphibians were only identified to the category of ‘frog’ or ‘toad’, as species identification was not usually possible. Animals on the verges were counted as roadkill, as we assumed that they may have been injured by a vehicle and moved off the road before dying. We did not remove animal carcasses, but we noted if they still remained on the road during the next survey. Carcasses that persisted on the roads were identified by GPS location, prior data, and photographs to prevent duplicate entries.

Environmental Characteristics of Roads

For all the roads, we obtained environmental variables: season (i.e., spring, summer), temperature (C°), humidity (%), precipitation (cm), and moon illumination (%). Spring was considered from April 14, 2019 (start of the surveys) to June 20, 2019. Summer was considered June 21, 2019 to September 22, 2019 (end of the surveys). Both daily precipitation and daily temperature were obtained from the NOAA weather data (https://ncdc.noaa.gov/). Moon illumination was obtained from timeanddate.com (https://www.timeanddate.com/moon/). In addition, humidity, temperature, and weather variables were recorded at the start of all surveys using a Brunton Atmosphere Pro handheld weather station.

Spatial and Structural Features of Roads

For all roads, we measured spatial and structural features including; canopy cover, vegetation cover, presence of understory, distance from natural area, land use type, traffic volume, road speed limit, and other road features. We measured canopy cover at fixed sampling points, every 400 m on both sides of the road and at roadkill points. On average, each transect had four fixed sampling points. At each point, four canopy cover measurements were taken and averaged together. Canopy cover measurements were taken once a month using HabitApp Version 1 (Scrufster, 2014), approximately in the middle of the month. At each roadkill point, three canopy cover measurements were taken and averaged together. Vegetation cover type (e.g., grasses, shrubs, berry bushes, etc.) and height of vegetation at each sampling point were also recorded in centimeters for the verges on both sides of the road and for the verges on both sides of the roadkill points. Vegetation height measurements were taken monthly, at the same time that canopy cover measurements were taken. Understory was considered the layer of plant growth between the forest floor and the forest canopy (Brookshire, 2018). Road distance from the protected areas was measured in Google Maps for each transect.

Roads were categorized based on the type of land use at the fixed points and at roadkill points. The land use types were simplified into four categories: natural, agriculture, developed, and mosaic. Natural was recorded when land use consisted of natural ecosystems (i.e., wet prairie, perennial ponds, upland savanna, wet shrubland, swamp forest, upland coniferous forest, upland deciduous forest, floodplain forest, sand barrens, Eurasian meadow, or upland prairie) on each side of the road. Agriculture was recorded when land use was inactive agriculture, active agriculture, fallow agriculture, or farm on each side of the road. Inactive agriculture was defined as fields that were left undisturbed throughout the spring and summer. Active agriculture was defined as fields that were plowed, seeded, had plant growth, and harvested throughout the spring and summer. Fallow agriculture was defined as fields that were plowed, but no plant growth or harvest was recorded throughout the spring and summer. Farm was defined as land with a barn present and/or fenced in pastures with livestock and/or poultry. Developed was recorded when land use was made up of suburban and/or urban development (housing, businesses, etc.). Mosaic was recorded when land use categories were different on each side of the road, i.e., natural and developed, natural and agriculture, or developed and agriculture. Land use types were originally identified using the Oak Openings Region land cover map (Root & Martin, 2018), then confirmed with Google Maps, and finally verified in the field.

Road features measured included traffic volume (AADT), road speed limit (km/h), length of road (km), width of road (m), road quality (newly paved, few cracks, tarred and chipped, many cracks and holes), road topography (raised, buried, level, mixed), presence of ditches, and presence of human structures (e.g., signs, telephone poles, fences, mailboxes, etc.). Traffic volume, annual average daily traffic (AADT), was obtained through the Ohio Department of Transportation (https://odot.ms2soft.com/tcds/tsearch.asp?loc=Odot&mod=). Road speed limits, in km/h, were recorded for each road during the study. Length of road, in km, was measured in Google Maps and confirmed with a cycling computer, Garmin Edge 25. Width of road, in m, was measured in Google Maps and confirmed with a Meter Man, Komelon Series 45 surveyor's wheel. Road quality or the degree of road deterioration was noted at the beginning of the survey and updated with any road construction changes (e.g., roads repaved, cracks sealed, holes patches, lines repainted, etc.).

At the beginning of the study, road topography was categorized at each fixed point based on one of six categories that refer to the cross section of the road (Clevenger et al., 2003) (Figure 2.7). Buried, raised, and level referred to when both sides of the roads were the same topography. Mixed was recorded when both sides of the road were not the same category (buried-raised, part-buried, part-raised). Buried-raised is defined, when both sides of the road are opposite of each other, but neither are level (i.e., one side buried and the other raised). Part-buried and part-raised, referred to when half of the road is level and the other half is either buried or raised, respectively. Presence of roadside ditches and human structures (e.g. signs, telephone poles, bridges, guardrails, etc.) were noted at both the fixed points and the roadkill points. We noted if any construction or repairs happened on or near roads that could change the road features.

Ephemeral Variables

During all surveys, we recorded the presence of ephemeral variables at roadkill points and for each road, including water presence (in ditches or fields), invertebrate presence (alive or dead), temporary vegetation presence (flowers or berries), presence of recycling or trash cans, and presence of living vertebrates. These were measured as they may all act as temporary resources for organisms.

Analysis

Totals, averages, minimums, maximums, and roadkill per km were all highly correlated, therefore, we created 200 m buffers around all the fixed sampling points to run statistical analysis using total roadkill per buffered sampling point. More information about the 200 m buffered fixed sampling points can be found in the analysis section under "Road Surveys". We also checked for association between variables and each individual taxon, except for reptiles, for which there was insufficient sample size. The Bonferroni correction was applied to the significance values to account for repeated statistical analyses.

For weekly temperature, precipitation, moon illumination, and canopy cover minimum, average, and maximum values were highly correlated, so analysis was run using maximum values for each variable. For humidity, only maximum and average humidity were highly correlated, therefore analysis was run using maximum and minimum humidity.

Road Surveys

We performed hot spot analysis using Getis-Ord Gi* in ArcGIS version 10.2 (Environmental Systems Research Institute, 2014). We chose Getis-Ord Gi* instead of Moran's I because not only did we want to know if our roadkill points were clustered, but more specifically we wanted to know whether or not there were clusters of high/low values. Following the methodology in Jonaitis (2017), we created 200 m buffers around each of the fixed sampling points for all transects; 200 m represented the midpoint between each of the fixed survey points. We assumed that the 200 m buffers accurately represented the variation in variables on and around the roads. The total number of roadkill found within each buffered point was summed for each month and season. The total number of roadkill from each taxon was also summed individually for each season. We did not perform a hot spot analysis for reptiles because of a low sample size. We used the spatial join tool in ArcGIS to sum the roadkill points within each buffer. This hot spot analysis (Getis-Ord Gi* statistic) identified where significantly high or low clusters of roadkill were found on the roads. The test is defined as:

$$\Sigma\, G_i{*}(d) = \Sigma\, W_{ij}\, (d)\, X_j / X_j$$

The Gi* statistic gives a z-score for each feature signifying the presence of a hot or cold spot and its level of significance. For a 90% confidence level or p-value of < 0.10 the z-score value is $\pm$ 1.65 away from zero. For a 95% confidence level or p-value of < 0.05 the z-score value is $\pm$ 1.96 away from zero. For a 99% confidence level or p-value of < 0.01 the z-score value is $\pm$ 2.58 away from zero. A high positive z-score value indicates high clusters of roadkill or hot spots and a low z-score value indicates low negative clusters of roadkill or cold spots (Mitchell, 2020).

Environmental Characteristics of Roads

We tested if the amount of total roadkill per survey week was associated to the environmental characteristics; season, temperature, humidity, precipitation, and moon illumination. To do this we used the Wilcoxon/Kruskal-Wallis Test in JMP version 15.0 (SAS Institute Inc., 2019). We also checked to see if there was a significant difference between the environmental characteristics over time (i.e., survey week, month, season). We used total roadkill per 200 m buffered fixed sampling points that were created during the hot spot analysis. We did this for total dead amphibians, birds, and mammals individually. The variation in range and average temperature, precipitation, humidity, and moon illumination by survey week can be found in Table 2.4.

Spatial and Structural Features of Roads

To analyze seasonal and monthly influence of canopy cover and vegetation cover, we averaged the seasonal and monthly measurements separately within each of the 200 m buffered fixed sampling points. We then looked for association between the total roadkill and maximum measurement for each fixed sampling point. To look for significance we used a nonparametric one-way analysis in JMP. These features were analyzed based on survey week, survey month, and survey season for amphibian, bird, and mammal roadkill. The variation in range and average canopy cover and vegetation by month can be found in Table 2.5.

In addition, we also explored the relationships between all spatial and structural features. We eliminated one of each pair of variables that were highly correlated with one another (Spearman $\rho \geq 0.700$). After removing the highly correlated variables, we utilized stepwise regression in JMP to check for significant relationships between multiple spatial and structural features and total roadkill per 200 m buffer points. We also ran a stepwise regression between all the variables and the individual taxon (except reptiles) based on survey week. The best model was based on the lowest Akaike's Information Criterion (AIC_c). The final model was checked for normality using the Shapiro-Wilks test.

Ephemeral Variables

We explored if there was a relationship between presence of ephemeral variables and month or season. The ephemeral variables that were recorded include water presence (in ditches or fields), invertebrate presence, temporary vegetation presence (flowers or berries), and presence of living vertebrates. We checked for significance in JMP, using a nonparametric one-way analysis, Wilcoxon/Kruskal-Wallis Test. Ephemeral variables were also included in the regression models in JMP after highly correlated variables (Spearman $\rho \geq 0.700$) were removed. The best model was based on the lowest AIC_c. The final model was checked for normality using the Shapiro-Wilks test.

Results

Road Surveys

We surveyed roadways for 12 weeks, covering a total of 706 km over 100 hours between April 14, 2019 - September 18, 2019. A total of 297 roadkill vertebrates were found on the 38 transects within and around the three major natural areas. Roadkill was found during each month throughout the study period (Table 2.6). Roadkill was highest in the summer with 219 (73.7%), with the rest in spring with 78 (26.3%). On average there were 24.8 dead animals per survey week, however, roadkill was not evenly spread across the survey period (Figure 2.8). April had eight (2.7%) roadkill, May had 28 (9.4%) roadkill, June had 47 (15.8%) roadkill, July had 51 (17.2%) roadkill, August had 101 (34.0%) roadkill, and September had 62 (20.9%) roadkill.

April had the lowest number of roadkill with an average of four roadkill found per survey week. Our hot spot analysis revealed 16 significant hot spots across six transects; South Berkey Southern Road (north), County Road 3, Archbold-Whitehouse Road, County Road F, County Road EF, and County Road 5. All hot spots were at a 99% confidence level ($z > 2.58$), except for two on County Road 3 which were at a 95% confidence level ($z > 1.96$) (Figure 2.9).

May had just under a tenth of all roadkill with an average of 14 roadkill found per survey week. Our hot spot analysis revealed 15 significant hot spots across seven transects; Frankfort Road (east), Old State Line Road, County Road 2, Archbold-Whitehouse Road, South Berkey Southern Road (south), Township Road EF, and County Road 5. Eight of the hot spots were at a

99% confidence level ($z > 2.58$), six of the hot spots were at a 95% confidence level ($z > 1.96$), and one hot spot was at a 90% confidence level ($z > 1.65$) (Figure 2.10).

June had an average of 23.5 roadkill found per survey week. Our hot spot analysis revealed 14 significant hot spots across nine transects; Bancroft Street, South Raab Road, South Schwamberger Road, Irwin Road, Old State Line Road, Archbold-Whitehouse Road, Neapolis-Waterville Road, County Road EF, and Jeffers Road. One hot spot was at a 99% confidence level ($z > 2.58$), six of the hot spots were at a 95% confidence level ($z > 1.96$), and seven of the hot spots were at a 90% confidence level ($z > 1.65$) (Figure 2.11).

July had the third highest roadkill percentage with an average of 25.5 roadkill found per survey week. Our hot spot analysis revealed 14 significant hot spots across six transects; County Road 5, County Road 2, County Road 3, Waterville-Swanton Road, Whitehouse Road, and County Road EF. Three hot spots were at 99% confidence level ($z > 2.58$), five were at 95% confidence level ($z > 1.96$), and six were at 90% confidence level ($z > 1.65$) (Figure 2.12).

August had the highest number of roadkill with an average of 50.5 roadkill found per survey week. Our hot spot analysis revealed nine significant hot spots across five transects; Bancroft Street, Frankfort Road (west), South Raab Road, County Highway 1-2, and County Road 4. Three hot spots were at 99% confidence level ($z > 2.58$) and six were at 95% confidence level ($z > 1.96$) (Figure 2.13).

September had the second highest percentage of roadkill with an average of 31 roadkill found per survey week. Our hot spot analysis revealed seven significant hot spots across four transects; Bancroft Street, Waterville- Swanton Road, Archbold-Whitehouse Road, and South Berkey Southern Road (south). Five hot spots were at 99% confidence level ($z > 2.58$) and two were at 95% confidence level (z > 1.96) (Figure 2.14). A hot spot map showing the frequency of occurrence of hot spots during the survey months can be found in Appendix B Figure 2.S1.

Roadkill was not evenly spread across the study site and there were changes in temporal hot spots, these temporal changes were also seen while looking at seasonal roadkill distribution by taxon. As a result of lower abundance of amphibian roadkill in spring, we only did a hot spot analysis for the summer. Our summer hot spot analysis revealed ten significant hot spots for dead amphibians across seven transects. Five of the hot spots were at a 99% confidence level (z > 2.58) and were located on two transects; Bancroft Street and Dorr Street (Figure 2.15).

Our spring hot spot analysis for bird roadkill revealed 12 significant hot spots across six transects. Three of the hot spots were at a 99% confidence level (z > 2.58) and were located on two transects; Old State Line Road and Archbold-Whitehouse Road (Figure 2.16). Our summer hot spot analysis revealed 10 significant hot spots across four transects. Seven of the hot spots were at a 99% confidence level (z > 2.58) and were located on four transects; Bancroft Street, Old State Line Road, Angola Road, and County Highway 1-2 (Figure 2.17).

Our spring hot spot analysis for mammal roadkill revealed 13 significant hot spots across six transects. Nine of the hot spots were at a 99% confidence level (z > 2.58) and were located on five transects; County Road 3, Archbold-Whitehouse Road, Neapolis-Whitehouse Road, South Berkey Southern Road (south), and County Road EF (Figure 2.18). Our summer hot spot analysis revealed 16 significant hot spots across six transects. Eight of the hot spots were at a 99% confidence level (z > 2.58) and were located on three transects; Waterville-Swanton Road, Archbold-Whitehouse Road, and Whitehouse Road (Figure 2.19).

Environmental Characteristics of Roads

The amount of roadkill (both total roadkill and total per taxon) did not vary significantly in relation to week, month, or season, although variations across these time scales were detected. Mammals made up the largest portion of all the roadkill, therefore similar trends were seen between mammalian roadkill and total roadkill. Because of the high correlation (Spearman $\rho \geq 0.700$) and the strong explanatory power mammalian roadkill had on total roadkill trends, we looked at individual roadkill taxon separately and not all roadkill together.

A total of 84 dead amphibians were found on or around the roads. Amphibians had a higher occurrence of roadkill in the summer than spring, with four roadkill being found in spring and 80 being found in summer. Summer had 20 times the amount of roadkill as spring. The number of dead amphibians peaked in August (Figure 2.20), with 72.6% of amphibian roadkill being found in August.

There was a total of 58 birds found on or near roadways. Birds had a higher occurrence of roadkill in summer, but not by much. We found 24 dead birds in spring and 34 dead birds in summer. The peak for bird roadkill occurred in June and July (Figure 2.20). During June and July, we found 14 birds on the road each. June and July made up 48.2% of all bird roadkill found.

We found a total of 148 mammalian roadkill. Mammals had a higher occurrence of roadkill in summer. In spring, we found 53 dead mammals and 95 dead mammals in summer. Summer had roughly 1.7 times the amount of roadkill as spring. Mammalian roadkill had two peaks, one in July and a second in September (Figure 2.20). July had 34 roadkill and September had 37 roadkill. Together July and September make up 47.9% of mammalian roadkill.

Environmental characteristic results were only reported for total roadkill per survey week and not month or season because of insufficient sample size for the larger temporal scales. Mammal, amphibian, and bird roadkill per week all had a nonsignificant positive relationship with maximum temperature. Maximum temperature and mammal roadkill were not significant after Bonferroni correction. As temperature increased, though, roadkill per week increased, and as the temperature decreased, so did the roadkill (Figure 2.21).

The total number of mammal roadkill per week showed a nonsignificant negative trend with minimum and maximum humidity. Bird roadkill also had a negative trend with minimum humidity, but it was nonsignificant. Amphibian roadkill, on the other hand, had a nonsignificant positive relationship with minimum humidity, but a nonsignificant negative relationship with maximum humidity (Figure 2.22). We found that minimum humidity and maximum moon illumination were highly correlated (Spearman $\rho \geq 0.700$), therefore, minimum humidity was removed from multivariate analysis.

Mammal and bird roadkill had a nonsignificant negative relationship with maximum precipitation and amphibians had a nonsignificant positive relationship with precipitation (Figure 2.23). As precipitation increases, mammal and bird roadkill per week decreases, whereas, amphibian roadkill increases. As precipitation decreases, mammal and bird roadkill per week increases, and amphibian roadkill decreases.

Moon illumination did not have any significant relationships with roadkill amount. All taxa had a weak positive relationship with maximum moon illumination.

Spatial and Structural Features of Roads

Weekly roadkill counts had a positive relationship with maximum canopy cover for all taxa. Mammal roadkill per week had a significant relationship (Wilcoxon signed-rank $p < 0.001$).

Bird roadkill had a nonsignificant positive relationship under Bonferroni correction. Vegetation height was only measured monthly and therefore we were unable to analyze based on survey week.

Ephemeral Variables

To accommodate a low occurrence of individual ephemeral variables (water presence, invertebrate presence, temporary vegetation presence, and presence of living vertebrates), they were grouped together in two categories; living vertebrates and all other ephemeral variables. Neither of these categories had significant associations to total roadkill by taxa. Bird roadkill per week had a positive trend with presence of living vertebrates on the roads, whereas amphibian and mammalian roadkill had a negative trend. Dead amphibians, birds, and mammals per week all had a positive association to the presence of ephemeral variables. The ephemeral variables showed no significant temporal differences over survey week, month, or season. For that reason, we removed ephemeral variables from the multivariate analysis.

Our multivariate analysis created models for each taxon with a variety of variable numbers. Models were created based on associations between each taxon and the listed variables based on survey week. We ran the noncorrelated variables and found the best models using one to six environmental and structural variables. We report the top models with AIC_c changes less than or equal to two. For dead amphibians, the models with one and two variables had the lowest AIC_c. The model with two variables had the highest r-squared adjusted value. The combination of variables that went into the best models included season for single variable models and season and maximum precipitation for two variable modes. For dead birds, the model with one variable had the lowest AIC_c and second highest r-squared adjusted value. The highest r-squared adjusted value came from the model with two variables. The variable that went into the best model was maximum canopy cover. For dead mammals, the model with three variables had the lowest AIC_c, and the third highest r-squared adjusted value. The highest r-squared adjusted values came from models with four and five variables. The variables that went into the best model was season, maximum precipitation, and maximum moon illumination. All models with values can be found in Table 2.7. All models passed the Shapiro-Wilks test.

Discussion

Road Surveys

Roadkill surveys help determine how the abundance, diversity, and distribution of terrestrial vertebrates change over time and if there are potential times of high concern. By including environmental characteristics, spatial and structural features, and ephemeral variables, we are better able to understand their effects on vertebrate mortality patterns over time. Understanding how road mortality patterns change over time can help better evaluate when animals are moving throughout the landscape. During our study, we found 297 vertebrates, comprising of all four terrestrial vertebrate taxa (amphibia, aves, mammalia, and reptilia). These were split across both seasons, spring and summer, and all six months (April - September). However, roadkill was not evenly spread across the survey period. Three months (July, August, and September) had 72% of all roadkill found during the entire study. These three months line up with the peak months for bird, amphibian, and mammal roadkill, respectively. These also tend to align with mating, breeding, and dispersal movements.

We had predicted that the primary peak for all roadkill would be in June, but that taxa specific roadkill peaks may vary. As a result of the abundance of amphibians found on the road, the total roadkill peak was actually in August, but as mentioned, roadkill peaks did vary based on taxa. We did not see the spring peak for amphibians that we were expecting, but given when surveys were initiated, we may have missed the spring movement. Both bird and mammal roadkill peaks seem to line up with potential juvenile dispersal. Juveniles have less experience with roadways and therefore may be hit more frequently during initial movement across the roads.

A study in southern Korea found that amphibian roadkill had three peak mortality times that coincided with reproduction and juvenile dispersal (Seo et al., 2015). We did not see multiple peaks in our amphibian roadkill, but that could be a result of starting the study after they had already moved in early spring. Therefore, we could have missed an early peak for amphibians. Similar to Langen et al. (2007), we did not start seeing dead amphibians on the road until late June/early July. We found one major peak for amphibian roadkill in mid-August. This increase of roadkill could be related to the juveniles dispersing from their breeding grounds.

Bird roadkill was present for all months, with an increase through June and then a decrease in roadkill after July. A variety of other studies across North America and South Korea have also reported similar increases in bird roadkill from May-July (Garrah et al., 2015; Seo et al., 2015; Vance et al., 2018). Vance et al. (2018), found this peak to be unique for passerines, while birds of prey had their peak in the winter. All bird roadkill found in our study were passerines. Seo et al. (2015), found that the late spring/early summer peak for bird roadkill coincided with juvenile dispersal.

Finally, mammal roadkill was the most consistent and spread out across the months. There were two peaks for mammalian roadkill, the first being in early July, followed by a decrease in roadkill, with the second peak happening at the end of September. In mid-July we noted that many of the dead racoons seemed smaller and were possibly juveniles, suggesting this peak may be from juvenile dispersal. Both Garrah et al. (2015) and Vance et al. (2018), also saw little variation for mammalian roadkill during their studies. Santos et al. (2017), suggests that this is because many mammals are highly mobile and generalist species, which would potentially lead to a more uniform distribution of roadkill over time. Mammal roadkill as a whole did not show a strong temporal trend, but a study by Smith-Patten and Patten (2008), showed that certain mammalian species may have a stronger temporal trend than others. In their study, raccoons showed little variation, but opossums had a seasonal increase of roadkill in spring. Vance et al. (2018) also had a species-specific roadkill peak. In their study, groundhogs had an increase of roadkill in June and July. In our study, racoons, opossums, and groundhogs were found dead on the roads more frequently in summer than spring. Racoons had a peak in road mortality in July and September, whereas opossums had a mortality peak in August and September and groundhogs showed little variation. For species of concern and rarer species, species specific research may better detect trends in roadkill.

Hot spot analysis was helpful in analyzing significant clusters of roadkill and how the hot spots changed based on month, season, and by taxa. These locations can help highlight areas and times of interest for varied goals such as management, restoration, and mitigation. Hot spots across the landscape help larger initiatives like Ohio's Green Ribbon Initiative (GRI) ("Green Ribbon Initiative", n.d.). The goal of the GRI is to conserve biodiversity and restore critical natural areas and connections among them (Woods, 2016). Hot spots could help pinpoint critical times that certain areas need protected. Taxa specific hot spots provide insight into how each individual taxon is affected over time, if their areas of concern move over time, and identify temporal hot spots for species of concern. By looking at temporal hot spots by taxa, land managers can look at taxa of interest and figure out if there is a certain month or time period that these organisms need more protection for movement than others. Temporal road closures or closing certain roads for a short period of time during known mass animal movement has been shown to improve habitat connectivity, restore habitat quality, and act as safe wildlife corridors (Beier & Gregory, 2012; Gibeau et al., 2001; Lamb et al., 2018; Marrotte et al., 2017; Whittington et al., 2019).

Environmental Characteristics of Roads

Though seasons did not have a significant influence on roadkill, the changes in environmental characteristics over time did appear to play a role and affected the taxa differently. In general, many roadkill studies across the world have seen an increase in roadkill counts during warmer and wetter seasons (spring and summer), and a decrease in roadkill numbers during cold and dry seasons (fall and winter) (Coelho et al., 2012; Langen et al., 2007; Morelle et al., 2013; Santos et al., 2017). Within this generalization, there are some taxa specific responses to the individual environmental variables. A study conducted by Carvalho et al. (2017) in Brazil found that reptile roadkill was significantly increased with increased humidity and precipitation. Mammal roadkill increased with increased precipitation, but bird roadkill showed no relationship to environmental characteristics. In contrast a study conducted in northeastern Spain by Garriga et al. (2017), found that reptile roadkill rates were associated with precipitation and temperature. They also found that mammal roadkill rates were only associated with temperature. Amphibian roadkill rates increased with humidity, whereas, bird roadkill rates decreased. In our study, we found all taxa roadkill positively associated with temperature per week, but bird and mammal roadkill had an inverse relationship with precipitation and humidity per week. These studies would suggest that to better understand influences to roadkill, future research should utilize a multi-taxa approach and potentially look at species specific results. As results appear to vary based on region, regional studies may be required to provide the best data.

Spatial and Structural Features of Roads

There was not a drastic variation in canopy cover percentage and vegetation height throughout our survey period, but we still found a general increase in roadkill with increased canopy cover. This is different than a previous study done within the Oak Openings Region. Jonaitis (2017) found that roadkill was associated with low to intermediate levels of vegetation cover. This difference could result from different surveying methods and/or different variation for the variables measured. Throughout the literature, canopy cover and vegetation height are generally looked at spatially, not how the changes over time influence roadkill (Canal et al., 2019; Farmer & Brooks, 2012). Many of the studies that include canopy cover are focused on arboreal species, since many of our roadkill species are not arboreal, the results are hard to compare (Chen & Koprowski, 2016; Goosem, 2007).

Our roadkill numbers are most likely underestimates of total roadkill due to carcass permanency and methodology. Though we did not track how long all roadkill remained on the roads, we did note if carcasses remained for multiple road surveys. Out of the 20 carcasses that were noted, all but two were larger mammals. For the roadkill that remained on the roads, permanency ranged from six days to 51 days, with a median of 13 days. According to Garrah et al. (2015), frogs, birds, and juvenile turtles lasted less than 24 hours on roads, whereas mammals, snakes, and adult turtles lasted for multiple days. Permanency can be impacted by predation and scavengers, traffic volume, and weather (Ratton et al., 2014). Though we used a standardized method (Collinson et al., 2014) and we traveled the roads more slowly on bikes, it is still possible that some roadkill was missed. To understand carcass permanency across the taxa, further studies are needed, which could help get a better estimate of mortality on the roadways.

This study was conducted over two seasons and six months, but there is quite a bit of variation among other roadkill surveys. In a literature review of 61 peer reviewed roadkill studies, studies ranged from collecting data from four days all the way to 168 months (Collinson et al., 2014). Throughout the literature there is a lot of variation in study length, but also climate. The Oak Openings Region, in the northern temperate zone, goes through all four seasons (spring, summer, fall, and winter), but many of the studies are performed in regions that are split between two seasons (dry and wet). In regions with four seasons, it can be harder to conduct surveys in winter months due to snow and ice, therefore some studies only survey during non-snow days spring through fall (Clevenger et al., 2003). These variations can change how taxa interact with their environment, how many resources are available, and the ability to detect roadkill. This can make it harder to compare results across roadkill studies. To understand a full picture of how animals are dying on roads and to identify consistent temporal hot spots, a multi-year study will be necessary.

Conclusion

This study provides insight into how roadkill changes over time and how changes in environmental and spatial variables influence different taxa in a heterogeneous but fragmented landscape. Based on our results, roadkill does not occur evenly across the months and does not occur evenly across taxa. Road surveys and hot spot analysis identified months of greater concern for each taxon. These “hot moments” potentially line up with natural dispersal patterns and times with potentially higher rates of movement. These results suggest that to better understand animal movement and mortality, a multi-taxa approach and a long-term study is required.

There are many variables that impact roadkill, many are spatial, but there are several that are temporal. These temporal variables help distinguish what drives the change in roadkill amounts. As we predicted, there was variation between when each taxon had a peak in roadkill numbers, but we were off a couple of months from when we thought total roadkill would peak. Roadkill peaks did seem to coincide with natural dispersal periods for each taxon, but these could be confirmed with a multi-year study within the Oak Openings Region.

Known times of high animal movement; migrations and dispersals, can be compared to temporal hot spots to help protect these movements. Using road surveys and hot spot analysis is a useful approach in identifying changes throughout the year in animal distribution, diversity, and abundance. This method is applicable and easily adaptable across all landscapes that are fragmented by roadways.

Literature Cited

Ashley, E. P., & Robinson, J. T. (1996). Road mortality of amphibians, reptiles and other wildlife on the Long Point Causeway, Lake Erie, Ontario. *The Canadian-Field Naturalist, 110*(3), 403-412.

Beier, P., & Gregory, A. J. (2012). Desperately seeking stable 50-year-old landscapes with patches and long, wide corridors. *PLoS Biology*, *10*(1). https://doi.org/10.1371/journal.pbio.1001253

Bennie, J. J., Duffy, J. P., Inger, R., & Gaston, K. J. (2014). Biogeography of time partitioning in mammals. *Proceedings of the National Academy of Sciences*, *111*(38), 13727-13732. https://doi.org/10.1073/pnas.1216063110

Breed, M. D., & Moore, J. (2016). *Animal behavior* (2nd ed.). https://doi.org/10.1016/B978-0-12-801532-2.00008-8

Brookshire, B. (2018, October 22). *Scientists say: Understory*. Retrieved May 15, 2020, from https://www.sciencenewsforstudents.org/article/scientists-say-understory

Canal, D., Camacho, C., Martin, B., de Lucas, M., & Ferrer, M. (2019). Fine-scale determinants of vertebrate roadkills across a biodiversity hotspot in southern Spain. *Biodiversity and Conservation*, *28*, 3239-3256. https://doi.org/10.1007/s10531-019-01817-5

Canal, D., Camacho, C., Martín, B., de Lucas, M., & Ferrer, M. (2018). Magnitude, composition and spatiotemporal patterns of vertebrate roadkill at regional scales: A study in southern Spain. *Animal Biodiversity and Conservation*, *41*(2), 281-300. https://doi.org/10.32800/abc.2018.41.0281

Canova, L., & Balestrieri, A. (2018). Long-term monitoring by roadkill counts of mammal populations living in intensively cultivated landscapes. *Biodiversity and Conservation*, *28*(1), 97-113. https://doi.org/10.1007/s10531-018-1638-3

Carvalho, C. F., Custodio, A. B. I., & Marcal-Junior, O. (2017). Influence of climate variables on roadkill rates of wild vertebrates in the cerrado biome, Brazil. *Bioscience Journal*, *33*(6), 1632-1641. https://doi.org/10.14393/BJ-v33n6a2017-39538

Chen, H. L., & Koprowski, J. L. (2016). Differential effects of roads and traffic on space use and movements of native forest-dependent and introduced edge-tolerant species. *PLoS One*, *11*(1). https://doi.org/10.1371/journal.pone.0148121

Chyn, K., Lin, T.-E., Chen, Y.-K., Chen, C.-Y., & Fitzgerald, L. A. (2019). The magnitude of roadkill in Taiwan: Patterns and consequences revealed by citizen science. *Biological Conservation*, *237*, 317-326. https://doi.org/10.1016/j.biocon.2019.07.014

Clevenger, A. P., Chruszcz, B., & Gunson, K. E. (2003). Spatial patterns and factors influencing small vertebrate fauna road-kill aggregations. *Biological Conservation*, *109*(1), 15-26. https://doi.org/10.1016/S0006-3207(02)00127-1

Climate data online. (n.d.). Retrieved October 1, 2019, from https://www.ncdc.noaa.gov/cdo-web/

Coelho, I. P., Teixeira, F. Z., Colombo, P., Coelho, A. V. P., & Kindel, A. (2012). Anuran road-kills neighboring a peri-urban reserve in the Atlantic Forest, Brazil. *Journal of Environmental Management*, *112*, 17-26. https://doi.org/10.1016/j.jenvman.2012.07.004

Collinson, W. J., Parker, D. M., Bernard, R. T. F., Reilly, B. K., & Davies-Mostert, H. T. (2014). Wildlife road traffic accidents: A standardized protocol for counting flattened fauna. *Ecology and Evolution*, *4*(15), 3060-3071. https://doi.org/10.1002/ece3.1097

Croteau, E. K. (2010). Causes and consequences of dispersal in plants and animals. *Nature Education Knowledge*, *3*(10). Retrieved from https://www.nature.com/scitable/knowledge/library/causes-and-consequences-of-dispersal-in-plants-15927714/

Dingle, H., & Drake, V. A. (2007). What is migration? *Bioscience*, *57*(2), 113-121. https://doi.org/10.1641/B570206

Farmer, R. G., & Brooks, R. J. (2012). Integrated risk factors for vertebrate roadkill in southern Ontario. *The Journal of Wildlife Management*, *76*(6), 1215-1224. https://doi.org/10.1002/jwmg.358

Garrah, E., Danby, R. K., Eberhardt, E., Cunnington, G. M., & Mitchell, S. (2015). Hot spots and hot times: Wildlife road mortality in a regional conservation corridor. *Environmental Management*, *56*, 874-889. https://doi.org/10.1007/s00267-015-0566-1

Garriga, N., Franch, M., Santos, X., Montori, A., & Llorente, G. A. (2017). Seasonal variation in vertebrate traffic casualties and its implications for mitigation measures. *Landscape and Urban Planning*, *157*, 36-44. https://doi.org/10.1016/j.landurbplan.2016.05.029

Gibeau, M. L., Herrero, S., McLellan, B. N., & Woods, J. G. (2001). Managing for grizzly bear security areas in Banff National Park and the Central Canadian Rocky Mountains. *Ursus*, *12*, 121-129. Retrieved from https://www.bearbiology.org/publications/ursus-archive/managing-for-grizzly-bear-security-areas-in-banff-national-park-and-the-central-canadian-rocky-mountains/

Goosem, M. (2007). Fragmentation impacts caused by roads through rainforests. *Current Science*, *93*(11), 1587-1595. Retrieved from https://www.jstor.org/stable/24099089

The Green Ribbon Initiative. (n.d.). Retrieved April 14, 2020, from https://www.oakopenings.org/about/

Grilo, C., Bissonette, J. A., & Santos-Reis, M. (2009). Spatial-temporal patterns in Mediterranean carnivore road casualties: Consequences for mitigation. *Biological Conservation*, *142*(2), 301-313. https://doi.org/10.1016/j.biocon.2008.10.026

Handwerk, B. (2019, April 19). Twelve epic migratory journeys animals take every spring. *Smithsonian Magazine*. Retrieved from https://www.smithsonianmag.com/science-nature/twelve-epic-migratory-journeysanimals-take-every-spring-180972001/

Jonaitis, L. (2017). *Using roadkill as a lens to understand animal movement and mortality.* (Electronic Thesis or Dissertation). Retrieved from https://etd.ohiolink.edu/

Kowalczyk, R., Zalewski, A., Jędrzejewska, B., Ansorge, H., & Bunevich, A. N. (2009). Reproduction and mortality of invasive raccoon dogs (Nyctereutes procyonoides) in the Białowieża Primeval forest (eastern Poland). *BioOne*, *46*(4), 291-301. https://doi.org/10.5735/086.046.0406

Lamb, C. T., Mowat, G., Reid, A., Smit, L., Proctor, M., McLellan, B. N., Nielsen, S. E., & Boutin, S. (2018). Effects of habitat quality and access management on the density of a recovering grizzly bear population. *Journal of Applied Ecology*, *55*(3), 1406-1417. https://doi.org/10.1111/ 1365-2664.13056

Langen, T. A., Machniak, A., Crowe, E. K., Mangan, C., Marker, D. F., Liddle, N., & Roden, B. (2007). Methodologies for surveying herpetofauna mortality on rural highways. *The Journal of Wildlife Management*, *71*(4), 1361-1368. https://doi.org/10.2193/2006-385

Lawson Handley, L. J., & Perrin, N. (2007). Advances in our understanding of mammalian sex-biased dispersal. *Molecular Ecology*, *16*(8), 1559-1578. https://doi.org/10.1111/j.1365-294X.2006.03152.x

Lin, Y.-P., Anthony, J., Lin, W.-C., Lien, W.-Y., Petway, J. R., & Lin, T.-E. (2019). Spatiotemporal identification of roadkill probability and systematic conservation planning. *Landscape Ecology*, *34*(4), 717-735. https://doi.org/10.1007/s10980-019-00807-w

Marrotte, R. R., Bowman, J., Brown, M. G. C., Cordes, C., Morris, K. Y., Prentice, M. B., & Wilson, P. J. (2017). Multi-species genetic connectivity in a terrestrial habitat network. *Movement Ecology*, *5*(21). https://doi.org/10.1186/s40462-017-0112-2

Meza-Joya, F. L., Ramos, E., & Cardona, D. (2019). Spatio-temporal patterns of mammal road mortality in Middle Magdalena Valley, Colombia. *Oecologia Australis*, *23*(03), 575-588. https://doi.org/10.4257/oeco.2019.2303.15

Mitchell, A. (2020). *The Esri guide to GIS analysis: Geographic patterns and relationships, Volume 1* (2nd ed.).

[Moon illumination for Toledo, Ohio]. (n.d.). Retrieved April 20, 2020, from https://www.timeanddate.com/moon/usa/toledo?month=4&year=2019

Morelle, K., Lehaire, F., & Lejeune, P. (2013). Spatio-temporal patterns of wildlife-vehicle collisions in a region with a high-density road network. *Nature Conservation*, *5*, 53-73. https://doi.org/10.3897/natureconservation.5.4634

Ohio Division of Wildlife. (2018). *Mammals of Ohio* [Pamphlet]. ODNR Division of Wildlife.

Prugnolle, F., & de Meeus, T. (2002). Inferring sex-biased dispersal from population genetic tools: A review. *Heredity*, *88*, 161-165. https://doi.org/10.1038/sj.hdy.6800060

Ratton, P., Secco, H., & da Rosa, C. A. (2014). Carcass permanency time and its implications to the roadkill data. *European Journal of Wildlife Research*, *60*(3). https://doi.org/10.1007/s10344-014-0798-z

Root, K. V., & Martin, A. K. (2018, June). *Oak Openings Region biodiversity model final report (phase II)*. Retrieved from http://karenroot.net/documents/Oak_Openings_Model_Report_Phase_II.pdf

Sandt, K. (2018, February 26). NPS will close river road to protect breeding amphibians. Retrieved May 4, 2020, from https://www.nps.gov/dewa/learn/news/amphibian-news-release.htm

Santos, R. A. L., Ascensao, F., Ribeiro, M. L., Bager, A., Santos-Reis, M., & Aguiar, L. M. S. (2017). Assessing the consistency of hotspot and hot-moment patterns of wildlife road mortality over time. *Perspectives in Ecology and Conservation*, *15*(1), 56-60. https://doi.org/10.1016/j.pecon.2017.03.003

Schwartz, A. L., Shilling, F. M., & Perkins, S. E. (2020). The value of monitoring wildlife roadkill. *European Journal of Wildlife Research*, *66*(18). https://doi.org/10.1007/s10344-019-1357-4

Seo, C., Thorne, J. H., Choi, T., Kwon, H., & Park, C.-H. (2015). Disentangling roadkill: The influence of landscape and season on cumulative vertebrate mortality in South Korea. *Landscape Ecological Engineering*, *11*, 87-99. https://doi.org/10.1007/s11355-013-0239-2

Smith, L. L., & Dodd, C. K., Jr. (2003). Wildlife mortality on U.S. highway 441 across Paynes Prairie, Alachua county, Florida. *Florida Academy of Sciences, Inc*, *66*(2), 128-140. Retrieved from https://www.jstor.org/stable/24321153

Smith-Patten, B. D., & Patten, M. A. (2008). Diversity, seasonality, and context of mammalian roadkills in the southern Great Plains. *Environmental Management*, *41*(6), 844-852. https://doi.org/10.1007/s00267-008-9089-3

Songer, M. (2019). Wildlife ecology. In *Encyclopedia of ecology* (2nd ed., Vol. 1, pp. 509-516). https://doi.org/10.1016/B978-0-12-409548-9.11177-7

Steen, D. A., Aresco, M. J., Beilke, S. G., Compton, B. W., Condon, E. P., Kenneth Dodd, C., Forrester, H., Gibbons, J. W., Greene, J. L., Johnson, G., Langen, T. A., Oldham, M. J., Oxier, D. N., Saumure, R. A., Schueler, F. W., Sleeman, J. M., Smith, L. L., Tucker, J. K., & Gibbs, J. P. (2006). Relative vulnerability of female turtles to road mortality. *Animal Conservation*, *9*(3), 269-273. https://doi.org/10.1111/j.1469-1795.2006.00032.x

Transportation data management system. (n.d.). Retrieved April 15, 2020, from https://odot.ms2soft.com/tcds/tsearch.asp?loc=Odot&mod=

Vance, J. A., Smith, W. H., & Smith, G. L. (2018). Species composition and temporal patterns of wildlife-vehicle collisions in southwest Virginia, USA. *Human-Wildlife Interactions*, *12*(3), 417-426. https://doi.org/10.26077/6a2c-cj16

Whittington, J., Low, P., & Hunt, B. (2019). Temporal road closures improve habitat quality for wildlife. *Scientific Reports*, *9*. https://doi.org/10.1038/s41598-019-40581-y

Winkler, D. W., Jorgensen, C., Both, C., Houston, A. I., McNamara, J. N., Levey, D. J., & Piersma, T. (2014). Cues, strategies, and outcomes: How migrating vertebrates track environmental change. *Movement Ecology*, *2*(10). https://doi.org/10.1186/2051-3933-2-10

Woods, S. (2016). Combining forces to protect the Oak Openings: The Green Ribbon Initiative. In J. Thieme (Ed.), *Living in the Oak Openings: A guide to one of the world's last great places* (3rd ed., pp. 50-55). Retrieved from http://whitehouseoh.gov/Forms/Public-Service/Oak-Openings-Guide-3rd-Edition-2016.pdf

Figures

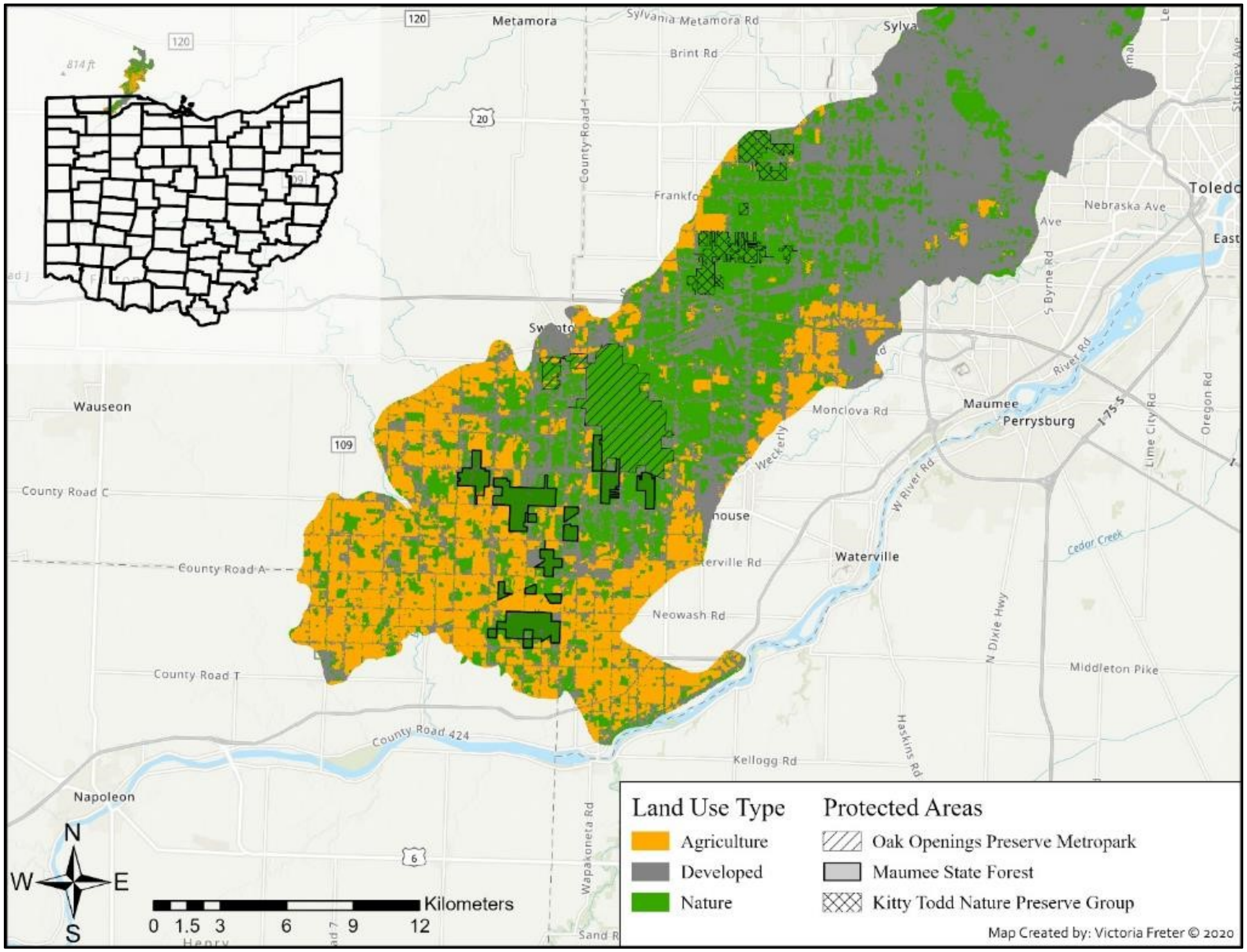

Figure 2.1. The Oak Openings Region in Northwest Ohio categorized by three land use types: agriculture (yellow), developed (gray), and nature (green) (Root & Martin, 2018). Oak Openings Preserve Metropark (diagonal), Maumee State Forest (black outline), and Kitty Todd Nature Preserve group (cross hatch) are highlighted.

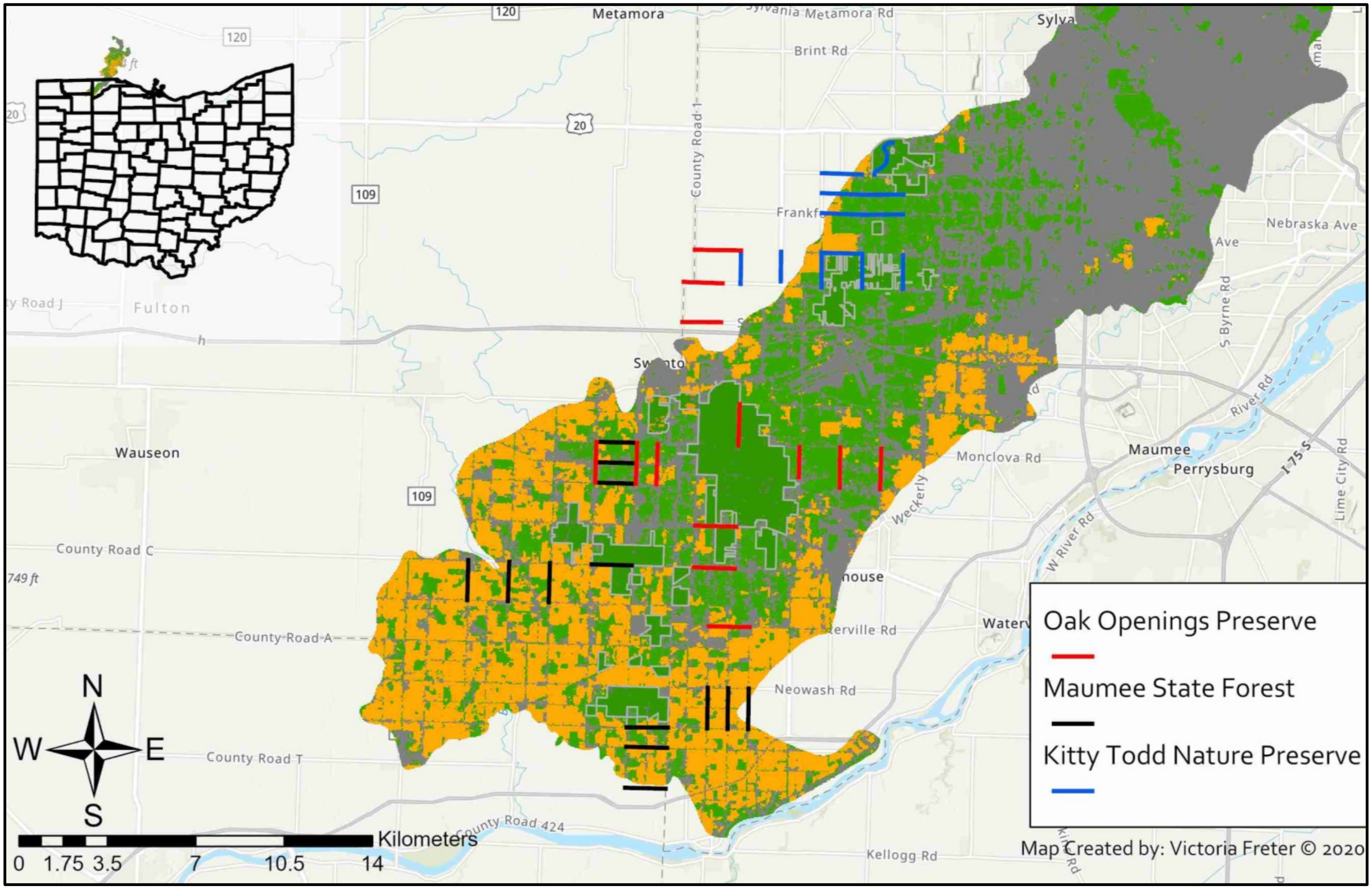

Figure 2.2. Map of surveyed road transects in and around Kitty Todd Nature Preserve (blue), Oak Openings Preserve (red), and Maumee State Forest (black) in the Oak Openings Region. Protected natural areas (gray outline) are highlighted (Root & Martin, 2018).

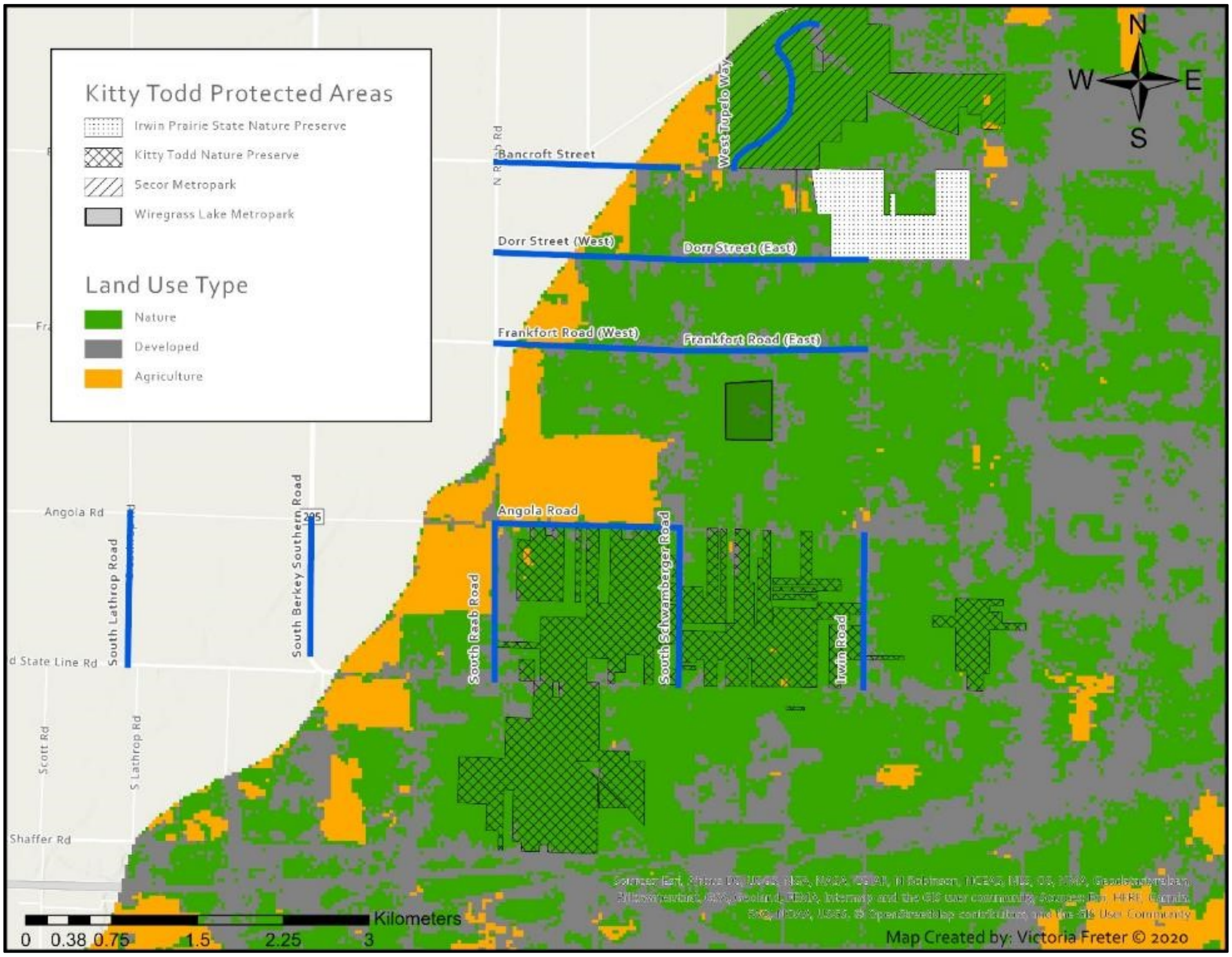

Figure 2.3. Map of surveyed road transects (blue) in and around Kitty Todd Nature Preserve. Irwin Prairie State Nature Preserve (dots), Kitty Todd Nature Preserve (cross hatch), Secor Metropark (diagonal), and Wiregrass Lake Metropark (black outline) are highlighted (Root & Martin, 2018).

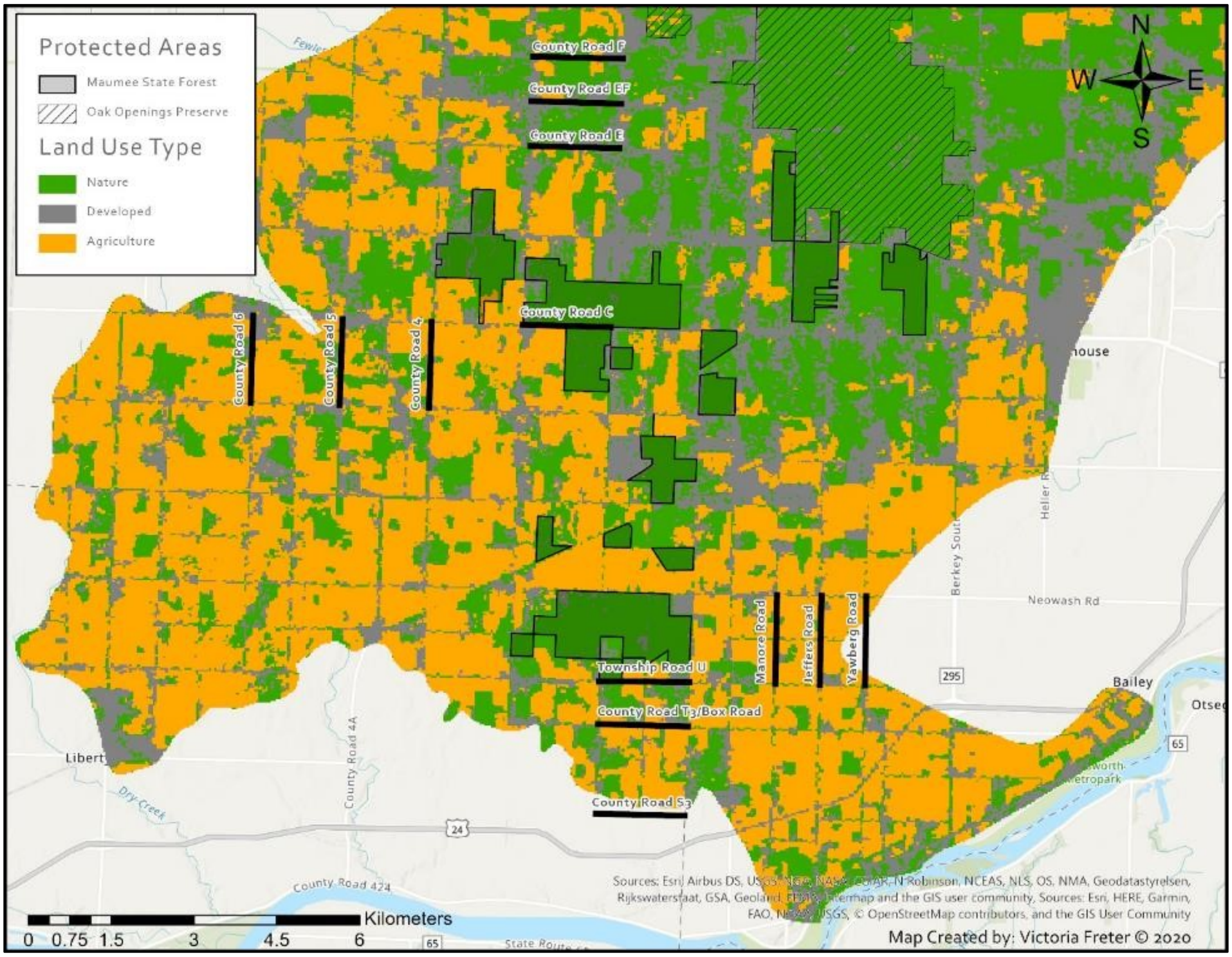

Figure 2.4. Map of surveyed road transects (black) in and around Maumee State Forest. Maumee State Forest (black outline) and Oak Openings Preserve (diagonal) are highlighted (Root & Martin, 2018).

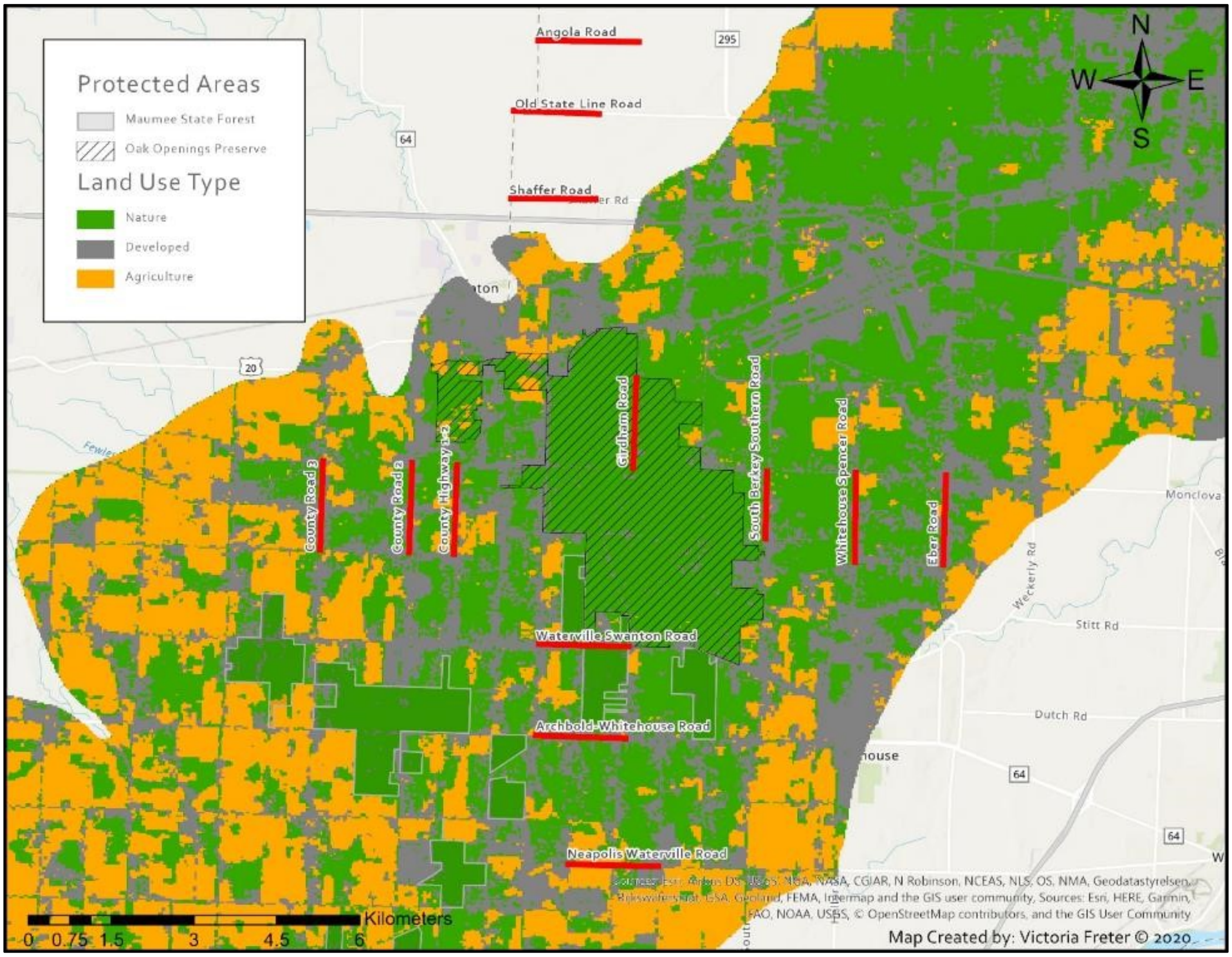

Figure 2.5. Map of surveyed road transects (red) in and around Oak Openings Preserve. Maumee State Forest (gray outline) and Oak Openings Preserve (diagonal) are highlighted (Root & Martin, 2018).

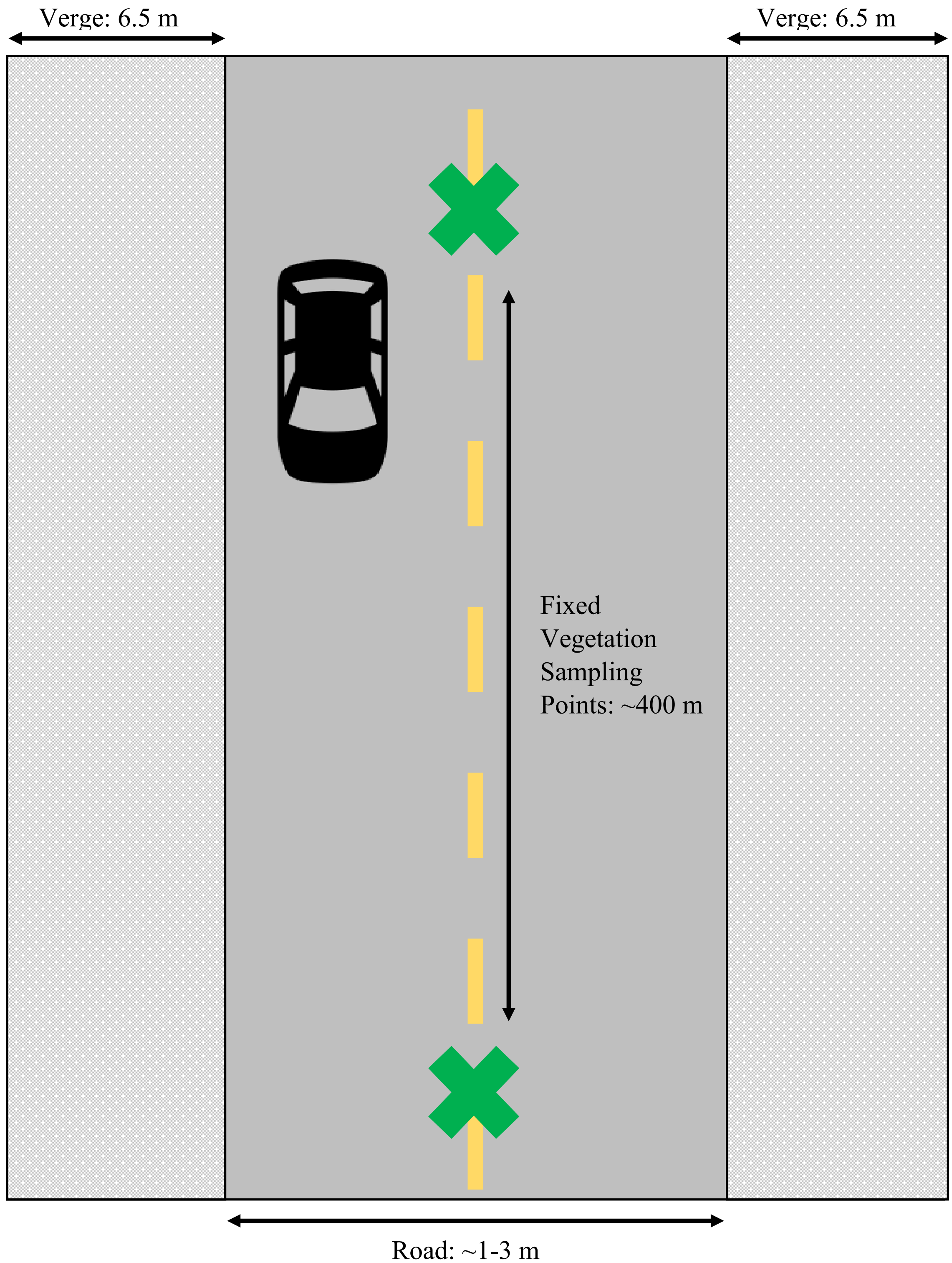

Figure 2.6. Road diagram showing approximate widths of roadways, verges, and distance between fixed vegetation points. Not to scale.

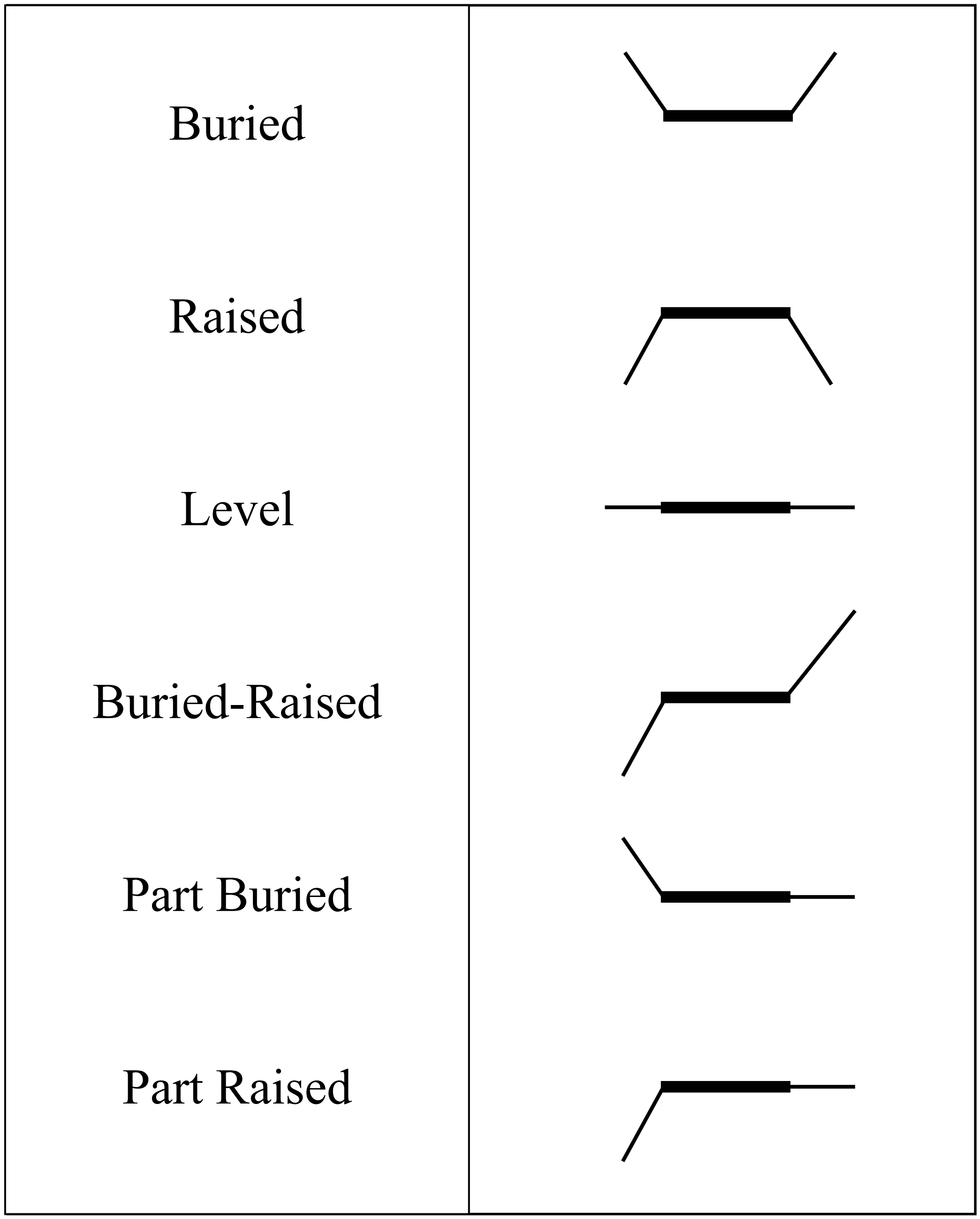

Figure 2.7. Categorization of road type based on road cross section. The thick black lines in the middle represents the road, the thin black lines on both sides represents the verges. Adapted from Clevenger et al. (2003).

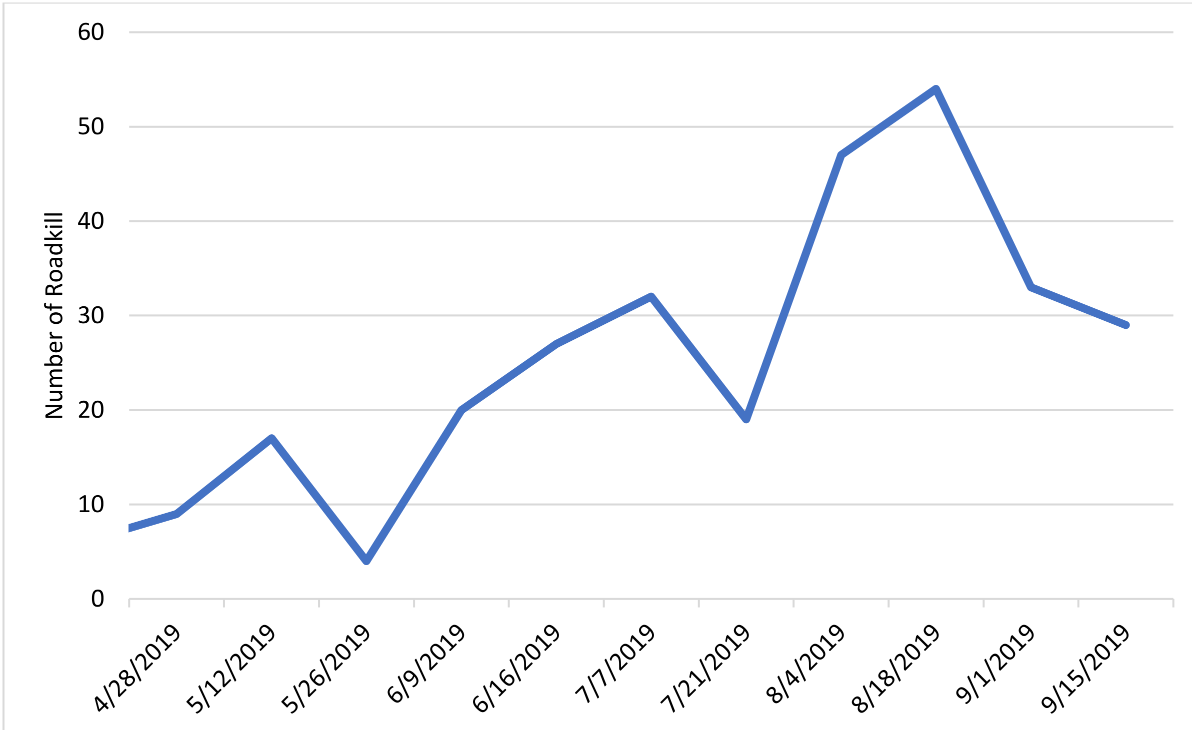

Figure 2.8. The weekly number of roadkill over the survey period from April - September 2019 in Northwest Ohio.

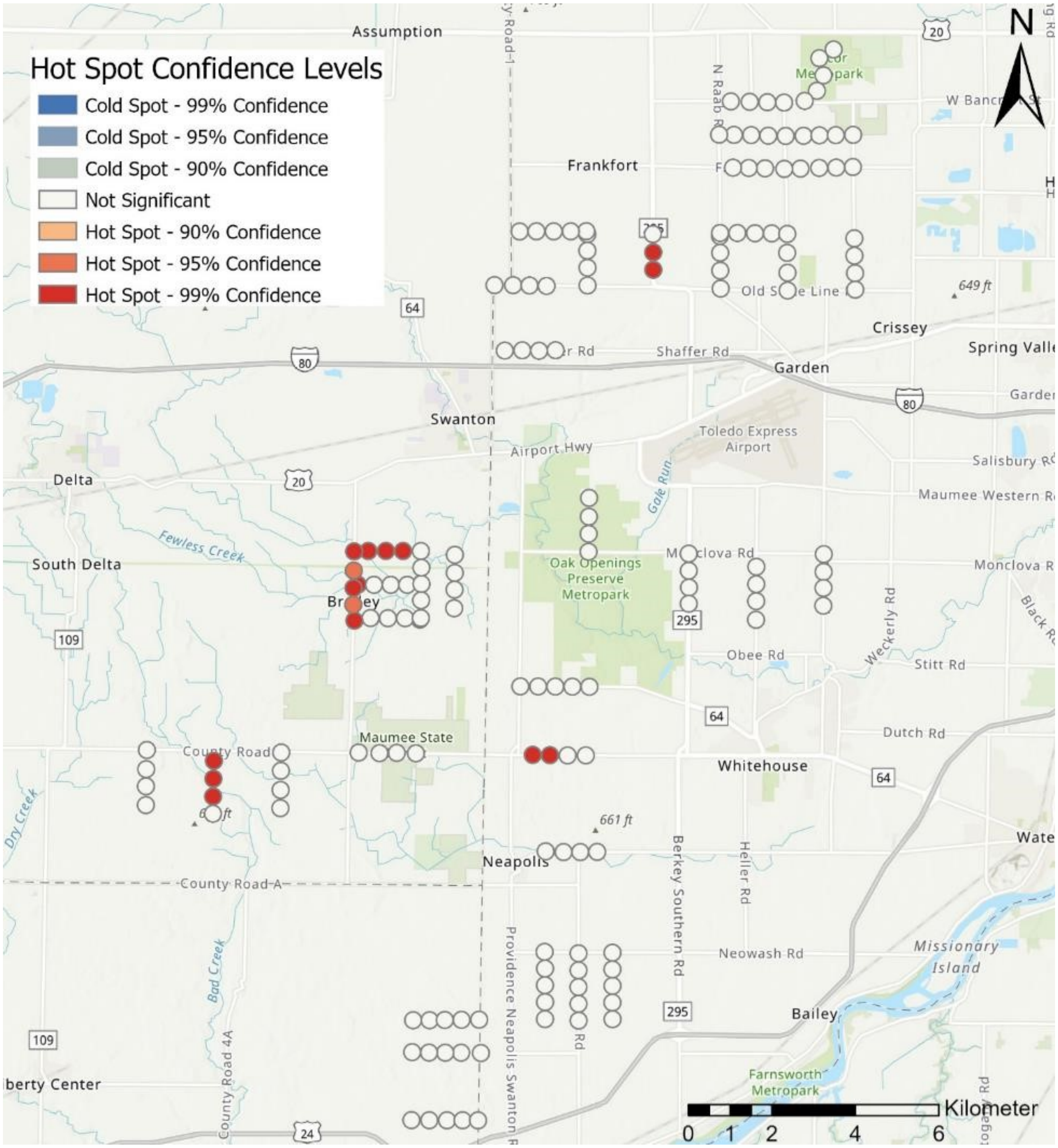

Figure 2.9. Getis-Ord Gi* hot spot analysis for all roadkill in April across the study area in Northwest Ohio. White circles show 200 m buffers that were not significant. Warm colored circles (orange-red) show buffers with 90-99% significance of being a hot spot. Cold colored circles (blue-green) show buffers with 90-99% significance of being a cold spot.

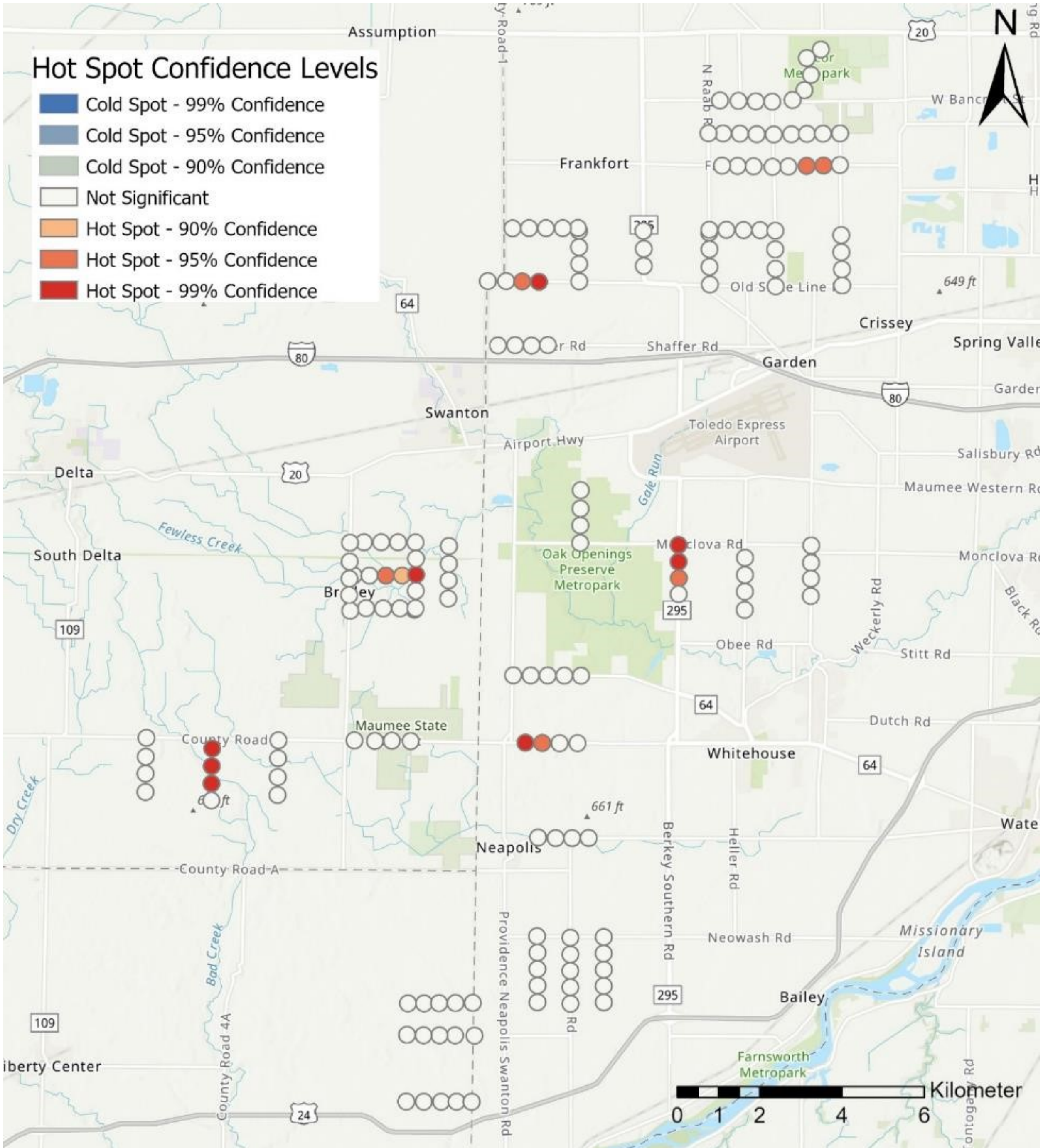

Figure 2.10. Getis-Ord Gi* hot spot analysis for all roadkill in May across the study area in Northwest Ohio. White circles show 200 m buffers that were not significant. Warm colored circles (orange-red) show buffers with 90-99% significance of being a hot spot. Cold colored circles (blue-green) show buffers with 90-99% significance of being a cold spot.

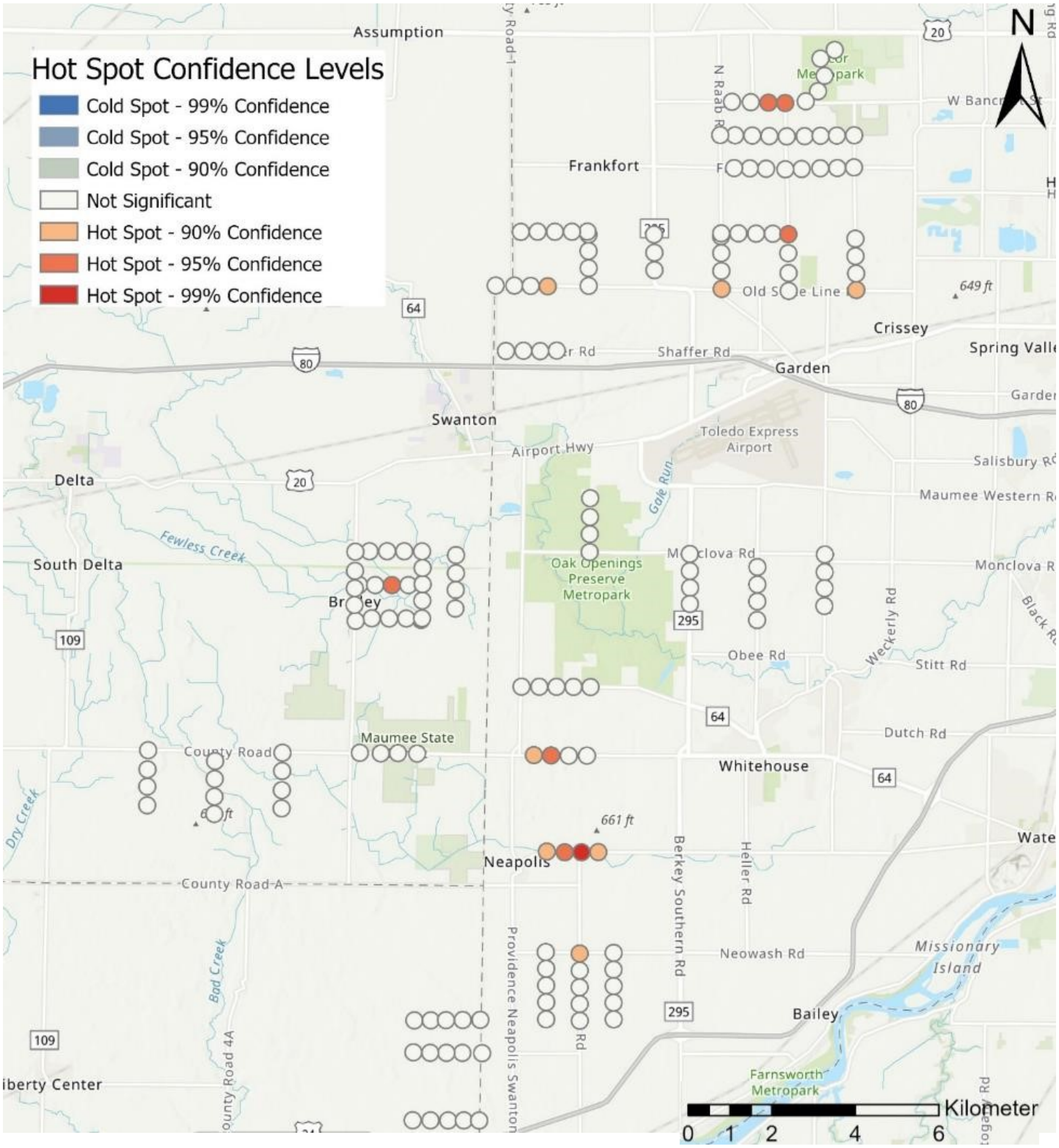

Figure 2.11. Getis-Ord Gi* hot spot analysis for all roadkill in June across the study area in Northwest Ohio. White circles show 200 m buffers that were not significant. Warm colored circles (orange-red) show buffers with 90-99% significance of being a hot spot. Cold colored circles (blue-green) show buffers with 90-99% significance of being a cold spot.

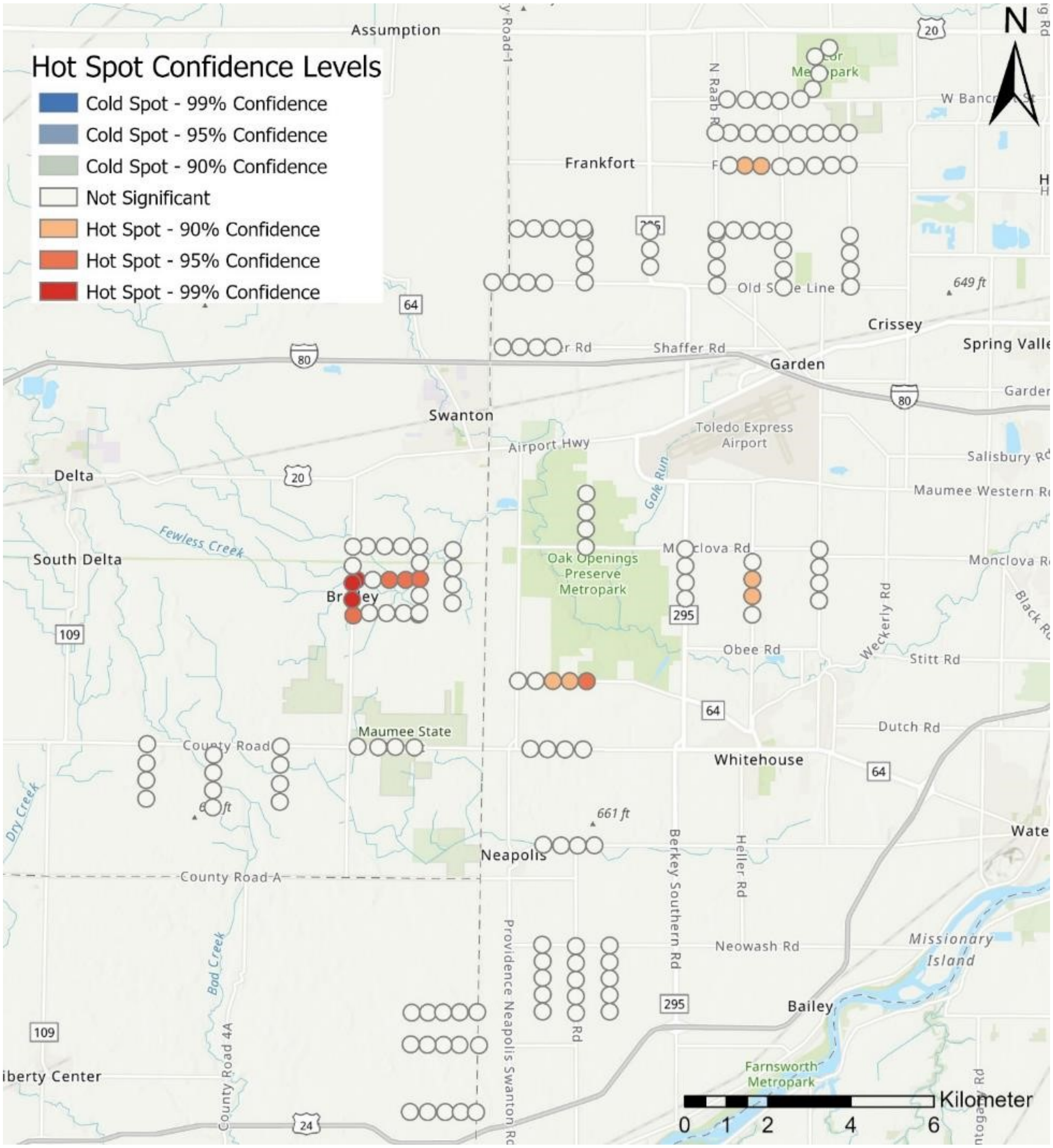

Figure 2.12. Getis-Ord Gi* hot spot analysis for all roadkill in July across the study area in Northwest Ohio. White circles show 200 m buffers that were not significant. Warm colored circles (orange-red) show buffers with 90-99% significance of being a hot spot. Cold colored circles (blue-green) show buffers with 90-99% significance of being a cold spot.

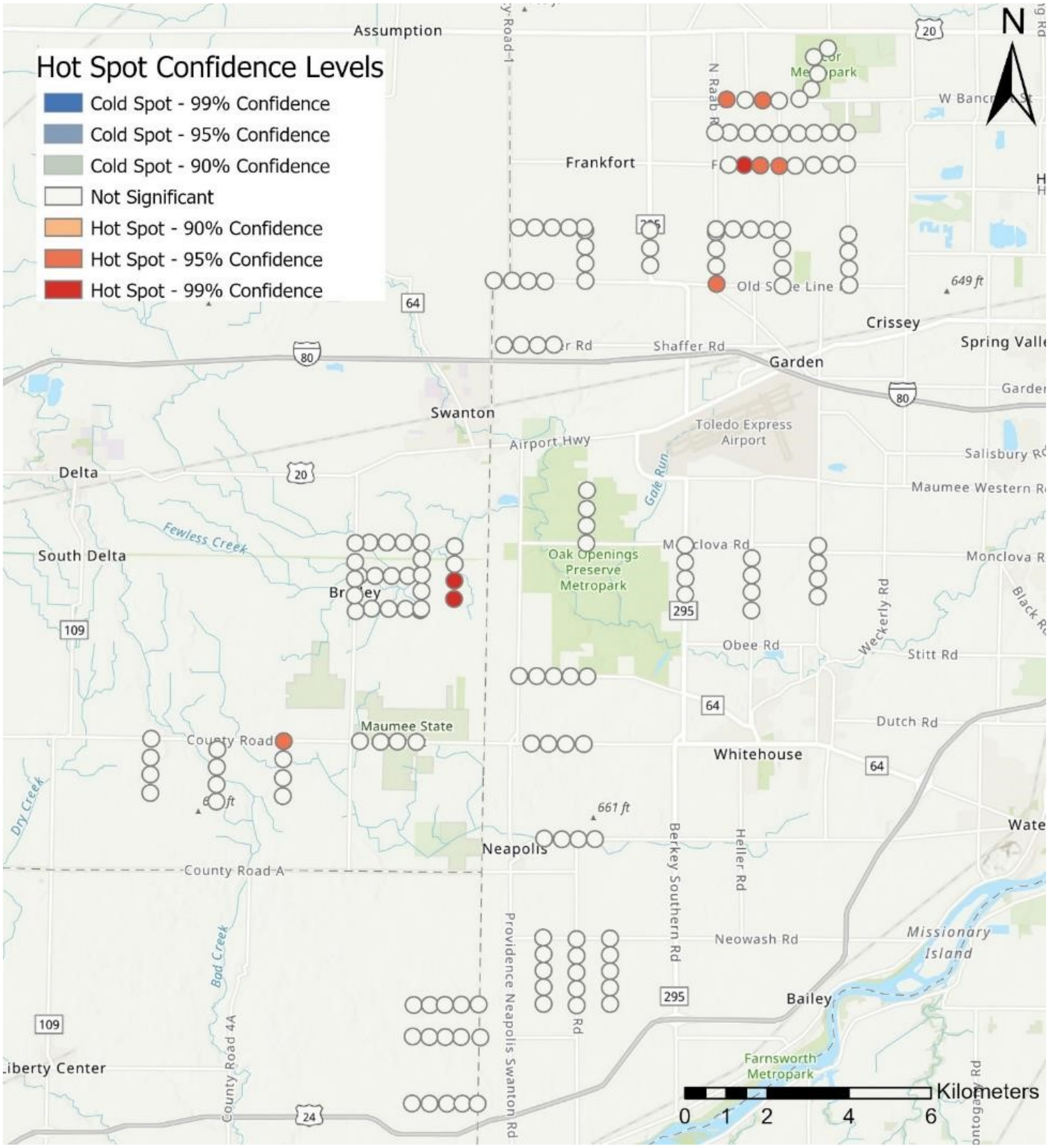

Figure 2.13. Getis-Ord Gi* hot spot analysis for all roadkill in August across the study area in Northwest Ohio. White circles show 200 m buffers that were not significant. Warm colored circles (orange-red) show buffers with 90-99% significance of being a hot spot. Cold colored circles (blue-green) show buffers with 90-99% significance of being a cold spot.

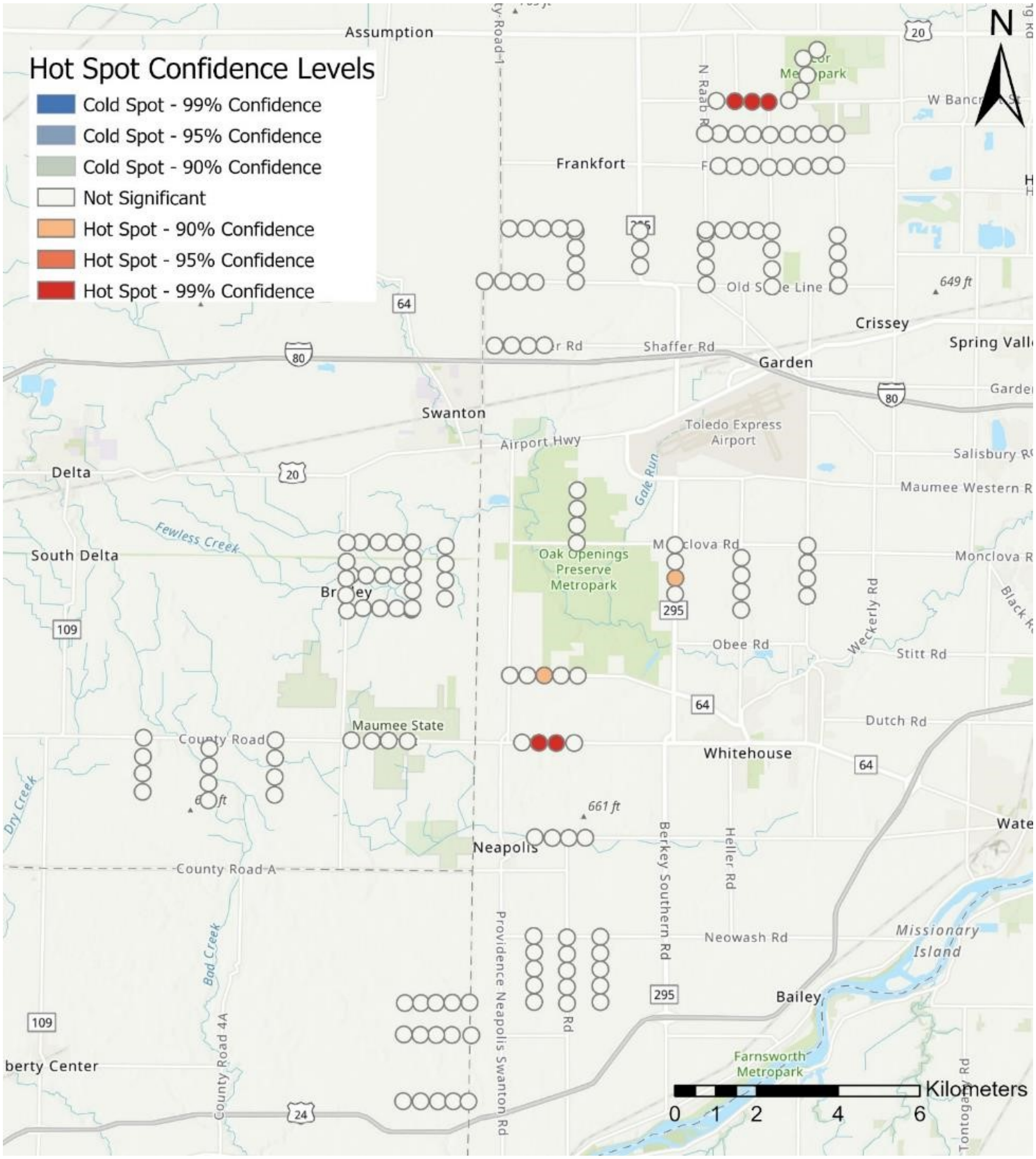

Figure 2.14. Getis-Ord Gi* hot spot analysis for all roadkill in September across the study area in Northwest Ohio. White circles show 200 m buffers that were not significant. Warm colored circles (orange-red) show buffers with 90-99% significance of being a hot spot. Cold colored circles (blue-green) show buffers with 90-99% significance of being a cold spot.

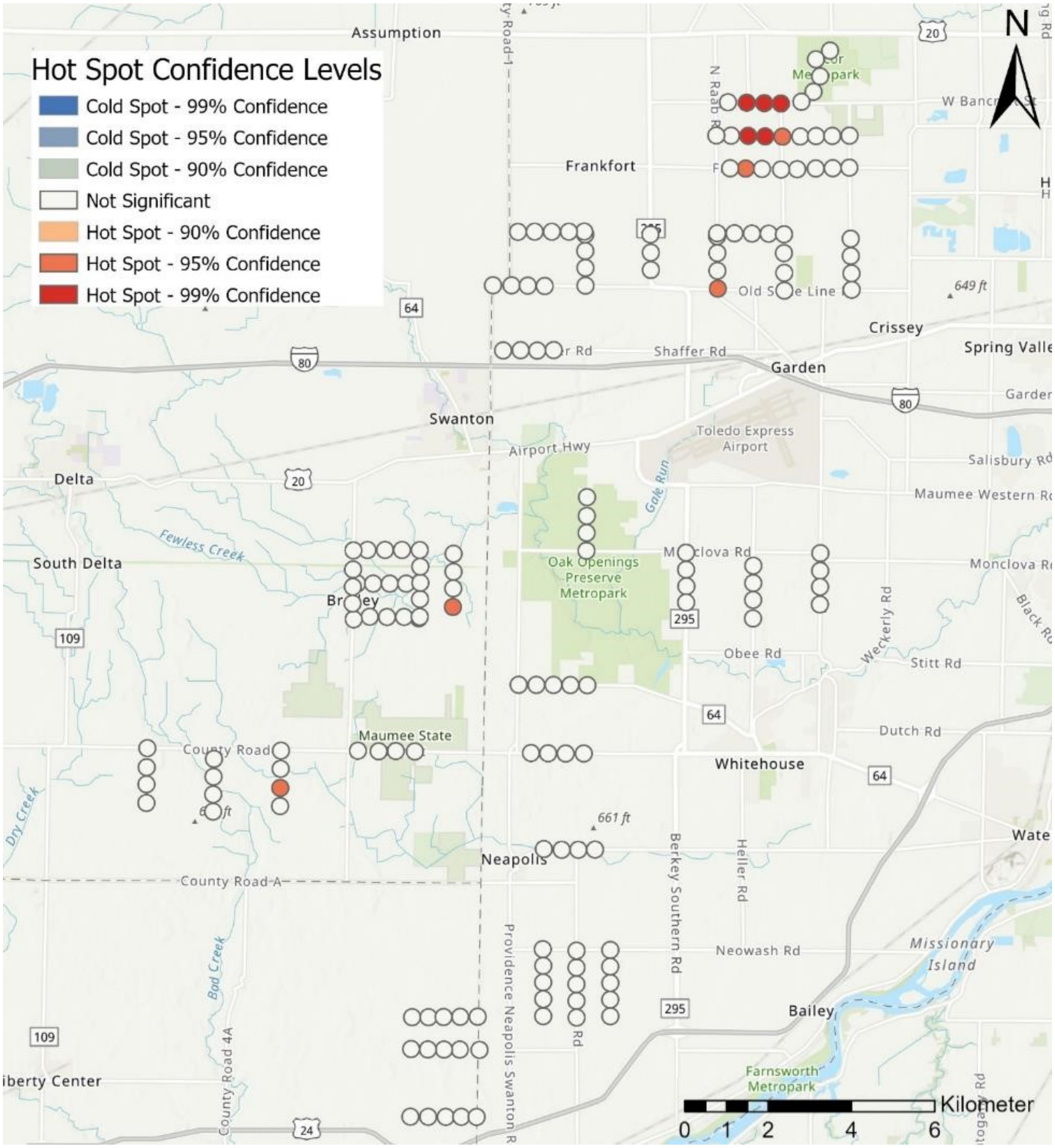

Figure 2.15. Getis-Ord Gi* hot spot analysis for all amphibian roadkill in summer across the study area in Northwest Ohio. White circles show 200 m buffers that were not significant. Warm colored circles (orange-red) show buffers with 90-99% significance of being a hot spot. Cold colored circles (blue-green) show buffers with 90-99% significance of being a cold spot.

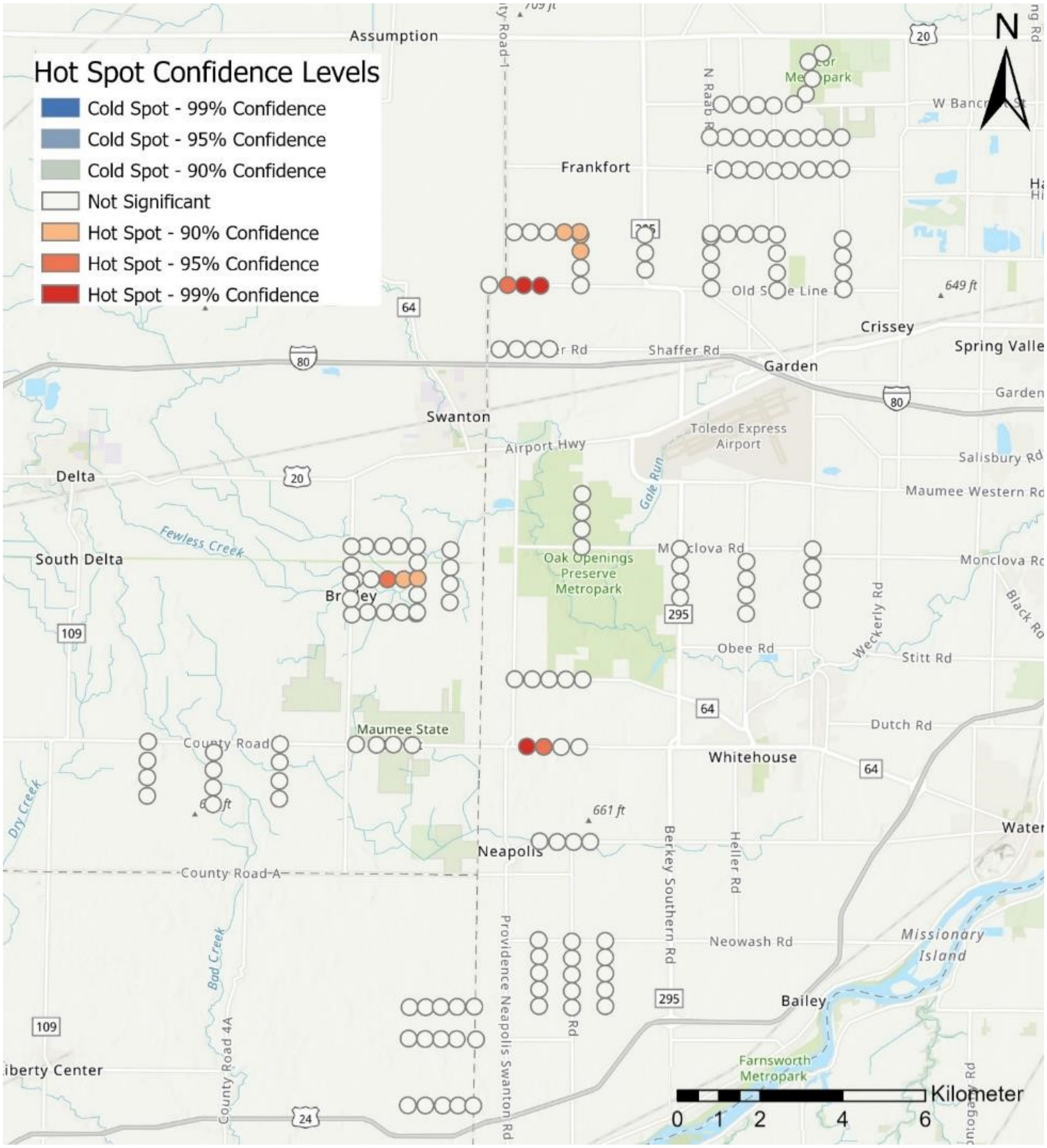

Figure 2.16. Getis-Ord Gi* hot spot analysis for all bird roadkill in spring across the study area in Northwest Ohio. White circles show 200 m buffers that were not significant. Warm colored circles (orange-red) show buffers with 90-99% significance of being a hot spot. Cold colored circles (blue-green) show buffers with 90-99% significance of being a cold spot.

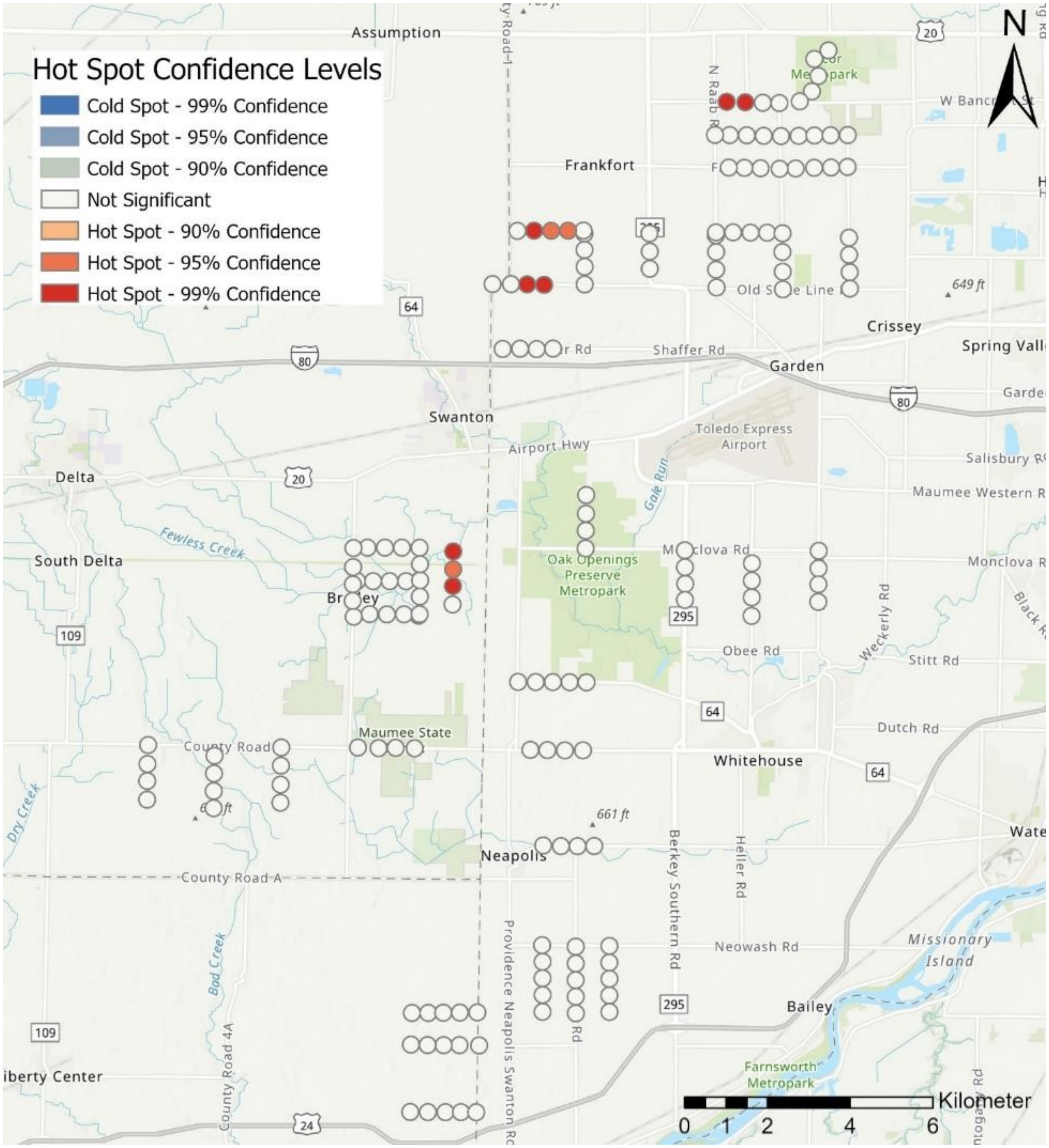

Figure 2.17. Getis-Ord Gi* hot spot analysis for all bird roadkill in summer across the study area in Northwest Ohio. White circles show 200 m buffers that were not significant. Warm colored circles (orange-red) show buffers with 90-99% significance of being a hot spot. Cold colored circles (blue-green) show buffers with 90-99% significance of being a cold spot.

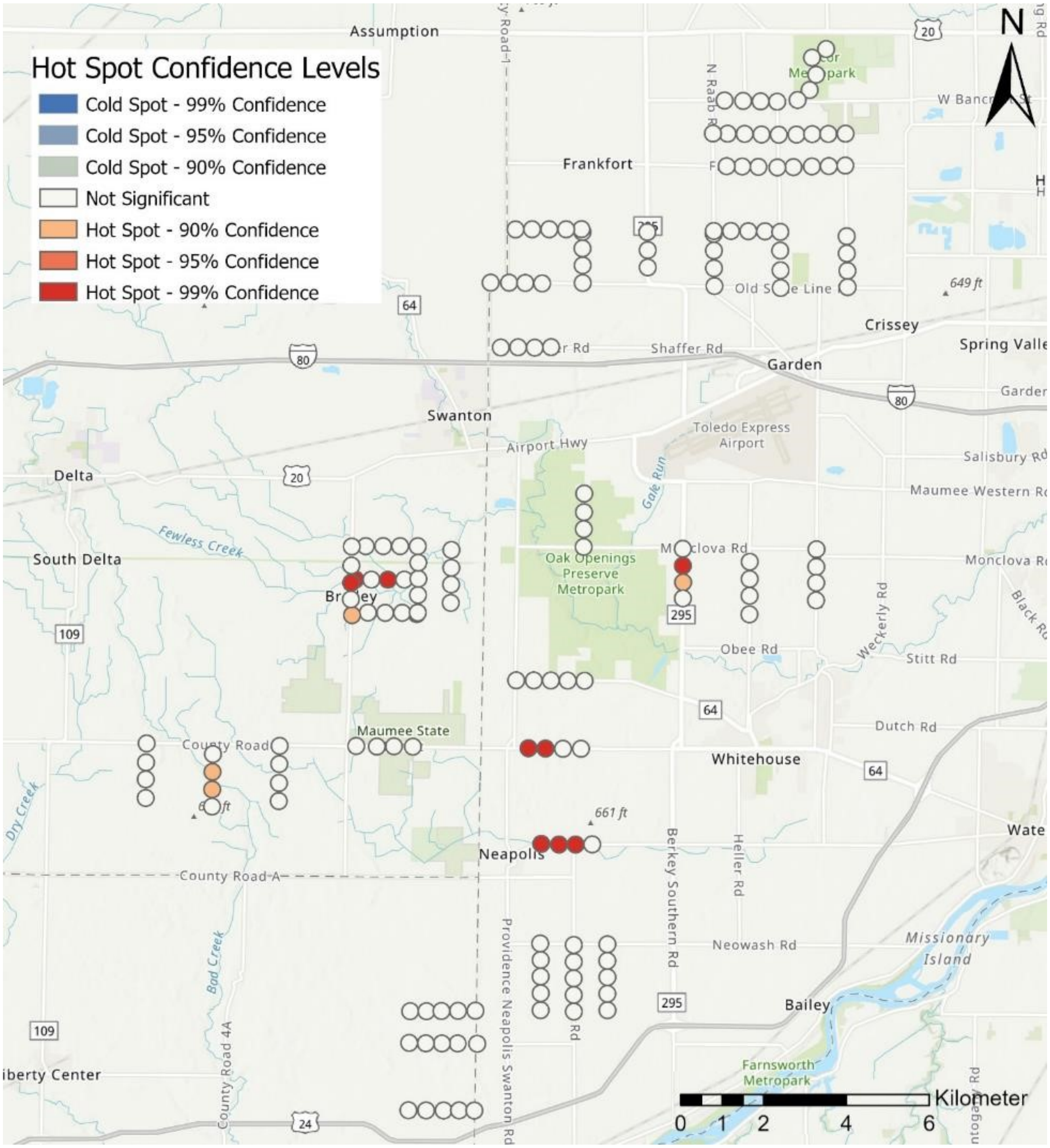

Figure 2.18. Getis-Ord Gi* hot spot analysis for all mammal roadkill in spring across the study area in Northwest Ohio. White circles show 200 m buffers that were not significant. Warm colored circles (orange-red) show buffers with 90-99% significance of being a hot spot. Cold colored circles (blue-green) show buffers with 90-99% significance of being a cold spot.

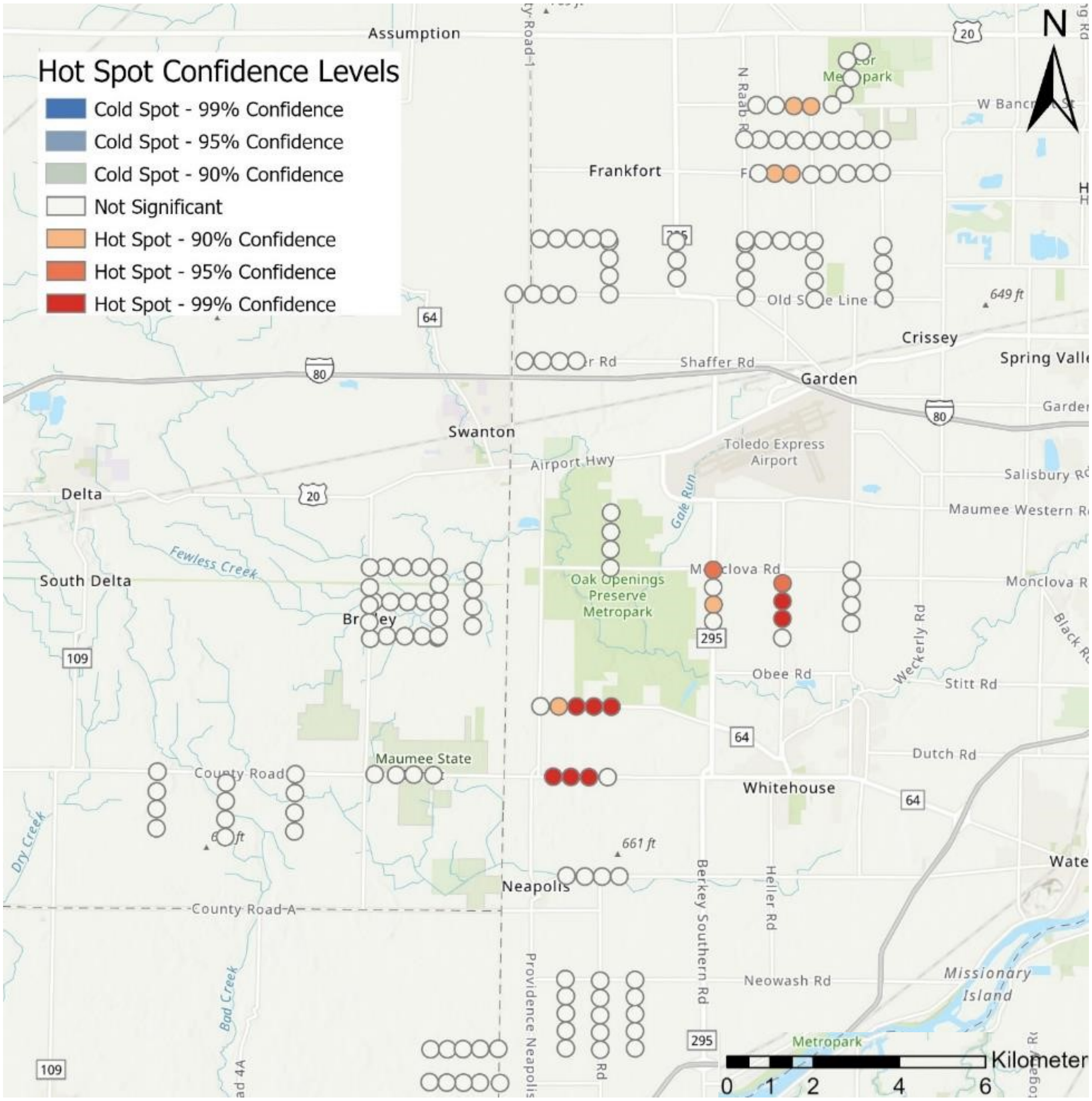

Figure 2.19. Getis-Ord Gi* hot spot analysis for all mammal roadkill in summer across the study area in Northwest Ohio. White circles show 200 m buffers that were not significant. Warm colored circles (orange-red) show buffers with 90-99% significance of being a hot spot. Cold colored circles (blue-green) show buffers with 90-99% significance of being a cold spot.

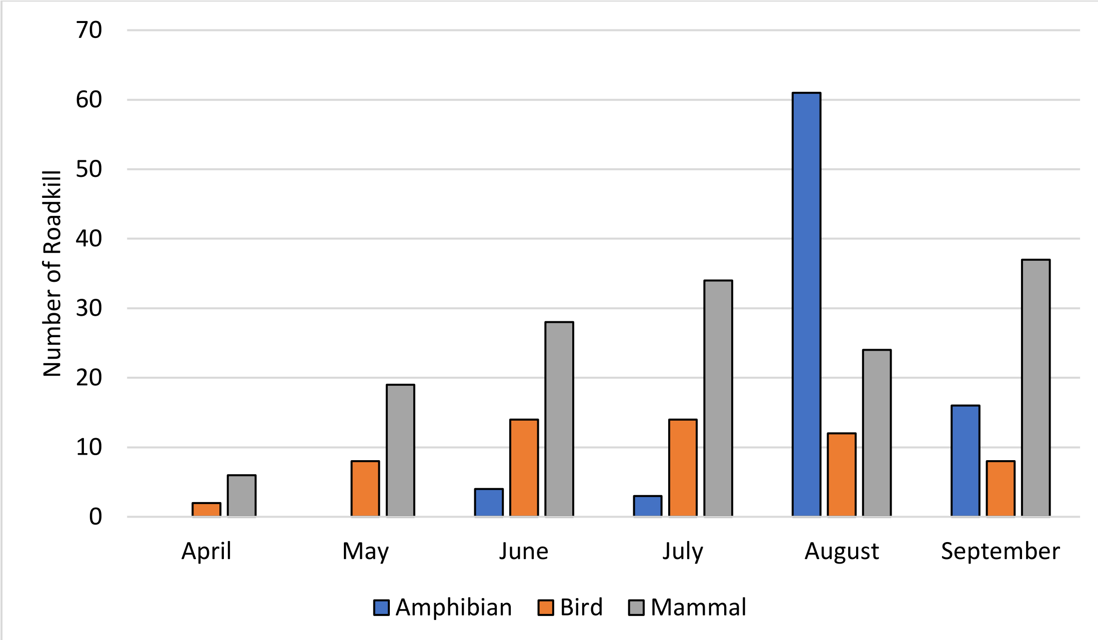

Figure 2.20. Roadkill per month by taxon that were found on the roadways from April - September 2019 in Northwest Ohio. Amphibian roadkill is represented by blue bars. Bird roadkill is represented by orange bars. Mammal roadkill is represented by gray bars.

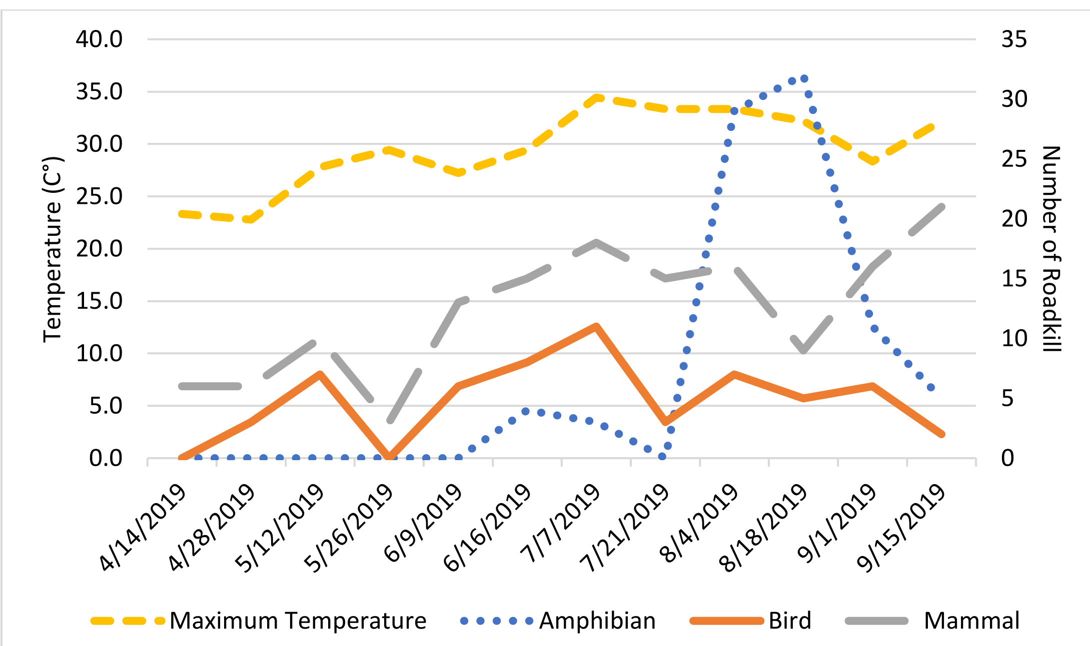

Figure 2.21. A comparison of trends in maximum temperature (C°) and the number of individual roadkill by taxon over the survey period per week. The blue dotted line represents temperature, the orange solid line represents amphibian roadkill, the gray dashed line represents bird roadkill, and the yellow dashed line represents mammal roadkill.

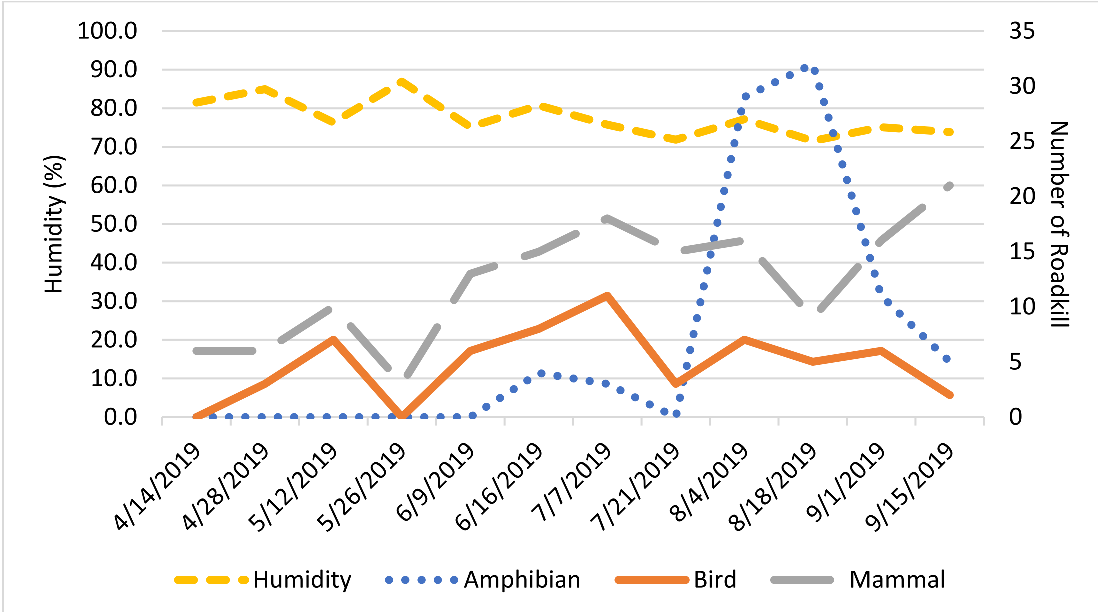

Figure 2.22. A comparison of trends in average humidity (%) and the number of each individual roadkill by taxon over the survey period per week. The yellow dashed line represents humidity, the blue dotted line represents amphibian roadkill, the orange solid line represents bird roadkill, and the gray dashed line represents mammal roadkill.

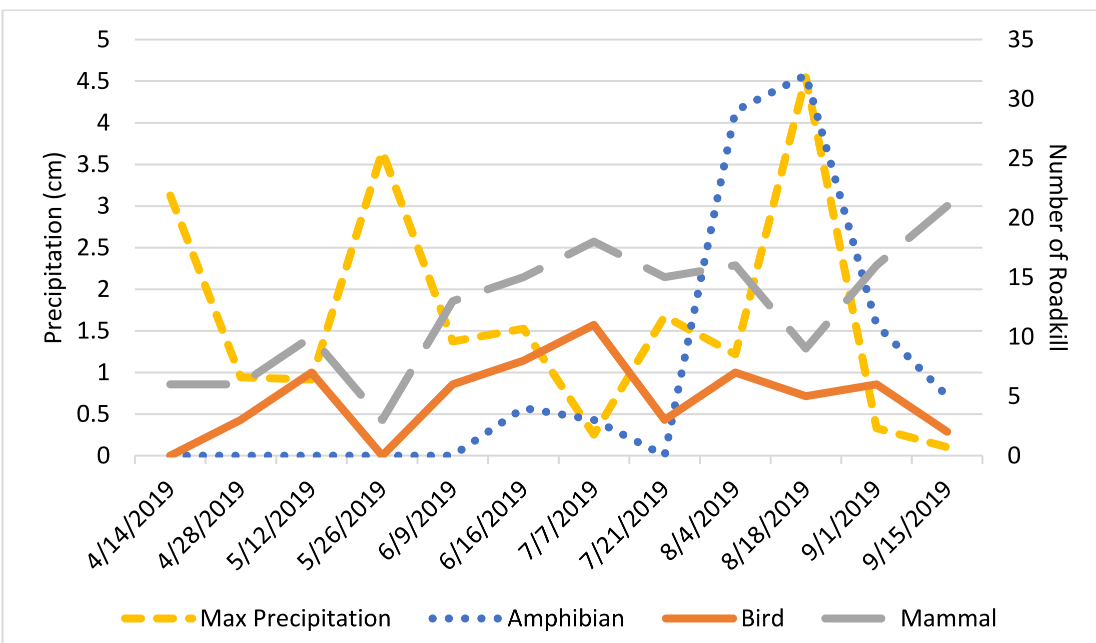

Figure 2.23. A comparison of trends in maximum precipitation (cm) and the number of each individual roadkill by taxon over the survey period per week. The yellow dashed line represents precipitation, the blue dotted line represents amphibian roadkill, the orange solid line represents bird roadkill, and the gray dashed line represents mammal roadkill.

Table

Table 2.1. The natural areas within the Oak Openings Region that were surveyed with key characteristics. Shown is the area in hectares and land manager for each protected area.

Natural Area	**Managed by**	**Size (hectares)**
Irwin Prairie State Nature Preserve	Ohio Division of Natural Areas and Preserve	83.8
Kitty Todd Nature Preserve	The Nature Conservancy	566.6
Maumee State Forest	Ohio Department of Natural Resources Division of Forestry	>1,254.5
Oak Openings Preserve Metropark	Metroparks Toledo	~2,023.4
Secor Metropark	Metroparks Toledo	237.1
Wiregrass Lake Metropark	Metroparks Toledo	~5

Table 2.2. All surveyed road transects broken down by natural area. Shown are the key characteristics for the roads: road name, transect number, length, width, traffic volume, speed limit, and distance from natural area.

Natural Area	Road Name	Transect Number	Length (km)	Width (m)	Traffic (AADT)	Speed Limit (km/h, mph)	Distance from Natural Area (km)
Kitty Todd Nature Preserve	West Tupelo Way	KT1	1.73	6.00	-	32.2, 20.0	0.00
	Bancroft Street	KT2	1.57	9.00	1132	88.5, 55.0*	3.15
	Dorr Street (west)	KT3	1.58	6.50	277	88.5, 55.0	2.35
	Dorr Street (east)	KT4	1.63	6.50	497	88.5, 55.0	2.35
	Frankfort Road (east)	KT5	1.62	6.50	869	88.5, 55.0*	1.55
	Frankfort Road (west)	KT6	1.59	6.50	734	88.5, 55.0*	1.55
	South Lathrop Road	KT7	1.32	6.00	209	88.5, 55.0	3.18
	South Berkey Southern Road	KT8	1.17	8.00	1124	88.5, 55.0	1.60
	South Raab Road	KT9	1.36	6.50	277	88.5, 55.0*	0.00
	Angola Road	KT10	1.61	6.00	300	88.5, 55.0*	0.00
	South Schwamberger Road	KT11	1.37	6.00	243	88.5, 55.0*	0.00
	Irwin Road	KT12	1.34	6.00	435	72.4, 45.0	0.00
Maumee State Forest	County Road F	M1	1.60	5.50	154	88.5, 55.0*	3.25
	Township Road EF	M2	1.61	6.00	412	88.5, 55.0*	2.44
	County Road E	M3	1.61	6.00	469	88.5, 55.0*	1.62
	County Road C	M4	1.59	6.50	958	88.5, 55.0*	0.00
	County Road 6	M5	1.58	6.50	245	88.5, 55.0*	3.63
	County Road 5	M6	1.55	6.00	320	88.5, 55.0*	1.62

	County Road 4	M7	1.54	5.50	199	88.5, 55.0*	0.00
	County Road S3	M8	1.60	6.00	100	88.5, 55.0*	2.40
	County Road T3/Box Road	M9	1.62	6.00	264	88.5, 55.0*	0.78
	Township Road U	M10	1.63	3.50	296	88.5, 55.0*	0.00
	Manore Road	M11	1.61	5.50	88	88.5, 55.0*	1.51
	Jeffers Road	M12	1.61	7.00	677	88.5, 55.0*	2.32
	Yawberg Road	M13	1.61	6.00	52	88.5, 55.0*	3.14
Oak Openings Preserve	Girdham Road	OO1	1.62	5.00	387	88.5, 55.0*	0.00
	Shaffer Road	OO2	1.52	6.50	850	88.5, 55.0*	2.36
	Old State Line Road	OO3	1.53	9.50	1659	88.5, 55.0*	3.90
	Angola Road	OO4	1.82	6.50	309	88.5, 55.0	5.22
	County Highway 1-2	OO5	1.55	6.00	1291	88.5, 55.0	1.57
	County Road 2	OO6	1.62	6.50	1451	88.5, 55.0*	2.40
	County Road 3	OO7	1.61	7.00	2410	56.3, 35.0	4.00
	Waterville Swanton Road	OO8	1.61	7.50	3105	88.5, 55.0	0.00
	Archbold-Whitehouse Road	OO9	1.61	8.50	1660	88.5, 55.0	1.67
	Neapolis Waterville Road	OO10	1.61	9.00	1500	88.5, 55.0	3.97
	South Eber Road	OO11	1.61	10.00	3057	88.5, 55.0*	3.24
	Whitehouse Road	OO12	1.61	6.50	532	88.5, 55.0*	1.62
	South Berkey Southern Road	OO13	1.20	8.00	2949	88.5, 55.0*	0.00

* Speed limit not posted, assumed 88.5 km/h (55.0 mph).

Table 2.3. Example of variables measured at each roadkill spot. In bold are the variables measured.

Roadkill 104:	Racoon
Transect:	M4 (County Road C)
Land Use Left/Right:	Inactive Ag/Forest
Structure Presence:	Telephone Lines, Sign
Ditch Presence:	Ditch Present
Ephemeral Presence:	None
Canopy Cover:	12%
Ground Vegetation Type/Height:	Grass/5.0 cm

Table 2.4. The variation in range and average temperature (C°), precipitation (cm), humidity (%), and moon illumination (%) within the study area from May - September 2019 by survey week.

Date	Temperature Range (C°)	Average Temperature (C°)	Precipitation Range (cm)	Average Precipitation (cm)	Humidity Range (%)	Average Humidity (%)	Moon Illumination Range (%)	Average Moon Illumination (%)
4/14	1.1 - 23.3	9.4	0.0 - 3.1	0.80	42.0 - 97.0	81.5	75.2 - 99.7	91.5
4/28	2.8 - 22.8	10.9	0.0 - 0.9	0.32	52.0 - 99.0	85.0	0.2 - 35.1	14.0
5/12	3.3 - 27.8	14.2	0.0 - 0.9	0.25	37.0 - 98.0	76.4	61.3 - 99.3	83.7
5/26	11.1 - 29.4	20.7	0.0 - 3.7	0.89	50.0 - 100.0	86.9	3.7 - 52.1	25.6
6/9	7.8 - 27.2	18.1	0.0 - 1.4	0.36	38.0 - 98.0	75.1	47.0 - 94.6	73.2
6/16	12.8 - 29.4	20.0	0.0 - 1.5	0.27	44.0 - 100.0	80.8	77.2 - 99.9	92.4
7/7	15.0 - 34.4	24.4	0.0 - 0.3	0.03	45.0 - 98.0	75.8	9.3 - 85.8	53.3
7/21	14.4 - 33.3	23.0	0.0 - 1.7	0.28	42.0 - 99.0	71.8	25.5 - 82.6	55.1
8/4	15.0 - 33.3	24.3	0.0 - 1.2	0.15	48.0 - 99.0	77.2	19.7 - 82.5	52.1
8/18	12.8 - 32.2	23.1	0.0 - 4.6	1.02	42.0 - 91.0	71.5	41.0 - 69.1	69.5
9/1	11.1 - 28.3	20.2	0.0 - 0.3	0.04	50.0 - 92.0	75.1	8.7 - 69.1	38.0
9/15	12.2 - 32.2	21.9	0.0 - 0.1	0.01	45.0 - 91.0	73.8	57.1 - 98.8	81.7

Table 2.5. The variation in range and average canopy cover (%) and vegetation height (cm) around the roadways from May - September 2019 by month.

Month	**Season**	**Canopy Cover Range (%)**	**Average Canopy Cover (%)**	**Vegetation Height Range (cm)**	**Average Vegetation Height (cm)**
May	Spring	0.0-73.0	6.9	5.0-50.8	14.9
June	Spring	0.0-73.0	8.0	5.0-94.0	17.7
July	Summer	0.0-79.0	9.8	2.5-94.0	17.9
August	Summer	0.0-93.0	10.0	2.5-137.2	17.4
September	Summer	0.0-78.0	9.0	2.5-137.2	13.7

Table 2.6. Frequency of all vertebrate taxa on the roadways in Northwest Ohio by month.

Month	**Amphibian**	**Bird**	**Mammal**	**Reptile**	**Total Roadkill**	**% of Roadkill**
April	0	2	6	0	8	2.7
May	0	8	19	1	28	9.4
June	4	14	28	1	47	15.8
July	3	14	34	0	51	17.2
August	61	12	24	4	101	34.0
September	16	8	37	1	62	20.9
Total	84	58	148	7	297	

Table 2.7. Stepwise models for amphibian, bird, and mammal roadkill based on variables per survey week. Only models with AIC_c changes of less than or equal to two were reported. The table includes number of variables (N), parameter estimate, significance (Prob > F), correlation (R^2 Adjusted), Akaike's Information Criterion (AIC_c), and change in AIC_c (ΔAIC_c). * indicates significant variables ($p < 0.05$). ** indicates significant variables after Bonferroni correction.

	N	Variables	Parameter Estimate	Prob > F	R^2 Adjusted	AIC_c	ΔAIC_c
Amphibian	1	Spring	-6.333333	0.0497*	0.265654	95.7219	0.0000
	2	Spring	-7.354949	0.0365*	0.414467	96.4544	0.7325
		Maximum Precipitation	9.148797				
Bird	1	Maximum Canopy Cover	0.0938144	0.0142*	0.414566	63.28253	0.0000
Mammal	3	Spring	-2.583472	0.0001**	0.879723	64.68438	0.0000
		Maximum Precipitation	-5.858456				
		Maximum Moon Illumination	0.101222				

GENERAL CONCLUSION AND MANAGEMENT IMPLICATIONS

Road surveys are a cheap and easy way to track where and when animals are dying on roadways. Using this information, land managers can easily track how distribution, abundance, and diversity of animals change over time. In addition, these studies provide a way to examine connectivity and movement through fragmented landscapes. We were able to analyze temporal patterns in roadkill and find similarity between those patterns and natural movement patterns (e.g., foraging, reproduction, dispersal, migration). Throughout this study, we were able to show that hot spots exist throughout the Oak Openings Region, that hot spots of road mortality vary based on taxa, and that hot spots change over time. There are many variables, both spatial and temporal, that need to be considered when identifying areas of potential concern.

Chapter 1. Our goal for chapter one was to identify vertebrate mortality hot spot locations across the study area for each taxon. We wanted to know where animals were dying on the roadways and what potential variables may be influencing where roadkill is found. Through road surveys and hot spot analysis we were able to locate 297 roadkill and hot spots for dead amphibians, birds, and mammals. By looking at a plethora of measured variables at fixed sampling points and roadkill points, we were able to find significant relationships between the variables and hot spot locations, as well as, the variables and individual roadkill taxon. Amphibian roadkill had the highest Spearman ρ correlation with the presence of mosaic land use type, presence of ditches, and presence of water. Bird roadkill had the highest Spearman ρ correlation with presence of mosaic land use type, presence of water, and presence of an understory. Mammal roadkill had the highest Spearman ρ correlation with presence of an understory, higher percentage of canopy cover, and presence of ditches. Using this information, land managers can work towards conservation of those organisms at high risk through mitigation of roadkill.

Chapter 2. Our goal for chapter two was to identify changes in roadkill abundance, distribution, and diversity over the survey time for each roadkill taxon. We conducted road surveys for six months, over two different seasons. Through hot spot analysis we located monthly hot spots for all roadkill and seasonal changes in hot spots for dead amphibians, birds, and mammals. We were able to identify changes in abundance, distribution, and diversity of roadkill during the survey period and pinpoint months of high road mortality for each roadkill taxon. Amphibian roadkill had its highest peak in August. Bird roadkill had a two-month peak between June and July. Mammal roadkill had two different peaks, one in July followed by one in September. Many of the roadkill peaks correlated with seasonal animal movement. Using this information, land managers are able to identify periods of time that are at higher risk for vertebrate mortality, as well as, protect these animals during their major seasonal movements.

Not only can this information be used to track changes in animal populations, but it can be used to protect these populations via mitigation of unsuccessful road crossing. Survey results can be especially helpful when looking at taxa or species of concern. Though there is not one main variable that drives roadkill, there are influential variables that can be altered by management. Structural features such as percentage canopy cover, height of vegetation, understory presence, etc. can be altered to reduce the number of dead animals. Our results suggested that higher amounts of vegetation along roadways may decrease visibility, therefore increasing roadkill. Managers could lessen the amount of roadside vegetation or create more areas of limited roadside vegetation to aid in animal movement.

For variables that cannot be easily altered such as traffic volume, weather, and season, mitigation methods can be adopted to try and reduce road mortality. This could be a combination of increasing human awareness, decreasing potential for getting hit, and access to safer crossing areas. Increased signage to remind humans of nearby wildlife, potentially could decrease driving speeds and increase driver vigilance. Temporarily closing roads or reducing speeds on roads can help reduce the risk of organisms getting hit on roads during periods of mass movement. The creation of wildlife crossing structures (e.g., land bridges, culverts) can provide a safe area for organisms to cross roads without impacting how humans use roadways.

To maximize the amount and effectiveness of mitigation techniques, we would recommend performing a multi-year, multi-taxa, and multi-scale survey. This would result in identification of consistent areas, taxa, and species of high concern. A multi-year study would also evaluate how environmental variables impact organisms differently throughout all seasons. The four taxa responded differently to different variables throughout our study, which is why a multi-taxa approach is recommended. Hot spots do move based on temporal scale (week, month, season), but also based on spatial scale (road, protected area, entire region). This would suggest a multi-scale approach is also necessary for the most accurate results. Different scales also allow different land managers and groups to focus on hot spots within their managed lands.

Road surveys and roadkill analysis is a recommended method to survey animal abundance, distribution, and diversity. Not only is it inexpensive, requires basic equipment, and is easy, it can also be done individually. This method is applicable and easily adaptable across all landscapes that are fragmented by roadways. These results are able to help mitigate death on the roads and increase conservation for local fauna.

APPENDIX A: CHAPTER 1 SUPPLEMENT

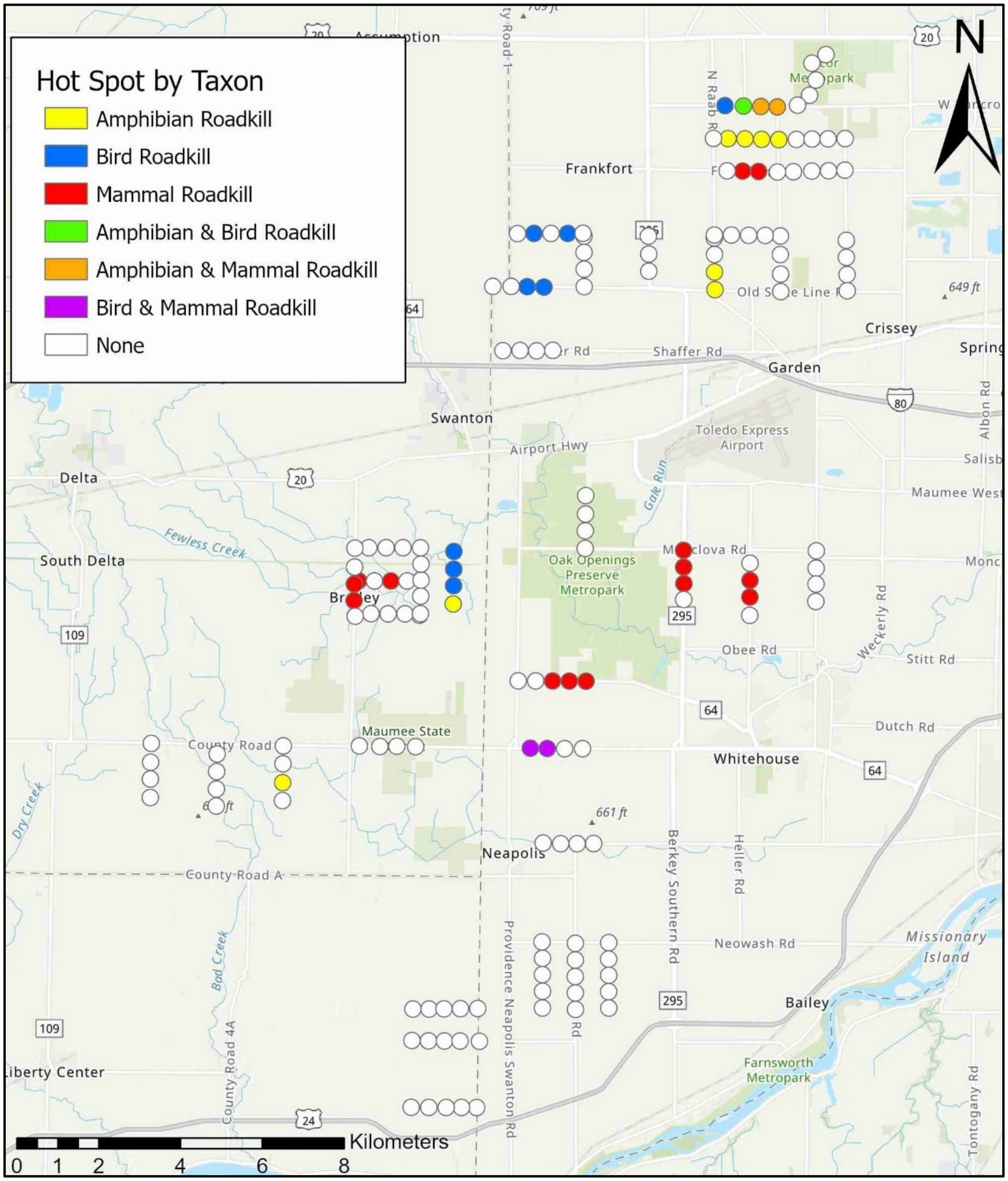

Figure 1.S1. The comparison between hot spots by roadkill taxon. White circles show 200 m buffers were never hot spots. Primary colors (yellow, blue, red) show 200 m buffers that were hot spots for only one roadkill taxon (amphibian, bird, or mammal). Secondary colors (green, orange, purple) show 200 m buffers that were hot spots for two of roadkill taxa (amphibian and bird, amphibian and mammal, or bird and mammal).

Table 1.S1. Breakdown of amphibian roadkill response to the four land use types. * indicates significant variables ($p < 0.05$). ** indicates significant variables after Bonferroni correction.

Land Use Type	**Spearman ρ**	**P-Value**
Agriculture	0.2419	0.0019**
Developed	0.1294	0.1009
Mosaic	0.3456	< 0.0001**
Nature	0.1655	0.0353*

Table 1.S2. Breakdown of bird roadkill response to the four land use types. * indicates significant variables ($p < 0.05$). ** indicates significant variables after Bonferroni correction.

Land Use Type	**Spearman ρ**	**P-Value**
Agriculture	0.1763	0.0248*
Developed	0.2089	0.0076*
Mosaic	0.3521	< 0.0001**
Nature	0.0861	0.2759

Table 1.S3. Breakdown of mammal roadkill response to the four land use types. * indicates significant variables ($p < 0.05$). ** indicates significant variables after Bonferroni correction.

Land Use Type	**Spearman ρ**	**P-Value**
Agriculture	0.0590	0.4558
Developed	0.3223	< 0.0001**
Mosaic	0.4756	< 0.0001**
Nature	0.3915	< 0.0001**

APPENDIX B: CHAPTER 2 SUPPLEMENT

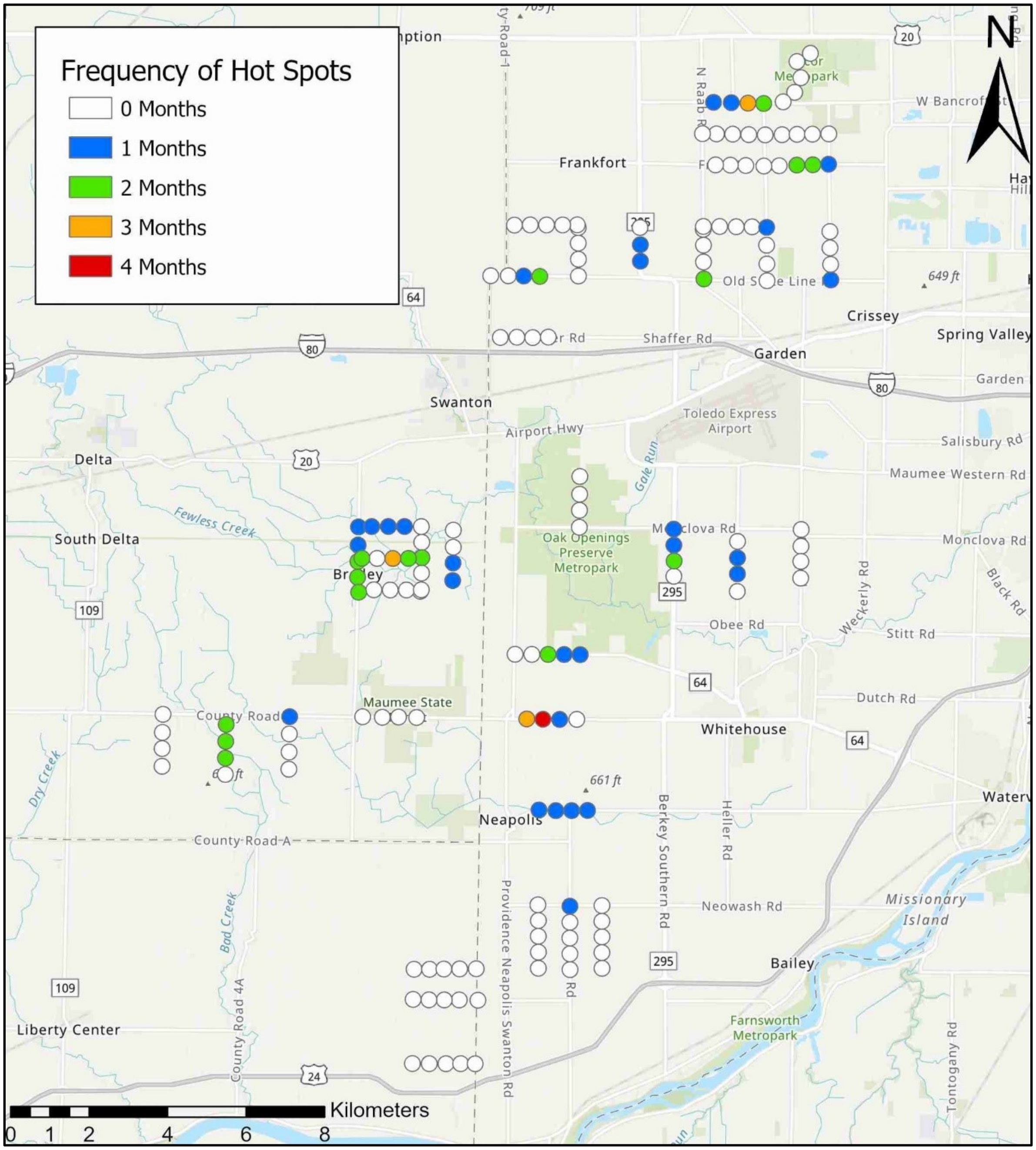

Figure 2.S1. Frequency of occurrence of hot spots during the survey period (April - September 2019). White circles show 200 m buffers were never hot spots. Blue circles show 200 m buffers that were hot spots for one month. Green circles show 200 m buffers that were hot spots for two months. Orange circles show 200 m buffers that were hot spots for three months. Red circles show 200 m buffers that were hot spots for four months.