USING ROADKILL AS A LENS TO UNDERSTAND ANIMAL MOVEMENT AND MORTALITY

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ABSTRACT

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This study took place within the Oak Openings Region, a biodiversity hotspot in northwest Ohio. The Oak Openings was created during the Cenozoic period, a time where glaciers continuously moved across Ohio, creating valleys and riverbeds. When the Wisconsin glaciers melted back from Ohio around 14,000 years ago, waters were released into multiple lakes with sandy beaches. Overtime, these sandy beaches started to become dunes characterized by rain water that could not drain past the clayey till and bedrock ground later. Water that would stand between the clay layer and sandy dunes provided moisture to support oak savanna habitat. Between the sand ridges, rain water would saturate the land, which created open areas of wet prairie (Higgins, 2003; Grigore, 2004). The combination of oak savanna habitat and open wet prairie gave this region the name of “Oak Openings” (Grigore, 2004).

Today, the Oak Openings looks quite different as a result of the economic development and agricultural pursuits along the Toledo-Detroit corridor. This area is highly fragmented by roads, agricultural fields and urban/residential development. Roughly 45% of the Oak Openings Region contains urban and suburban development and roughly a quarter of the region has been converted to areas of agricultural production (Schetter and Root, 2009). However, there are still remnants of wild lands that exist west and south of the city of Toledo. This area is Ohio’s largest single surficial sand covering which is 1-12 meters thick and consists of wet lowland and sand ridge terrain with elevations up to 210 meters above sea level. This region is approximately 8 kilometers wide and 32 kilometers long (Higgins, 2003; Figure 1) which contains oak savanna,

oak woodland and wet prairie habitats on post glacial beach ridges and swales and covers 345 km^2 (The Nature Conservancy, 2001; Grigore, 2004).

The Oak Openings Region contains one third of all of Ohio's endangered plant communities as well as many rare animals and early successional ecosystems. However, only about 10% of the Oak Openings Region is in protected areas (Abella et al., 2007). Early successional ecosystems in this area (e.g. oak savannas and prairies) were historically disturbed by fire. Now, these disturbances are mimicked by management activities such as prescribed fires and thinning. In Ohio there are two preserves known as the Oak Openings Preserve and Maumee State Forest, which are the largest protected areas and support many different vertebrate populations and species. However, these two preserves are also highly fragmented by agricultural fields, roadways, railways, and trails that may limit animal movement and increase road mortality. Therefore, it is critical to better understand animal movement and road mortality in this area to promote viable populations, increase our knowledge of vertebrate movement and prevent the killing of vertebrate species by vehicle collisions.

This thesis has two foci, each as the topic of stand-alone chapters. The overarching goal is to predict animal movement and identify the features that may be managed to reduce road mortality, which is likely to be applicable to other reserves in human-dominated landscapes. The objective of the first chapter is to understand what factors influence animal movements and road mortality. Specifically, the first chapter examines how structural features of roads, environmental variables, spatial factors and land cover types can influence vertebrate movement (e.g. mammals, birds, reptiles, amphibians) and road mortality on roads surrounding and within the Oak Openings Preserve and Maumee State Forest. Road surveys as well as visual surveys on trails within both protected areas were utilized to account for animal movement, dispersal and mortality.

The second chapter focuses on mammal movement patterns and road mortality on roads surrounding and within the Oak Openings Preserve and Maumee State Forest. Similar to chapter I, road and visual surveys along trails were utilized to understand mammal movement and mortality. Mammals were found as roadkill more than any other taxa, so it is important to understand what factors influence mortality and movement. Again, structural features of roads, environmental variables, spatial factors and land cover types were all analyzed to understand what influences mammal movement and mortality.

This work is dedicated to my family who have always believed in me, supported me and who have helped me accomplish anything I put my mind to.

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GENERAL INTRODUCTION

This study took place within the Oak Openings Region, a biodiversity hotspot in northwestern Ohio. The Oak Openings was created during the Cenozoic period, a time where glaciers continuously moved across Ohio, creating valleys and riverbeds. When the Wisconsin glaciers melted back from Ohio around 14,000 years ago, waters were released into multiple lakes with sandy beaches. Overtime, these sandy beaches started to became dunes inundated by rainwater that could not drain past the clayey till and bedrock ground layer. Water that would stand between the clay layer and sandy dunes provided moisture to eventually support oak savanna habitat. Between the sand ridges, rainwater would saturate the land, which created open areas of wet prairie (Higgins, 2003; Grigore, 2004). The combination of oak savanna habitat and open wet prairie gave this region the name of "Oak Openings" (Grigore, 2004).

Today, the Oak Openings looks quite different as a result of the economic development and agricultural pursuits along the Toledo-Detroit corridor. This area is highly fragmented by roads, agricultural fields and urban/residential development. Roughly 45% of the Oak Openings Region contains urban and suburban development and roughly a quarter of the region has been converted to areas of agricultural production (Schetter and Root, 2011). However, there are still remnants of wild lands that exist west and south of the city of Toledo. This area is Ohio's largest single surficial sand covering which is 1-12 meters thick and consists of wet lowland and sand ridge terrain with elevations up to 210 meters above sea level. This region is approximately 8 kilometers wide and 32 kilometers long (Higgins, 2003; Figure 1) and contains oak savanna, oak woodland and wet prairie habitats on post glacial beach ridges and swales and covers 478 km^2 (The Nature Conservancy, 2001; Grigore, 2004; Schetter and Root, 2011).

The Oak Openings Region contains one third of all of Ohio's endangered plant communities as well as many rare animals and early successional ecosystems. However, only about 10% of the Oak Openings Region is in protected areas (Abella et al., 2007). Early successional ecosystems in this area (e.g. oak savannas and prairies) were historically maintained by disturbances such as fire. Now, these disturbances are mimicked by management activities such as prescribed fires and thinning. In Ohio there are two preserves known as the Oak Openings Preserve and Maumee State Forest, which are the largest protected areas and support many different vertebrate populations and species. However, these two preserves are also highly fragmented by agricultural fields, roadways, railways, and trails that may limit animal movement and increase road mortality. Therefore, it is critical to better understand animal movement and road mortality in this area to promote viable populations, increase our knowledge of vertebrate movement and prevent the killing of vertebrate species by vehicle collisions.

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CHAPTER I. PREDICTING VERTEBRATE MOVEMENT AND ROAD MORTALITY INFLUENCED BY ENVIRONMENTAL VARIABLES AND STRUCTURAL FEATURES OF ROADWAYS

Abstract

In the United States, road networks are vast and most effects of roads on native species are negative. Roads, which can reduce population connectivity or structural features along roadways can affect vertebrate movements by acting as barriers, attractants or a source of mortality. In order to reduce negative impacts of roads, it is important to understand how structural features (e.g. vegetation density, canopy cover, road features, and land cover types) spatial factors (e.g. traffic density, speed) and environmental variables (e.g. temperature, humidity, and rain) relate to animal life history, road mortality and movement across vertebrate taxa (e.g. amphibians, reptiles, birds and mammals). This study uses roadkill as a lens to study animal movement and identify structural features of roads that may be managed to reduce road mortality. In the Oak Openings Region, a biodiversity hotspot in Northwest Ohio, we surveyed roads surrounding and within the Oak Openings Preserve and Maumee State Forest, the largest protected areas, for all vertebrate taxa for the period of May-October 2016. We also performed daytime and nighttime surveys within the parks to account for sources of animal dispersal and dispersal corridors. We found 292 vertebrates dead on roads surrounding protected areas, including more mammals than any other taxa. Almost 44% of road mortality was found on one roadway that appeared to be an area of frequent vertebrate crossings and mortality from vehicle collisions as confirmed by animal movement in visual surveys primarily in areas adjacent to that road. Intermediate levels or no canopy on roads increased the number of road killed vertebrates for all surveyed months (Wilcoxon, $p<0.02$). Road structural features (e.g. presence of

agricultural fields, vertical road signs, grassy fields, verge slope, forest and water) significantly influenced vertebrate road morality, but the effects varied among species and months. Land cover types along the roads and edges also influenced animal movement and road mortality for mammals and birds during all surveyed months. Our approach predicted animal movement and identified the features that may be managed to reduce road mortality, which is likely to be applicable to other reserves in human-dominated landscapes.

Introduction

Land cover changes caused by the increase of land use by humans threaten the long-term viability of natural areas, and land use will have a large global impact on biodiversity (Schetter and Root, 2011). Part of this great transformation involves the vast networks of roads that are continuously being constructed. In the United States, road networks encompass almost 6.6 million km of public roadway spread out so that 80% of the land area is within 1 km of a road (Gagne, et al., 2015). By examining how roads can affect wildlife, we can learn about some of the potential impacts of these human modifications and how to mitigate their impacts on native vertebrates. Most effects of roads on wildlife are negative; in which roads can reduce population connectivity as a result of habitat fragmentation and animals may dramatically change their behavior e.g., road avoidance (Andrews et al. 2008; Rotholz and Mandelik, 2013; Fahrig et al., 1995; Forman, 1998; Shepard et al., 2008). Structural features along roadways can affect vertebrate populations and their movements by acting as barriers, increase movement in generalist species, attract species in search of certain resources (e.g. agricultural fields) or cause wildlife-vehicle collisions resulting in animal mortality or population decline (Cuyckens, et al., 2015). Examination of the different effects roadways and traffic have on wildlife has led to the rise of a discipline called road ecology in which research has focused on wildlife-vehicle collisions, basic variables such as traffic volume, speed and location and on mitigation efforts to reduce road mortality (D'Amico, et al. 2015; Forman, 1998; Keken et al., 2016). However, it is important to understand how structural features (e.g. vegetation density, canopy cover, road characteristics, and land cover types) spatial factors (e.g. traffic density, speed) and environmental variables (e.g. temperature, humidity, and rain) relate to animal life history, movement and road mortality across all terrestrial vertebrate taxa (i.e. amphibians, reptiles, birds and mammals). This study utilizes roadkill as a lens to study animal movement and identify structural features of roads that may be managed to reduce road mortality. Specifically, spatial factors, environmental variables and structural features of roads could potentially affect animal movement and mortality in human-dominated landscapes such as the Oak Openings Region of northwest Ohio.

In Ohio, the increase of roadways, as a result of agricultural expansions and urban development, has both directly and indirectly affected wildlife by causing habitat fragmentation within remnant natural habitats. In northwest Ohio, there is a biodiversity hotspot known as the Oak Openings Region. This extraordinary set of habitats consist of a mosaic of oak savanna, wet praire, oak woodland, oak barrens and floodplain forest, and is critically important to maintain the wildlife in this region (Lawrence et al., 2004; Brewer and Vankat, 2006). At present, it is a suite of remnant natural areas that has been fragmented by roadways, railways, trails and anthropogenic land alterations.

The Oak Openings Region supports many diverse animal and plant communities. Nearly one-third of all Ohio's endangered plants along with many rare animals are found within the Oak Openings Region (Grigore, 2004). Early successional ecosystems, such as prairies and oak savannas are only maintained by disturbances that are now often mimicked by management activities such as prescribed fires. The Metroparks of Toledo Area manage canopy cover and sapling densities using prescribed fires and thinning that mimic the disturbances that were historically essential to many of the ecosystems of the Oak Openings Region (Albell et al., 2004). In addition to the human alterations to the landscape, there are significant alterations to the disturbance regime that may threaten native vertebrate populations. To preserve and maintain such a special area, considered by Nature Conservancy as ecologically important as the Florida Everglades and one of the 200 "last great places on earth" (Anonymous, 2016), we need to maintain viable populations, become more knowledgeable about vertebrate movement and prevent the killings of vertebrate species by vehicle collisions on roadways.

Previous research collected by Amanda Kuntz, a former doctoral student with Dr. Root, demonstrated an effect of roadways on vertebrate communities within the Oak Openings Preserve (Unpublished data, 2010). Amanda surveyed roadways surrounding and within Oak Openings Preserve for roadkill by identifying what species were killed as well as where and when. Data collection began in May and continued through November 2010 with surveys conducted at random and approximately four to five times a month. About 165 roadkill specimens were identified on the roadways surrounding and within the Oak Openings Preserve. It was evident that one specific roadway on the border of Oak Openings Preserve had higher road mortality than the other roadways with 59% of the road mortalities recorded on that roadway. It is likely that this specific roadway, Waterville Swanton Road is an area for frequent vertebrate crossing as it also borders another protected area known as known as Maumee State Forest (Unpublished work, 2010). This area could potentially affect how vertebrate communities move between the Oak Openings Preserve and Maumee State Forest and resulting vertebrate road mortality may have detrimental effects on local vertebrate population sizes. These data suggest it is important to understand how structural features located on roadways surrounding and in Oak Openings Preserve and Maumee State forest relate to vertebrate movement and mortality.

According to Beebee (2013), the development of roadways in many countries constitutes the most significant barrier to the movement of wildlife and creates what is known as the barrier effect by road avoidance when traffic density is high (Grilo et al., 2015; Clevenger et al., 2003; Forman, 1998). For example, the barrier effect can be evident in roadways that bisect a wetland which amphibians may avoid or risk crossing. Roads that bisect wetlands tend to have higher road mortality of amphibians since amphibians have life cycles in which juvenile stages rely on water (Langen et al., 2009; Glista et al., 2008; Woltz et al., 2008). Also, roadways may act as barriers to many reptile species such as turtles, as they move from feeding grounds to nesting sites and back to feeding grounds. If roadways bisect feeding grounds and nesting sites during times of extensive movements than this can cause high mortality in these species (Obbard and Brooks, et al., 1980). Roadways may act as barriers to birds in which pollution, visual stimuli and noise are known to be critical factors that cause birds to avoid roadways and decrease bird densities near roadways (Reijnen et al., 1997). Lastly, roads may affect mammals by acting as a barrier by reducing access to food resources since some mammal species may avoid noisy or high traffic density roadways. However, roadways can also increase food sources for certain species like white-tailed deer by providing grassy edges increasing the chance of these animals getting hit by vehicles (Oxley, et al, 1974). Roads could have certain structural features that attract vertebrates (e.g. agriculture fields) or restrict the movement of vertebrate communities between patches as organisms take advantage of the different resources available in these separated areas.

Road edge can also restrict or encourage animal movement and animal-vehicle collisions. Road edge is defined as the area that is in between a road and a certain land cover type (e.g. forests). Road edges can encourage animal movement by operating as corridors in which edge specialists like white-tailed deer or coyote can move more easily through an area. This can increase the chance of animal-vehicle collisions (Oxley, et al, 1974). Also, road edges can provide habitat for some species, like amphibians that are known to use roadside ditches when filled with water (Spellerberg, 1998; Gibbs, 1998). Road edge can restrict animal movement as species may avoid edges to avoid predation from other edge specialists like red-tailed hawks. Lastly, species may avoid road edges due to traffic density, traffic noise pollution or other anthropogenic disturbances (Grilo et al., 2015; Clevenger et al., 2003; Forman, 1998).

To better distinguish vertebrate movement patterns, Amanda conducted nighttime surveys within the Oak Openings Preserve to identify which species reside within the preserve and note where species were moving (Unpublished work, 2010). Understanding animal dispersal can help us better predict if, when, and where, an animal might cross a road. According to Howard (1960), innate animal dispersal is important to species because it increases the spread of new genes, supports out breeding, allows a species to spread its range as favorable habitats are created, and helps species invade areas that may have been depopulated due to disturbances or stochastic events (Howard, 1960). Since the Oak Openings Region is intermittently disturbed (e.g. fires, thinning, mowing, invasive species removal, spraying) to maintain the historic oak savanna habitat animals may disperse out of newly disturbed areas and thus, increase the chances of road crossings and collisions with vehicles. Animals may also be moving to take advantage of resources that may be temporally or spatially limited.

To understand the effect roadways have on vertebrate communities, temporal and spatial factors need to be considered. Variation in movement occurs across time. If we understand these variations across time, managers could anticipate when roadkill would be more likely. For example, temperature and rainfall can be linked to peaks in road kill patterns (Clevenger, et al., 2003). Spatial variations in road structural features can also be associated with roadkill frequency (D'Amico et al. 2015; Clevenger, et al., 2003). An example of how temporal and spatial factors led to a better understanding of how roadways affect vertebrate communities is found in a study conducted by Grilo et al. (2015) in Portugal. This study used a large spatio-temporal scale to better evaluate the response of several vertebrate species (barn owl, red fox, hedgehog, and rabbit) to increasing traffic density for 2003-2009. The results indicated that traffic intensity on roadways affected each species differently. For instance, the maximum probability of road mortality due to traffic volume was 3,000 vehicles/day for hedgehogs, 5,000 vehicles/day for red foxes and 2,000 vehicles/day for rabbits and barn owls (Grilo et al., 2015).

Similarly, a study conducted by D'Amico et al. (2015) in Doñana Natural Area (a biodiversity hotspot) investigated life history as well as temporal and spatial factors that can predict casualty patterns such as seasonal behaviors, climatic factors, road features and traffic volume. The authors found that vertebrate taxa respond differentially to spatial and temporal factors. They also discovered that temporal and spatial patterns could be identified to decrease road mortality. This study (D'Amico et al., 2015) found that the phenology could explain why lizards, birds and small mammals were more likely to be hit by vehicles. They also discovered that climatic predictors (e.g. rainfall or temperature) lead to an increase of road mortality for both amphibians and snakes. Road mortality of amphibians decreased with high temperature, but with snakes the opposite occurred. Similarly, they discovered that wintering birds had higher road mortality after an increase of precipitation. For spatial factors, they found that amphibians were more likely to be hit by vehicles near water bodies; but for snakes, lizards and small passerines the opposite was true. For road features, they found that the presence of culverts decreased the road mortality of snakes, whereas the presence of wildlife crossing structures decreased lizard road mortality but increased snake and small mammal road mortality. Finally, the presence of vertical road signs increased road mortality of snakes and birds (D'Amico et al., 2015). The research conducted by Grilo et al. (2015) and D'Amico et al. (2015) demonstrate that it is important to incorporate a temporal and spatial analysis into our study of road mortality to provide a more effective prediction of the potential impacts.

By conducting road kill surveys, while including environmental variables, spatial factors and structural features of roads, better mitigation efforts can be developed to potential lower road mortality on roadways. In this research, we combined road surveys with visual surveys for vertebrates within protected areas to examine the distribution and movement of vertebrates in a highly fragmented, human-impacted landscape. We hypothesized that vertebrates are likely to be crossing at high numbers between protected areas concentrated on specific road sections, such as Waterville Swanton Road, which is a likely corridor between Oak Opening and Maumee State Forest. We also predicted that certain environmental variables (e.g. rainfall, humidity, and temperature), spatial factors (e.g. traffic density, speed) and structural features of roads could help predict road kill probability of these areas. Lastly, we sought to predict animal movements throughout both preserves and identify which areas have a high density of animals in close proximity to roadways. Using roadkill as a lens can help predict vertebrate movement and to identify road features that may be managed to reduce road mortality, which is likely to be applicable to other reserves in human-dominated landscapes.

Methods

Study Location

Our research was conducted in Oak Openings Preserve and Maumee State Forest within the Oak Openings Region in northwestern Ohio, Figure 1. The Oak Openings Preserve is roughly 1,214 hectares comprised of oak savanna, wet prairie, oak woodland, oak barrens, and floodplain forest and is managed by Metroparks of the Toledo Area. Maumee State Forest is roughly 1,292 hectares and is composed of hardwood forest, swamp forest, pine forest and wet prairie that is managed by Ohio Department of Natural Resources (ODNR) Division of Forestry.

Road Surveys

We surveyed roadways that surrounded Oak Opening Preserve and Maumee State Forest for vertebrates alive and dead. The roads that surrounded Oak Opening Reserve include: Airport Highway US HWY 20A, Wilkins Rd, Monclova Rd., Southern Berkley Rd., and Waterville Swanton Rd., which roughly constituted a road length of 20 kilometers (13 miles) around Oak Openings Preserve. The roads parallel to Waterville Swanton Rd. were also surveyed in this study; these roads were Fulton-Lucas Rd. and Archbold-Whitehouse Rd. The roads that were surveyed surrounding Maumee State Forest include: County Rd. D (which turns into Waterville Swanton Rd.), Providence Neapolis Swanton Rd., County Rd. 3, and County Rd. C., which roughly constituted 12.87 kilometers (8 miles) (Figure 2).

Three roads within the Oak Openings Preserve were surveyed, which included: Girdham Rd., Jeffers Rd., and Monclova Rd. (surveying of Monclova Rd. started in July). The paved bike trail was surveyed within the Oak Openings Preserve, which is known as the Wabash Cannonball Trail (Figure 3). This bike trail is a converted railway line that was similar in size and composition to a single land road with non-motorized traffic. There were no accessible roads within Maumee State Forest to survey.

Vertebrates across all terrestrial taxa were counted (e.g. amphibians, reptiles, birds and mammals). Roads were chosen at random while allowing for logistical constraints, but surveyed at the same time within each week. Total number of roadkill for each taxon was recorded. We performed a vehicle survey driving 32-48 kph (20-30 mph) down each road 2-3 times a week. For each specimen found we recorded the location with a handheld GPS (Garmin Etrex), identified the species (if possible otherwise at least identified by vertebrate class), noted what side of the road it was found on and took a photograph. We included animals found on the verges since a vehicle may have injured them, but later succumbed to the injury. Animals detected crossing the road were also identified to species, and we noted the direction of movement and what road was crossed. Lastly, a handheld GPS was used to track the movement of the observer on each road surrounding the parks.

Environmental Variables of Roads

For all roads we measured environmental variables, which included: season, rainfall, humidity and temperature. May-June 20nd 2016 was considered spring, June 21st-September 22nd was considered summer and September 23rd-November 1st was considered fall. Rainfall in centimeters for each survey day was obtained from the NOAA weather data (https://www.ncdc.noaa.gov/). Humidity, as a percentage, was measured and recorded at the start and end of each survey using a handheld weather station (Burton Atmosphere Pro). Air temperature, in degrees Celsius, was measured each survey day using NOAA weather data and the handheld weather station device throughout the survey until the completion of a survey.

Spatial Factors and Structural Features of Roads

Spatial factors and structural features of roads were estimated for all of the roads, which included: canopy cover, vegetation cover, road features, land cover types, traffic volume and speed. Canopy cover was measured at fixed sampling points, every 0.48 kilometers of the road and within 6.5 meters of either side of the road. The measurement of 6.5 meters was based on the average measurement of all verges on the roads. Moreover, 6.5 meters was used since many verges merged onto private property. We created a canopy scale system using light meter estimates (Digital Lux Meter, Model: LX1330B). The scale system ranged from 0-5 with a score of a 0 being no canopy cover and a score of a 5 being dense canopy cover (Table 1; Figure 3). Canopy cover was measured for all roads in the months of April (spring), June (summer) and October (fall) to account for changes in canopy cover due to change of season. Vegetation cover at each sampling point was also scored on a scale from 0-5, with 0 representing bare ground vegetation and 5 equal to dense vegetation cover (Table 1; Figure 4).

Road features such as length and width of the road, verge (length and width) and verge slope were collected using a surveyor's wheel (Meter Man, Komelon Series 45). Slope refers to the tangent of the angle of that surface, which exist on hills or ditches. Verge is the narrow strip of grass or plants located between the roadway, curb or boundary of the road. Usually verges will end where forest and vegetation begins. Roadside ditches were noted for the presence of water, and if present, water depth was recorded (i.e. shallow (2.5 cm), mid-deep (15 cm), and deep (30 cm)). Wildlife road crossing signs and bridges were marked on all roads using GPS. Speed limits were recorded for each road as well as exact location of speed limit signs (latitude and longitude, GPS) to explore the relationship in amount of road kill with speed limit sign location and road speed. At fixed sampling points, every 0.48 kilometers along each road, we calculated what proportion of houses, agricultural fields, forest patches, grass patches and water were found within a 250m buffer on ArcGIS Software (version 11). Lastly, land cover types (i.e. turf, wet praire, residential, asphalt, water, oak savanna, shrub/scrub, swamp forest, conifers, upland forest, floodplain forest, barrens, eurassian meadow, prairie, cropland) were noted for each sampling point, every 0.48 kilometers along roads and road edges using a land cover map in ArcGIS (Schetter and Root, 2013). Land cover types were defined as follows: Turf was characterized as a grassy area. Wet prairie was characterized as lowland grassland that occurs on saturated or seasonally flooded steam and river floodplains or lake boundaries (Hewes, 1951). Asphalt was characterized as a mixture of dark colored sand or gravel, used to surface roads. Oak savanna was characterized as a rare area that is lightly forested with oak trees and is historically maintained by wild fires or grazing animals. Shrub/scrub land cover was characterized by bush and shrub plant communities, but could also contain grasses and herbs. Swamp forests were characterized as forests that were flooded with freshwater permanently or seasonally and normally occurred around rivers or lakes. Conifers forests were characterized as containing predominantly coniferous trees. Upland forests were characterized by hardwood trees and had a distinct over story of shade-tolerant deciduous trees with an understory of woody shrubs (Host, et al., 1987). Floodplain forests were characterized as deciduous forest communities that occupied areas adjacent to rivers or streams and endured occasional flooding cycles. Barrens were characterized by thin soil and sand and, in general, had little vegetation cover. Eurasian meadows were characterized as “mesic to dry cool-season grassland and old fields dominated by Eurasian species” (Schetter & Root, 2013). Tall grasses and mixed or short grasses depending on soil quality and rainfall were characterized as prairies. Lastly, croplands were characterized as areas of lands used for crop production and harvest.

To measure traffic volume, we conducted random traffic counts for each road by choosing three time periods during the day to count the number of cars that pass in a half hour time span (as in Langen et al. 2009). For each road, we measured traffic during two weekdays and one weekend day. We also obtained the average 24-hour traffic data was obtained from the Ohio Department of Transportation website (http://www.dot.state.oh.us/pages/home.aspx, 2017).

Finally, we did not remove the carcasses, but we noted how many days a body remained on the road or the immediate vicinity. Carcasses were marked by GPS and were spray painted green to mark the animal.

Visual Surveys within the Protected Areas

Daytime and nighttime walking surveys were conducted in Oak Opening Preserve and Maumee State Forest. Daytime surveys from mid mornings to afternoons. Nighttime surveys were conducted from 5:00 pm (EST) onward. We used these surveys to estimate animal distribution and movement patterns within the parks and identify which areas had a high density of animals with proximity to roadways. We selected the Boyscout trail to survey the entire area to of the park in relatively close proximity to roadways. This trail was split into two sections by the Wabash Cannonball Trail. The north portion of the Boyscout trail we will refer to as the Boyscout #1 North and the south portion of the Boyscout trail will be referred to as the Boyscout #2 South (Figure 5). Boyscout #1 North was roughly 14.48 km (9 miles) and Boyscout #2 South was approximately 14.8 km (9.2 miles). In Maumee State Forest, the ATV trail was surveyed, which was approximately 11.3 km (7 miles) (Figure 6).

Daytime and nighttime walking surveys were conducted at the same time for each week. For example, if at the start of the week a daytime survey began at 12:00pm then the same time was used for the other surveys of that week. For each trail, we noted the amount of time it took to walk the specific trail and maintained the same speed each time so that we could take into account sampling effort. The Oak Openings trail halves took approximately five hours each to complete. In Maumee State Forest the ATV trail took approximately 2 ½ hours to complete. Trails were rotated each survey to guarantee that each trail was completed at different times of the day.

The visual surveys included all terrestrial vertebrate taxa (i.e. amphibians, reptiles, birds and mammals) identified to the species level. When an animal was seen, we recorded the species, what direction it was moving, what portion of the park it was located, if it was near a roadway, what kind of habitat it was found in, anthropogenic noise (categorized as mild, medium or severe) and canopy/vegetation scores 0-5 (Table 1; Figure 3; Figure 4). One day and one night survey were conducted for each month from mid-March to the end of October 2016.

Environmental Variables for Visual Surveys

Temporal factors were measured during each visual survey. Rainfall in centimeters for each survey date was obtained from NOAA weather data (https://www.ncdc.noaa.gov/). Humidity, as a percentage, was measured and recorded periodically throughout each survey using a handheld weather station (Brunton Atmosphere Pro). Air temperature, in degrees Celsius, was measured each survey day using NOAA weather data and periodically throughout surveys using a handheld weather station. Lastly, a GPS (Garmin Etrex) was used to track the movement of the observer on each trail during visual surveys within the parks.

Spatial Factors and Structural Features for Visual Surveys

Spatial factors were estimated for all trails, which included: canopy cover and vegetation cover. Canopy cover was measured at fixed sampling points, every 500 meters of the trail and within 6 meters of either side of the trail path. The trail width varied in size with an average width of 6 meters. We used the average trail width for sampling to include the trail and along its edge. The same canopy cover scale system was used for the trails as in the road surveys (Table 1; Figure 3). Similar to road survey canopy cover measurements, a scale system from 0-5 was used with a score of a 0 representing no canopy cover and a score of a five representing dense canopy cover (Table 1). Also, ground vegetation cover was scored similar to road vegetation coverage at the sampling points with a score of a 0 representing bare ground and a score of a 5 representing dense vegetation (Table 1; Figure 4).

Lastly, land cover types (e.g. turf, wet praire, residential, asphalt, water, oak savanna, shrub/scrub, swamp forest, conifers, upland forest, floodplain forest, barrens, eurassian meadow, prairie, cropland) were noted for each sampling point using a land cover map in ArcGIS (Schetter and Root, 2013).

<u>*Statistical Analysis*</u>

Road Surveys

We created 250 m buffers around each fixed sampling location along each road and summed the total number of roadkill found within each buffer for all surveyed months combined. We chose 250 m because it was the halfway point between each survey point location. We used the spatial statistics toolbox and performed a Getis-Ord G_i* statistic on each 250 m buffer. This statistical analysis identified where clusters of high and low values of roadkill were located on each road. The test is defined as:

$$\sum G_i*(d) = \sum W_{ij}\ (d)\ X_j / X_j \qquad \text{Equation 1}$$

$G_i*(d)$ is the statistic for point i where with distance d from i, $W_{ij}(d)$ is the spatial weight matrix at distance d in dimensions i and j and X_j is the total roadkill found throughout all surveyed months. The analysis summed the values of the target point with its neighboring points and divided it by the sum of all the features. A z score was calculated where significant clusters were present in each 250 m buffer. For a significant z score value, the score varied more than 1.96 away from zero, corresponding to a confidence level of 95% (Mitchell 2005).

Environmental Variables of Roads

A nonparametric one-way analysis (Wilcoxon/Kruskal-Wallis Test) was used in JMP Statistical Software to test if the daily amount of roadkill per survey day was linked to the average rainfall, season, humidity and temperature per survey day.

Spatial Factors and Structural Features of Roads

To look for monthly influence of canopy and vegetation cover, we averaged the scores for each month within the 250m buffer of each fixed sampling point location. We summed the roadkill found within each buffer and identified the average canopy and vegetation score associated with that buffer (Figure 7). To test for spatial autocorrelation, we ran a Moran's I test on each 250 m buffer fixed sampling point for each surveyed month and noted if p-values were ≤ 0.05. We used a nonparametric one-way analysis (Wilcoxon Kruskal-Wallis test) in JMP to test for significant relationships. We analyzed each surveyed month separately (June-October 2016).

To examine the relationship between roadkill and the presence of road structural features such as vertical road signs, road verge slope (downhill or uphill), houses, agricultural fields, grassy fields, water and forests we used logistic regression in JMP. We analyzed each surveyed season separately (e.g. spring was May 1st-June 21st, summer was June 22nd to September 21st and fall was September 22nd to October 31st) and the number of roadkill within each season. We used a Spearman's rank correlation coefficient in JMP for a nonparametric measure of statistical dependence amoung all structural features and eliminated structural features that were highly correlated with one another (ρ=>0.7). Lastly, we used stepwise logistic regression to test the relationship between roadkill and combinations of structural features in JMP.

Within our 250 m buffers we identified structural features within its borders. We computed a principal components analysis for total number of roadkilled mammals across all surveyed months in JMP and examined their relationship with structural features (e.g. houses, water, agricultural fields, grass fields, forests, speed signs, wildlife road crossing signs) found within a 250 m buffers. Similarly, we computed a principal components analysis for total number of roadkilled birds across all surveyed months in JMP and examined their relationship with structural features within a 250 m buffers. Roadkilled reptiles and amphibians had too few roadkill numbers to compute a principal components analysis.

We assessed the association among roadkilled vertebrates species and the presence of the following land cover type within 250 m buffers of the fixed sampling points: turf, wet praire, residential, asphalt, water, savanna, shrub/scrub, swamp forest, connifers, upland forest, floodplain forest, barrens, eurassian meadow, prairie and cropland (Schetter and Root, 2013). We ran Moran I test on each 250 m buffer fixed for all seasons combined and noted if p-values were $\leq$0.05. We used a logistic regression comparing the relationship of each land cover type within each buffer to total roadkill within each buffer in JMP. Next, we used a Spearman's rank correlation coefficient in JMP for a nonparametric measure of statistical dependence among all land cover types and eliminated land cover types that were highly correlated with one another (ρ=>0.7). We used a stepwise logistic regression to test the relationship of roadkill with combination of land cover types in JMP. We analyzed all seasons combined (e.g. spring, summer, fall). We computed a principal components analysis in JMP for total number of roadkilled mammals and examined their relationship with land cover types within 250 m buffers of the fixed sampling pont locations we stopped at along each road. Similarly, we computed a principal components analysis in JMP for total number of roadkilled birds and examined their relationship with land cover types within the 250 m buffers. Again, roadkilled reptiles and amphibians had too few roadkill numbers to compute a principal components analysis.

We also assessed if certain land cover types (e.g turf, wet praire, residential, asphalt, water, oak savanna, shrub/scrub, swamp forest, conifers, upland forest, floodplain forest, barrens, eurassian meadow, prairie, cropland) along the edge of the road were related to roadkilled vertebrates. In ArcGIS we created a 250 by 100 m rectangular window within our 250 m buffers to identify land cover types within its borders. We used 100 m because edge effects often extend up to 50 m in fragmented landscapes and we wanted to account for both sides of the road (Murcia, 1995). Within our 250 by 100 m rectangular window, we evaluated what land cover types were present on road edges and the total number of roadkill per tax within each rectangular window. Using a logistic regression in JMP, we evaluated if land cover types along the edge were associated with certain roadkilled taxa. Next, we used a Spearman's rank correlation coefficient in JMP for a nonparametric measure of statistical dependence among all land cover types along road edges and eliminated land cover types that were highly correlated with one another (ρ=>0.7). We used a stepwise logistic regression to test the relationship of roadkill associated with combinations of land cover types on road edges in JMP. We computed a principal components analysis in JMP for total number of roadkilled mammals and examined their relationship with land cover types along the edge of the road within the 250 m by 100 m rectangular window. Lastly, we computed a principal components analysis in JMP for total number of roadkilled birds and examined their relationship with land cover types along the edge of the road within 250 m by 100 m rectangular window. Roadkilled reptiles and amphibians had too few roadkill numbers to compute a principal components anaysis.

We evaluated if all structural features along each road (e.g. vertical road signs, houses, average canopy score across all surveyed months, average vegetation score across all surveyed months, land cover types) were associated with terrestrial vertebrate diversity. Again, we used a 250 by 100 m rectangular window in ArcGIS to identify the presence of structural features and vertebrate taxa within its borders. We used an stepwise logistic regression to test the relationship of animal diversity associated with the various structural features across all months.

Lastly, we anlayzed traffic on each road to see if they were correlated with one another. Next, we analysed traffic volume, speed, road width and length to evaluate the influence on the amount of roadkill found daily across all surveyed months. To do so we calculated the number of vehicles per kilometer and roadkill per kilometer per surveyed road. We used a nonparametric one-way analysis (Wilcoxon Kruskal-Wallis test) in JMP to test for significant relationships.

Visual Surveys within the Protected Areas

From our visual survey data, we analyzed what direction each animal was moving by calculating what percentage of animals were moving in cardinal and intracardinal points (i.e. N, E, S, W, NE, NW, SE, and SW) in Oak Openings Preserve and Maumee State Forest.

Environmental Variables for Visual Surveys

To test if animal movement within the parks was linked to the environmental variables of daily rainfall, humidity and temperature, we used a nonparametric one-way analysis (Wilcoxon/Kruskal-Wallis Test) of the total number of each taxa within both preserve per survey day across all trails in JMP. We were unable to test if animal movement was linked to monthly changes of environmental variables since we only surveyed twice a month.

Spatial Factors and Structural Features for Visual Surveys

Next, we created a 100 m buffer in ArcGIS around each canopy and vegetation fixed sampling point along each trail in Oak Openings Preserve and Maumee State Forest. We used a 100 m buffer around each canopy and vegetation point location because edge effects often extend up to 50 m in fragmented landscaped and we wanted to account for both sides of the trail (Murcia, 1995). Within each 100 m buffer we noted the presence of structural features (e.g. average canopy and vegetation score of all surveyed months combined), land cover types, and terrestrial vertebrate taxa. Next, we used a logistic regression to test the relationship between structural features and land cover types to terrestrial vertebrate diversity along the trails, similar to the roadkill analysis. After, we used a Spearman's rank correlation coefficient in JMP for a nonparametric measure of statistical dependence among structural features and land cover types and eliminated those that were highly correlated (ρ=>0.7). Lastly, we used a stepwise logistic regression to assess whether taxa presence was associated with the various structural features and land cover types along the trails.

Results

Road Surveys

In total, 292 vertebrates were found dead on roads surrounding Oak Openings Preserve and Maumee State Forest, including more mammals than any other taxa (Figure 8). A total number of 255 mammals, 26 birds, 7 reptiles and 4 amphibians were found dead on roads from May-October (Table 2). This represented an average of 7 dead animals per 1 km of road over that time period. We found that 44% of the roadkill was located on one roadway along the NW portion of Oak Openings preserve, Waterville Swanton Rd., which is likely to be a corridor for animal movement (Figure 9). Also, we identified 3 clusters of high values from the Getis-Ordinal Gį* test that were significant at the 99% confidence level ($z > 1.96$) and 2 clusters of high values from the Getis-Ordinal Gį* test at the 95% confidence level ($z> 1.96$) suggesting these clusters are areas of frequent terrestrial vertebrate road mortality. All 5 clusters were located on Waterville Swanton Rd. There were two clusters at the 90% confidence level that were located on Waterville Swanton Rd. and Berkley Southern Rd. (Figure 10). The number of roadkill on roadways surrounding Oak Openings Preserve was significantly greater than near Maumee State Forest (Wilcoxon, $p<0.01$). Lastly, June had the highest number of roadkill (Figure 8).

Environmental Variables of Roads

Daily rainfall, temperature and humidity did not significantly influence the amount of roadkill. However, roadkill numbers appeared to track temperature; numbers decreased, but not significantly, with decreasing temperatures (Figure 11).

Spatial Factors and Structural Features of Roads

The spatial autocorrelation (Moran's I) test revealed no spatial autocorrelation on each 250 m buffer fixed sample point location. Roadkill increased with low or intermediate levels of vegetation cover (average scores 1 and 2) present on road verges in June, July, August, September and October (Wilcoxon, $p \leq 0.01$). May was excluded because vegetation and canopy was not measured for that month. Similarly, roadkill increased with low or intermediated levels (average scores 1 and 2) or no canopy (average score 0) present along roadways in all surveyed months, June-October 2016 (Wilcoxon, $p \leq 0.01$).

Next, we analyzed average canopy and vegetation scores along the road associated with different taxa in spring, summer and fall. For mammals, the number of roadkill increased with low or intermediate levels (average score 1 and 2) or no vegetation (average score 0) present along road verges for all surveyed months (Wilcoxon, $p \leq 0.01$). Similarly, for birds the number of roadkill increased with low or intermediate levels (average scores 1 and 2) or no canopy (average score 0) present along roadways for spring and summer (Wilcoxon, $p \leq 0.04$), but there was no significant relationship in the fall. There were too few roadkilled reptiles and amphibians to test the relationship of average vegetation and canopy score with road mortality (Table 3).

We looked at the relationship between roadkilled terrestrial vertebrates and the presence of structural features (e.g. vertical road signs, verge slope, house, bridges, agricultural fields, grassy fields, forests and water) along roads for each season. In spring months, the presence of agricultural fields, corresponded with an increased mammal road mortality (Chi-Square, $p<0.01$). However, there were no other significant influences of these structure features for spring months for all other taxa (Table 4).

In summer months, mammal road mortality increased in the presence of houses and agricultural fields (Chi-square, $p \leq 0.04$). The presence of vertical signs corresponded with a significant increased bird road mortality (Chi-square $p < 0.02$). Structural features did not have significant influence for summer months for all taxa (Table 4).

In fall months, houses and agricultural fields were significantly positively related to mammal road mortality (Chi-Square, $p \leq 0.01$). No other taxa showed a significant influence of structural features on road mortality in fall months (Table 4).

The principal component analysis of the relationship between total number of roadkilled mammas and structural features across all months within the 250 m buffer revealed a principal component of 1 that explained 27% and was positively associated with grassy fields, while principal component 2 explained 21.1% and was positively associated with houses (Figure 12; Figure 13; Table 7) The principal component analysis for total number of roadkilled birds associated with structural features across all months and within the 250 m buffers revealed a principal component 1 that explained 28.8% and was positively associated with wildlife road crossing signs and speed signs (vertical road signs in general), while principal component 2 explained 24.1% and was positively associated with houses (Figure 14; Figure 15; Table 8).

We examined if the presence of land cover types around each fixed point location influenced road mortality for all surveyed months combined. The spatial autocorrelation test revealed no spatial autocorrelation among each 250 m buffer fixed sample point location. Increased numbers of roadkilled mammals were associated with turf, savanna, and upland forest (Chi-Square, ≤ 0.03). For birds, the presence of floodplains was positively associated with roadkill (Chi-Square, $p < 0.04$). No other land cover types showed any influence on road mortality of vertebrate taxa (Table 5). The principal component analysis for total number of roadkilled mammals associated with land cover types revealed a principal component 1 that explained 16.7% and was positively associated with upland forests, while principal component 2 explained 14.3% and was positively associated with savanna and prairie but negatively associated with croplands (Figure 16; Figure 17; Table 9). The principal component analysis for total number of roadkilled birds associated with land cover types revealed a principal component 1 that explained 18.7% and was positively associated with swamp forests, while principal component 2 explained 16.5% and was positively associated with savanna, but negatively associated with asphalt (Figure 18; Figure 19; Table 10).

We also examined if the presence of land cover types along the edge of the road influenced the number of roadkilled vertebrates. Bird road mortality was associated with cropland along the edge of the road (Chi-Square, $p<0.01$). No other taxa or land cover types along the edge of the road had a significant relationship. The principal component analysis for total number of roadkilled mammals associated with land cover types along the road edge revealed a principal component 1 that explained 17.6% and was positively associated with floodplains, while principal component 2 explained 11.6% and was positively associated with asphalt (Figure 20; Figure 21; Table 11). The principal component analysis for total number of roadkilled birds associated with land cover types along the edge of the road revealed a principal component 1 that explained 19.4% and was positively associated with prairies, while principal component 2 explained 14% and is positively associated with turf (Figure 22; Figure 23; Table 12).

We also found no relationship between structural features and animal roadkill diversity (all taxa).

Similarly, we found no significant relationship between roadkill per kilometer per road and road width, vehicles per kilometer or road speeds for all surveyed months for any taxa. Traffic between roads was not significantly correlated.

Visual Surveys within the Protected Areas

In total, 865 vertebrates were seen within both parks during visual surveys. Specifically, 271 mammals, 563 birds, 20 amphibians and 11 reptiles were observed overall (Table 2). This represents an average of 21 animals seen per 1 km of trail. During our visual surveys, 56% of animals were seen moving in the NW and SW direction within the Oak Openings Preserve. The NW and SW area of the preserve runs parallel to Waterville Swanton Rd., which had the highest amount of roadkill than any other surveyed road (44%). In Maumee State Forest, 46 % of animals were seen moving in the NW and SW direction. In the NW and SW area of Maumee State Forest is another large protected area. This area was not surveyed.

We did not find that animal diversity along the trails was significantly related to structural features in either park.

Lastly, animal movement within the parks was not influenced by environmental variables of daily rainfall, humidity and temperature.

Our models that explored combinations of variables had very little explanatory power (e.g. r^2 ranged from 0.05-0.47) and few or no significant relationships, which suggest a very complex response to factors across species or one that was not detectable with our data (Table 6).

Discussion

Road Surveys

By analyzing environmental variables, spatial factors and structural features of roads, our study assessed the effects roads and traffic had on terrestrial vertebrate taxa. Similarly, by using roadkill as a lens and performing visual surveys within protected areas, we were able to better understand vertebrate movement patterns in a highly fragmented human-dominated landscape. Throughout our study, 292 vertebrates were found dead on roads surrounding Oak Openings Preserve and Maumee State Forest, including more mammals than any other taxa. Specifically, mesopredators (e.g. raccoons, opossums, and skunks) were found dead in the highest numbers. According to Frey and Conover (2006), many mesopredators tend to prefer to live near roads, levees or other linear features in the landscape if the habitat around them remains suitable (Frey and Conover, 2006). Since the Oak Openings Region consists of many different habitat types, mesopredators may take advantage of the different resources available to them in the area. Also, roads in general provide many resources including spilled grains, roadsides plants, insects, other basking vertebrates, roadkill, agricultural fields and road salt that may attract mesopredators since they are scavengers (Smith-Patten and Patten, 2008). Likewise, the Oak Openings Region consists of many urban and suburban areas in which mesopredators are known to take advantage of artificial food resources that are relatively reliable and spatially stable (Prange, et al., 2004).

There were a small number of vertebrates such as birds, amphibians and reptiles discovered during roadkill surveys. This may be influenced by our sampling technique of vehicle surveys. However, in a study conducted by Garriga et al. (2012), both vehicle surveys reaching 20 km/h and walking surveys were used to detect roadkill. They discovered that 7-67% of the total number of road victims were misdetected in foot surveys (Grigga et al, 2012). Also, smaller vertebrates may be removed from the road by other scavenging species or by traffic volume. According to Ratton et al. (2014), carcass permanency on the road depends on three main factors, which include: scavengers, weather and traffic volume. All three factors can influence roadkill removal differently depending on the time at which the roadkill occurs. In the study conducted by Ratton et al. (2014), 92% of installed carcasses were removed within 42 hours of the experiment, and 89% were removed within the first 24 hours. These results suggest that in order to correctly estimate roadkill rates, there is need to consider carcass removal from scavengers, weather and traffic volume (Ratton et al., (2014). Within our study area, majority of smaller vertebrates (e.g. chipmunks, squirrels, amphibians) were observed to be removed from the road by scavengers within one day of vehicle collisions. Larger vertebrates, such as raccoons, were observed to stay on the road as long as 23 days. However, due to a small sample size of birds, amphibians and reptiles, we cannot accurately evaluate differences on carcass removal rates between taxonomic groups or seasons, so further study is needed. Therefore, our results suggest that our roadkill numbers are minimum estimates since we are likely to have undercounted due to carcass removal from scavengers, weather and traffic volume.

As we predicted, 44% of all roadkill occurred on Waterville Swanton Road, which suggests that this road was a likely corridor between the Oak Openings Preserve and Maumee State Forest. Both protected areas are the largest in the region; therefore vertebrates may risk crossing the road to access resources available in other parks. Also, these two protected areas are sporadically managed to mimic historic disturbances that were essential to the Oak Openings Region (Albell et al., 2004). Consequently, vertebrates may disperse to take advantage of or to avoid a newly disturbed and depopulated area and may risk crossing the road (Howard, 1960). Based on our visual surveys within the Oak Opening Preserve, we detected more vertebrates moving in the NW and SW direction of the park, which is adjacent to Waterville Swanton Road. For this reason, Waterville Swanton Rd. may have had the highest number of roadkilled vertebrates. Also, roads around Oak Openings Preserve had a significantly greater number of dead vertebrates than roads near Maumee State Forest. Previous work suggests that there is a greater abundance of animals in the Oak Openings Preserve than Maumee State Forest, although further research is needed to confirm this. The Oak Opening Preserve is a mosaic of many different habitat types, including early succession habitat, while Maumee State Forest is more forested and fragmented, so there may be different resources available between the two protected areas that foster movement of vertebrates. The roadkill hotspot analysis also revealed that three out of the four roadkill hotspots were located on Waterville Swanton Rd., which further confirms that this area is a likely corridor for animal movement.

The month of June had the highest amount of roadkill than any other surveyed month, with more mammals than any other taxa (Figure 8). This was most likely a result of changes in the activities of mammal populations, e.g. reproduction (Merritt et al., 2001). Also, June is a time when juvenile animals may become independent, and disperse further in search of resources or mates, resulting in a greater probability of crossing a road. Birds, reptiles and amphibians had low roadkill sample sizes, so we could not accurately assess which months would have the highest roadkill numbers. However, according to a vertebrate roadkill study conducted by Coelho and Kindel (2008), summer months had the highest numbers of roadkilled birds and reptiles. Similar to mammals, bird activity may be highest in these months due to breeding and dispersal activities, which has been linked to peaks in road mortality. Since reptiles are ectotherms, warmers months increase activity and movement; therefore more reptiles may risk crossing the road risking vehicle collisions (Coelho and Kindel, 2008).

Environmental Variables of Roads

We did not find a significant influence on daily roadkill by daily average rainfall, temperature and humidity. However, the influence of environmental variables on roadkill seems to vary by climatic zones. A study conducted by Garriga et al. (2017) within Catalonia found that roadkill numbers seemed to track rainfall and humidity, in which numbers would peak during rainfall events and decrease during times of drought (Garriga et al, 2017). However, Catalonia has high climatic variability, with annual rainfall ranging from 400 -1,000 mm and temperatures ranging from 16°C to 26°C. Within our study, the total amount of rainfall across our surveys was 4.3 cm and daily average temperatures reached a maximum of 33 °C and a low of 8°C. Our study contradicted Garriga et al. (2017) in which roadkill numbers appear to track temperature, although not significantly. These results suggest that temperature, humidity and rainfall may affect each taxa or species differently due to life history or phenology and these effects may vary by location. For example, in a roadkill study conducted by D'Amico et al. (2015) in Doñana Natural Area characterized by a Mediterranean climate, higher temperatures were associated with an increase in reptile road morality, but the opposite pattern occurred in amphibians. They also found that an increase in precipitation was associated with an increase in roadkilled birds (D'Amico et al., 2015).

Spatial Factors and Structural Features of Roads

We found that an increase in roadkill was associated with low to intermediate levels of vegetation cover on road verges in June, July, August, September and October. Low levels of vegetation cover may promote movement since it may be less of a barrier and easier for an animal to move through. Vegetation structure and cover can allow an animal to remain hidden from a predator and can influence escape decisions and perceived risk across many taxonomic groups (Camp et al., 2012). In a study conducted by Camp et al. (2012) analyzing pygmy rabbits, concealment by vegetation structure was the most important functional property that influenced perceptions of predation risk, and flight initiation distance was lowest with maximum concealment and minimum visibility (Camp et al, 2012). So, if there is thicker vegetation along road verges, animals may not risk crossing the road since the habitat is suitable and provides protection against predators, like red-tailed hawks or coyotes which are edge-specialists, or it may be difficult to travel through.

It seems that certain structural features present along the roads (i.e vertical road signs, verge slope, house, agricultural fields, grassy fields, forests and water) were associated with roadkilled vertebrates, but the effects varied by season. However, for mammals, across all seasons, road mortality was associated with the presence of agricultural fields present along the roads. Agricultural fields can be an attractant for many species, providing food or refuge. According to Hodara and Busch (2006), agricultural fields can provide high food resources and shelter during high plant growth and cover periods (Hodara and Busch, 2006). Also, the presence of houses along the road positively influenced mammal road mortality in spring and summer months (Chi-square, $p \leq 0.01$). As previously stated, mesopredators comprised the largest number of roadkill, and these scavenging species may have been attracted to houses because of artificial food resources that humans provide that are relatively reliable and spatially stable (Prange, et al., 2004). Lastly, there was an increase in road morality of birds associated with the presence of vertical road signs along the road in summer months. The principal component analysis also suggested that roadkilled birds were positively associated with vertical road signs *(*i.e. wildlife road crossing signs and speed signs). These results were similar to the results from a study conducted by D’Amico et al. (2015), in which they also found that the presence of vertical road signs was associated with an increase in bird road mortality (D'Amico et al., 2015). Spring and fall months had the lowest number of roadkilled birds, so the relationship of roadkilled birds should be interpreted with caution because of a low sample size (Figure 8).

The presence of land cover along the road was related to road mortality for all surveyed months combined, but the effects varied among taxa. Turf, savanna and upland forest were positively associated with mammal road mortality. Again, mammals had the highest road mortality, and many mammal species roadkilled were generalist species (e.g. raccoon, opossum, and skunk) that can survive in many different habitats. Upland forests provide many different resources and can include different types of trees and dense canopy, which offer shelter for generalist species. Turf generally runs along the edge of the road, and has been known to provide habitat for insect species that generalist species may prey upon (Altamirano et al., 2016). Savannas provide variable environments, with very little tree canopy, but a highly diverse understory, which can provide refuge, shelter and food resources for generalist species. Therefore, mammals may risk crossing the road to access these different land cover types.

Bird road mortality was associated with the presence of flood plains along the road. Birds may be attracted to floodplains because they provide fertile soils for plan growth and the richest habitats for wildlife. Floodplains can host food resources for birds, with a diversity of insects, resting areas for migrating birds and act as a water source (Anonymous, 2017). Previous research by USGS in the Upper Mississippi River found that mature floodplain forests are critical habitat for breeding birds, many that are a species of concern to the U.S. Fish and Wildlife Service found in which we also have in the Oak Openings Region (e.g. Bald eagles (*Haliaeetus leucocephalus*), Red-shouldered hawks (*Buteo lineatus*) and cerulean warblers

(*Dendroica cerulean*); Swenson and Nelson, 2002). Therefore, birds may risk crossing the road to access flood plain locations in Oak Openings Preserve and Maumee State Forest.

We also examined the influence of the land cover found on the immediate edge along the roads on the influence on vertebrate roadkill. Roadkilled birds were more likely to be found where there was cropland along the edge of the road. According to Filloy and Bellocq (2007) mix-farming scenarios play an important role in seasonal population dynamics in birds, and birds may favor particular components of croplands, which can provide food, shelter or nesting sites. However, different species of birds can respond to croplands positively or negatively and bird species may differ in their sensitivity to crop-production (Filloy and Bellocq, 2007). Therefore, birds may risk crossing the road to access croplands, increasing the chance of vehicle collisions. Croplands may be attractive to some bird species but not others, but we did not analyze separate species because of a small sample size. Also, the principal components analysis revealed that bird road mortality was positively associated with prairies along the edge of the road. Many grassland birds may use prairies as a source of refuge, shelter and food resources and therefore may be an attractant for some species, which can increase collisions with vehicles.

We did not find a significant relationship between the animal diversity found along roads and structural features (e.g. vertical road signs, houses, average canopy cover, average vegetation cover, land cover) because the response of individual species to these features may vary tremendously and different taxa may be drawn to different resources. Our results suggest that there was no single variable that explained the diversity of animals found in a specific location. According to Garriga et al. (2012), road mortality does not affect all taxonomic groups in the same way, and it is difficult to identify specific variables that influence road mortality in all taxonomic groups (Garriga et al., 2012). Similarly, our models with combinations of variables had very little explanatory power and suggest a very complex response to factors temporally and across species.

Finally, roadkill per kilometer per road was not related to road widths, vehicles per kilometer or road speeds for all surveyed months. This is in contrast to other studies that found road width, traffic and speed to be associated with road mortality. For example, in a study conducted by Jaeger et al, (2005) in which they analyzed the effects of roads and traffic on animal populations by creating models, it was discovered that small roads (one lane in each direction) or large roads (two or more lanes in each direction) with high traffic intensity resulted in higher road avoidance in animal populations. So, roads that have less traffic, with less noise pollution may increase animal vehicle collisions (Jaeger et al, 2005). Also, according to Trombulak and Frissell (2000) in a review of the ecological effects of roads, high speed roads (96 -120 kph) usually are associated with less roadkill than on medium-speed roads (72-88 kph) because high speed roads have cleared vegetation along the edge, which creates less attractive habitat and greater visibility for both animals and drivers. In our study area, road speed limit was between 80 kph (50 mph) and 88 kph (55 mph) for all roads, with as low as 12 to as high as 1,092 vehicles per hour. Roads with high traffic density (1,092 per hour) had low total roadkill numbers (32 dead animals) whereas roads with medium traffic density (250 per hour) had the highest total roadkill numbers (128 dead animals). Our results are similar to the results of Jaeger et al, (2005) and Trombulak and Frissell (2000) since high traffic roads had fewer roadkill suggesting higher road avoidance and medium speed roads had higher roadkill numbers than high speed roads.

Visual Surveys within the Protected Areas

During our visual surveys, 56% of animal movement was primarily seen in the NW and SW portion of the Oak Opening preserve, which runs parallel to Waterville Swanton Road. Waterville Swanton Rd. had the highest number of roadkilled vertebrates, which suggest that animals may risk crossing the road in order to access Maumee State Forest, which is in the SW direction. Therefore, Waterville Swanton Rd. may be a corridor for animal movement. Also, the hotspot analysis revealed that three out of the four roadkill hotspots were on Waterville Swanton Road, confirming that many animals cross this road. In Maumee State Forest, 46% of animal movement was primarily in the NW and SW area of the preserve. In the NW and SW direction of Maumee State Forest, there was a large patch of protected natural area, so animals could be moving to access other resources in this location. However, these areas were not surveyed.

As we found in our road surveys, within the parks there were no significant relationships between structural features and detected animal diversity. Since different taxa may be drawn to different resources, it is not surprising that no single variable is clearly associated with all taxa in protected areas. Our models with combinations of variables had very little explanatory power and suggest a very complex response to factors temporally and across species. More research is needed to further identify features that are important for the individual groups or species.

Lastly, animal movement within the parks was not influenced by environmental variables of daily rainfall, humidity and temperature. This may be because we did not survey enough to see daily or monthly relationships of animal movement to environmental variables. Within out study, the climate was quite variable with temperatures as high as 33 °C and as low as 8 °C, and rainfall across all surveys totaled 4.3 cm, which could be why there were no significant relationships. However, other studies suggest that animal movement is related to environmental variables. For example, a study conducted by Webb et al. (2009) in Oklahoma found that monthly rainfall was linked to an increase in movement of white-tailed deer because of increased abundance of forage (Webb et al., 2009). Another study conducted by Vasconcelos and Calhoun, (2004) in Maine found that amphibian movement has been shown to increased with increasing temperatures and precipitation (Vasconcelos and Calhoun, 2004). Lastly, a study conducted by Shepard et al, (2008) in Illinois found that turtle and snake movements increased with precipitation and higher temperatures. Therefore, we may need to survey more often to understand if animal movement within our study area is related to environmental variables or sample throughout the whole year to get a full range of temperatures.

The stepwise models with combinations of variables had very little explanatory power and suggest a very complex response to factors and across taxa, which could be caused by several reasons. First, there could be high variation of structural features and land cover types between roads and trails that we did not successfully capture within the study areas. Second, we may have needed to sample more often along roads and trails for certain variables, like canopy or ground vegetation cover. Third, the scale at which we sampled may have not been able to accurately capture a combination of variables to understand animal movement and road mortality. Lastly, sample sizes of certain variables or taxa may have been too small to accurately assess animal movement and road mortality across the landscape. Therefore, while some significant relationships were detected caution is warranted, as there is temporal and spatial complexity within these two protected areas, which is typical of human-dominated landscapes.

Conclusion and Management Implications

Our results indicate that in order to understand animal movement and road mortality it is important to incorporate road and visual surveys as well as consider environmental variables, spatial factors and structural features of roads. In this study, we used roadkill as a lens to better understand vertebrate movement and mortality in a highly fragmented human dominated landscape. Based on our results, there is no single variable that influences animal movement and mortality, and the influence of variables change over time and space as well as among terrestrial vertebrate taxa. However, management can alter structural features of roads to reduce road morality and animal movement. For example, allowing dense vegetation or canopy along roads may reduce animal-vehicle collisions. Denser vegetation along roads may provide more suitable habitat and protection against predators, therefore decreasing animal movement and road crossing events. Denser canopy along roads has also been linked to a decrease in roadkill because denser canopy allows arboreal movements to cross the road (Chen and Koprowski, 2016). Likewise, mangers can identify structural features that may deter species presence or road crossings to prevent animal-vehicle collisions. Lastly, we identified certain land cover types along the edge of the road and within the reserves that animals may move to and risk crossing the road. Managers can identify the locations of these land cover types and can create wildlife crossing structures, (e.g. culverts, land bridges) or wildlife road crossing signs to allow for successful animal road crossing and increase human awareness. Our approach is useful for predicting animal movement and identifying structural features that may be managed to reduce road mortality, and this approach is likely to be applicable to other protected areas in human-dominated landscapes.

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Literature Cited

Albella, S. R., Jaeger, J. F., & Brewer, L. G. (2004). Fifteen years of plant community dynamics during a northwest Ohio oak savanna restoration. *The Michigan Botanist*, *43*(2).

Altamirano, A., Valladares, G., Kuzmanich, N., & Salvo, A. (2016). Galling insects in a fragmented forest: incidence of habitat loss, edge effects and plant availability. *Journal of insect conservation*, *20*(1), 119-127.

Andrews, K. M., Gibbons, J. W., Jochimsen, D. M., & Mitchell, J. (2008). Ecological effects of roads on amphibians and reptiles: a literature review. *Herpetological Conservation*, *3*, 121-143.

Anonymous, (2017). "Benefts of Floodplain by Design." The Nature Conservancy. Retrieved from: https://www.nature.org/ourinitiatives/habitats/riverslakes/benefits-ofhealthy-floodplains.xml

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

Beebee, T. J. (2013). Effects of road mortality and mitigation measures on amphibian populations. *Conservation Biology*, *27*(4), 657-668.

Brewer, L. G., & Vankat, J. L. (2004). Description of Vegetation of the Oak Openings of Northwestern Ohio at the Time of Euro-American Settlement1. *The Ohio Journal of Science*, *104*(4), 76.

Brewer, L. G., & Vankat, J. L. (2006). Richness and diversity of oak savanna in northwestern Ohio: proximity to possible sources of propagules. *The American midland naturalist*, *155*(1), 1-10.

Burnham, K. P., & Anderson, D. R. (2004). Multimodel inference understanding AIC andBIC in model selection. *Sociological methods & research*, *33*(2), 261-304.

Camp, M. J., Rachlow, J. L., Woods, B. A., Johnson, T. R., & Shipley, L. A. (2012). When to run and when to hide: the influence of concealment, visibility, and proximity to refugia on perceptions of risk. *Ethology*, *118*(10), 1010-1017.

Chen, H. L., & Koprowski, J. L. (2016). Barrier effects of roads on an endangered forest obligate: influences of traffic, road edges, and gaps. *Biological Conservation*, *199*, 33 40.

Coelho, I. P., Kindel, A., & Coelho, A. V. P. (2008). Roadkills of vertebrate species on two highways through the Atlantic Forest Biosphere Reserve, southern Brazil. *European Journal of Wildlife Research*, *54*(4), 689.

Coffin, A. W. (2007). From roadkill to road ecology: a review of the ecological effects of roads. *Journal of transport Geography*, *15*(5), 396-406.

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.

Cuyckens, G. A. E., Mochi, L. S., Vallejos, M., Perovic, P. G., & Biganzoli, F. (2016). Patterns and Composition of Road-Killed Wildlife in Northwest Argentina. *Environmental management*, *58*(5), 810-820.

D'Amico, M., Román, J., de los Reyes, L., & Revilla, E. (2015). Vertebrate road-kill patterns in Mediterranean habitats: Who, when and where. *Biological Conservation*, *191*, 234-242.

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.

Filloy, J., & Bellocq, M. I. (2007). Patterns of bird abundance along the agricultural gradient of the Pampean region. *Agriculture, ecosystems & environment*, *120*(2), 291-298.

Forman, R. T. (1998). Road ecology: a solution for the giant embracing us. *Landscape Ecology*, *13*(4), III-V.

Frey, S. N., & Conover, M. R. (2006). Habitat use by meso-predators in a corridor environment. *Journal of Wildlife Management*, *70*(4), 1111-1118.

Gagné, S. A., Bates, J. L., & Bierregaard, R. O. (2015). The effects of road and landscape characteristics on the likelihood of a Barred Owl (Strix varia)-vehicle collision. *Urban Ecosystems*, *18*(3), 1007-1020.

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*(4), 874-889.

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 and Conservation*, *21*(11), 2761-2774.

Gibbs, J. P. (1998). Amphibian movements in response to forest edges, roads, and streambeds in southern New England. *The Journal of Wildlife Management*, 584-589.

Glista, D. J., DeVault, T. L., & DeWoody, J. A. (2008). Vertebrate road mortality predominantly impacts amphibians. *Herpetological Conservation and Biology*,

Grigore, M. T. (2004). *Living in the Oak Openings: a homeowner's guide to one of the world's last great places*. Homewood Press.

Grilo, C., Ferreira, F. Z., & Revilla, E. (2015). No evidence of a threshold in traffic volume affecting road-kill mortality at a large spatio-temporal scale. *Environmental Impact Assessment Review*, *55*, 54-58.

Hewes, L. (1951). The northern wet prairie of the United States: Nature, sources of information, and extent. *Annals of the Association of American Geographers*, *41*(4), 307-323.

Higgins, J.B (2003). Emergy analysis of the Oak Openings region. *EcologialEngineering*,21(1), 75-109.

Hobday, A. J., & Minstrell, M. L. (2008). Distribution and abundance of roadkill on Tasmanian highways: human management options. *Wildlife Research*, *35*(7), 712 726.

Hodara, K., & Busch, M. (2006). Return to preferred habitats (edges) as a function of distance in Akodon azarae (Rodentia, Muridae) in cropfield-edge systems of central Argentina. *Journal of ethology*, *24*(2), 141-145.

Host, G. E., Pregitzer, K. S., Ramm, C. W., Hart, J. B., & Cleland, D. T. (1987). Landform mediated differences in successional pathways among upland forest ecosystems in northwestern Lower Michigan. *Forest Science*, *33*(2), 445-457.

Howard, W. E. (1960). Innate and environmental dispersal of individual vertebrates. *American Midland Naturalist*, 152-161.

Jaeger, J. A., Bowman, J., Brennan, J., Fahrig, L., Bert, D., Bouchard, J., & von Toschanowitz, K. T. (2005). Predicting when animal populations are at risk from roads: an interactive model of road avoidance behavior. *Ecological modelling*, *185*(2), 329348.

Keken, Z., Kušta, T., Langer, P., & Skaloš, J. (2016). Landscape structural changes between 1950 and 2012 and their role in wildlife–vehicle collisions in the Czech Republic. *Land Use Policy*, *59*, 543-556.

Langen, T. A., Ogden, K. M., & Schwarting, L. L. (2009). Predicting hot spots of herpetofauna road mortality along highway networks. *The Journal of Wildlife Management*, *73*(1), 104-114.

Merritt, J. F., Lima, M., & Bozinovic, F. (2001). Seasonal regulation in fluctuating small mammal populations: feedback structure and climate. *Oikos*, *94*(3), 505-514.

Mitchell, A. (1999). *The Esri guide to GIS Analysis: geographic patterns & relationships* (Vol. 1). ESRI, Inc.

Murcia, C. (1995). Edge effects in fragmented forests: implications for conservation. *Trends in ecology & evolution*, *10*(2), 58-62.

Niemi, M., Jääskeläinen, N. C., Nummi, P., Mäkelä, T., & Norrdahl, K. (2014). Dry paths effectively reduce road mortality of small and medium-sized terrestrial vertebrates. *Journal of environmental management*, *144*, 51-57.

NOAA, (2017). Retrieved from: https://www.ncdc.noaa.gov/

Obbard, M. E., & Brooks, R. J. (1980). Nesting migrations of the snapping turtle (*Chelydra serpentina*). *Herpetologica*, 158-162.

Ohio Department of Transportation (2017). Retrieved from http://www.dot.state.oh.us/Divisions/Planning/TechServ/traffic/Pages/TrafficCount-Reports-and-Maps.aspx

Oxley, D., Fenton, M. B., & Carmody, G. R. (1974). The effects of roads on populations of small mammals. *Journal of Applied Ecology*, 51-59.

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.

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), 543-546.

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*(4), 567-581.

Rotholz, E., & Mandelik, Y. (2013). Roadside habitats: effects on diversity and composition of plant, arthropod, and small mammal communities. *Biodiversity and conservation*, *22*(4), 1017-1031.

Schetter, T. A., Root, K. (2009). "The Oak Openings from space". *Living in the Oak Openings: A homeowner's guide to one of the world's last great places*. Homewood Press, 2009.

Schetter, T. A., T. Walters and Root, K.V. 2013. A multi-scale spatial analysis of native and exotic plant species richness within a mixed-disturbance oak savanna landscape. *Environmental Management* 52(3): 581-594

Schetter, T. A., & Root, K. V. (2013). Assessing an imperiled oak savanna landscape in northwestern Ohio using landsat data. *Natural Areas Journal*, *31*(2), 118-130.

Seibert, H. C., & Conover, J. H. (1991). Mortality of vertebrates and invertebrates on an Athens County, Ohio, highway.

Shepard, D. B., Kuhns, A. R., Dreslik, M. J., & Phillips, C. A. (2008). Roads as barriers to animal movement in fragmented landscapes. *Animal conservation*, *11*(4), 288-296.

Shepard, D. B., Dreslik, M. J., Jellen, B. C., & Phillips, C. A. (2008). Reptile road mortality around an oasis in the Illinois corn desert with emphasis on the endangered eastern massasauga. *Copeia*, *2008*(2), 350-359.

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.

Spellerberg, I. A. N. (1998). Ecological effects of roads and traffic: a literature review. *Global Ecology and Biogeography*, *7*(5), 317-333.

The Nature Conservancy, 2001. Oak Openings Region Information Publication.

Teixeira, F. Z., Coelho, A. V. P., Esperandio, I. B., & Kindel, A. (2013). Vertebrate road mortality estimates: effects of sampling methods and carcass removal. *Biological Conservation*, *157*, 317-323.

Trombulak, S. C., & Frissell, C. A. (2000). Review of ecological effects of roads on terrestrial and aquatic communities. *Conservation biology*, *14*(1), 18-30.

Urich, R., Swenson, G., & Nelson, E. (2002). Upper Mississippi River and Illinois River floodplain forests. *Upper Mississippi River Conservation Committee, Rock Island, IL*.

Vasconcelos, D., & Calhoun, A. J. (2004). Movement patterns of adult and juvenile *Rana sylvatica* (LeConte) and *Ambystoma maculatum* (Shaw) in three restored seasonal pools in Maine. *Journal of Herpetology*, *38*(4), 551-561.

Webb, S. L., Riffell, S. K., Gee, K. L., & Demarais, S. (2009). Using fractal analyses to characterize movement paths of white-tailed deer and response to spatial scale. *Journal of Mammalogy*, *90*(5), 1210-1217.

Zuberogoitia, I., del Real, J., Torres, J. J., Rodríguez, L., Alonso, M., de Alba, V., & Zabala, J. (2015). Testing pole barriers as feasible mitigation measure to avoid bird vehicle collisions (BVC). *Ecological Engineering*, *83*, 144-151.

Table 1. Canopy cover scores as it relates to light meter estimates. Canopy cover was scored from 0-5 with 0 being no canopy and 5 being dense canopy.

Canopy Score	**Light Meter (LUX, lumen/m^2)**
0	1450 or higher
1	1250 - 1449
2	950 - 1249
3	640 - 949
4	400 - 639
5	399 or below

Table 2. Shown are the number of animals observed taxa per season killed on roads surrounding and within Oak Openings Preserve and Maumee State Forest in Northwest Ohio. Also, shown are the number of animals observed per taxa per season seen on trails during visual surveys within both preserves.

		Killed on Roads		Visual Surveys	
		Oak Openings	Maumee	Oak Openings	Maumee
Spring					
	Amphibians	2	0	5	3
	Birds	8	4	163	58
	Mammals	55	21	84	3
	Reptiles	3	0	1	0
Summer					
	Amphibians	2	0	9	0
	Birds	9	3	186	59
	Mammals	69	41	103	29
	Reptiles	4	0	6	2
Fall	Amphibians	0	0	3	0
	Birds	0	2	53	44
	Mammals	44	25	42	10
	Reptiles	0	0	2	0

Table 3. Significant nonparametric one-way analysis (Wilcoxon Kruskal-Wallis test) of average canopy and vegetation scores along each road per season associated with roadkill terrestrial vertebrates are shown. Mammals and birds were the two taxa that showed a relationship with average vegetation and canopy scores, therefore, reptiles and amphibians were eliminated.

Season	***Mammal***		***Birds***	
	Veg	*Canopy*	*Veg*	*Canopy*
Spring	Score: 0,1,2 **p≤0.01**	NS	Score: 0, 1, 2 **p≤0.04**	Score: 0 **p≤0.04**
Summer	Score: 0, 1, 2 **p≤0.01**	NS	Score: 1, 2 **p≤0.04**	Score: 0 **p≤0.04**
Fall	Score: 0, 1, 2 **p≤0.01**	NS	NS	NS

Table 4. Significant logistic regression values of structural features along the road associated with, the indicated taxa for each season based on the Chi-square test. Significant values displayed in bold.

	Vertical Road Sign	*Verge Slope*	*House*	*Ag. Fields*	*Grassy Fields*	*Forest*	*Water*
Spring	NS	NS	NS	**Mammal X^2=$\leq$0.04 p<0.01**	NS	NS	NS
Summer	**Bird $X^{2=}\leq$0.04 p<0.02**	NS	NS	**Mammal X^2=$\leq$0.04 p<0.01**	NS	NS	NS
Fall	NS	NS	**Mammal X^2=<0.02 p<0.01**	**Mammal X^2=<0.02 p<0.01**	NS	NS	NS

Table 5. Significant logistic regression values of land cover types along the road associated with the indicated taxa for all surveyed months based on the Chi-square test. Significant values displayed in bold. Land cover types with no significant relationship to any roadkilled taxa were excluded.

Land cover type	*Mammals*	*Birds*	*Reptiles*	*Amphibians*
Turf	**X^2=<0.05** **p≤0.03**	NS	NS	NS
Savanna	**X^2=<0.01** **p≤0.03**	NS	NS	NS
Upland Forest	**X^2=<0.01** **p≤0.03**	NS	NS	NS
Floodplains	NS	**X^2=<0.04** **p<0.04**	NS	NS

Table 6. Model results, including degrees of freedom (DF), chi-square (X^2), significance (p-value), correlation (R^2) and Akaike's Information Criterion (AICc) for each model that examined all structural features and land cover types affecting presence of roadkilled vertebrate taxa in the Oak Openings Preserve and Maumee State Forest of Northwest Ohio. Amphibians were eliminated due to a small sample size.

	Model	DF	X^2	P <0.05	R^2	AICc
All Vertebrate Taxa	Shrub/Scrub + Swamp Forest	2	13.7	<0.0010	0.09	136.3
Mammals	Turf + Savanna + Swamp Forest + Flood Plain + Cropland	5	32.2	<0.0001	0.47	49.07
Birds	Floodplain + Water	2	9.8	<0.0071	0.14	83.3
Reptiles	Shrub/Scrub + Swamp Forest + Barrens + Houses + Grassy Fields + Vertical Road Sign	6	33.5	<0.0001	0.9	18.5

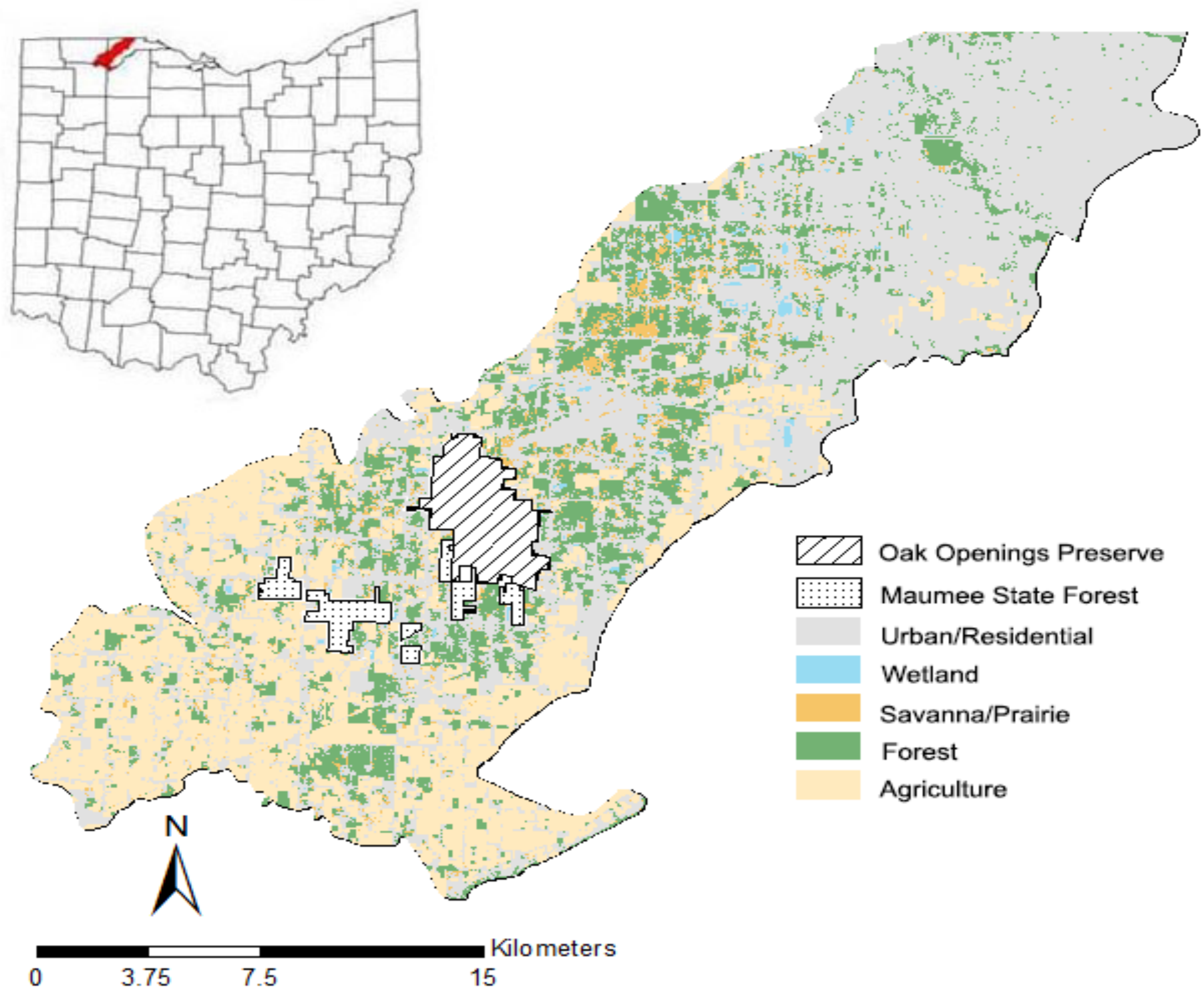

Figure 1. Oak Openings Region (red) in Northwest Ohio is a mosaic of remnant forest, savannas, and wetlands within an agricultural and urban/residential matrix (Schetter and Root, 2011). Oak Openings Preserve and Maumee State Forest are shown in hatched and dot patterns, respectively.

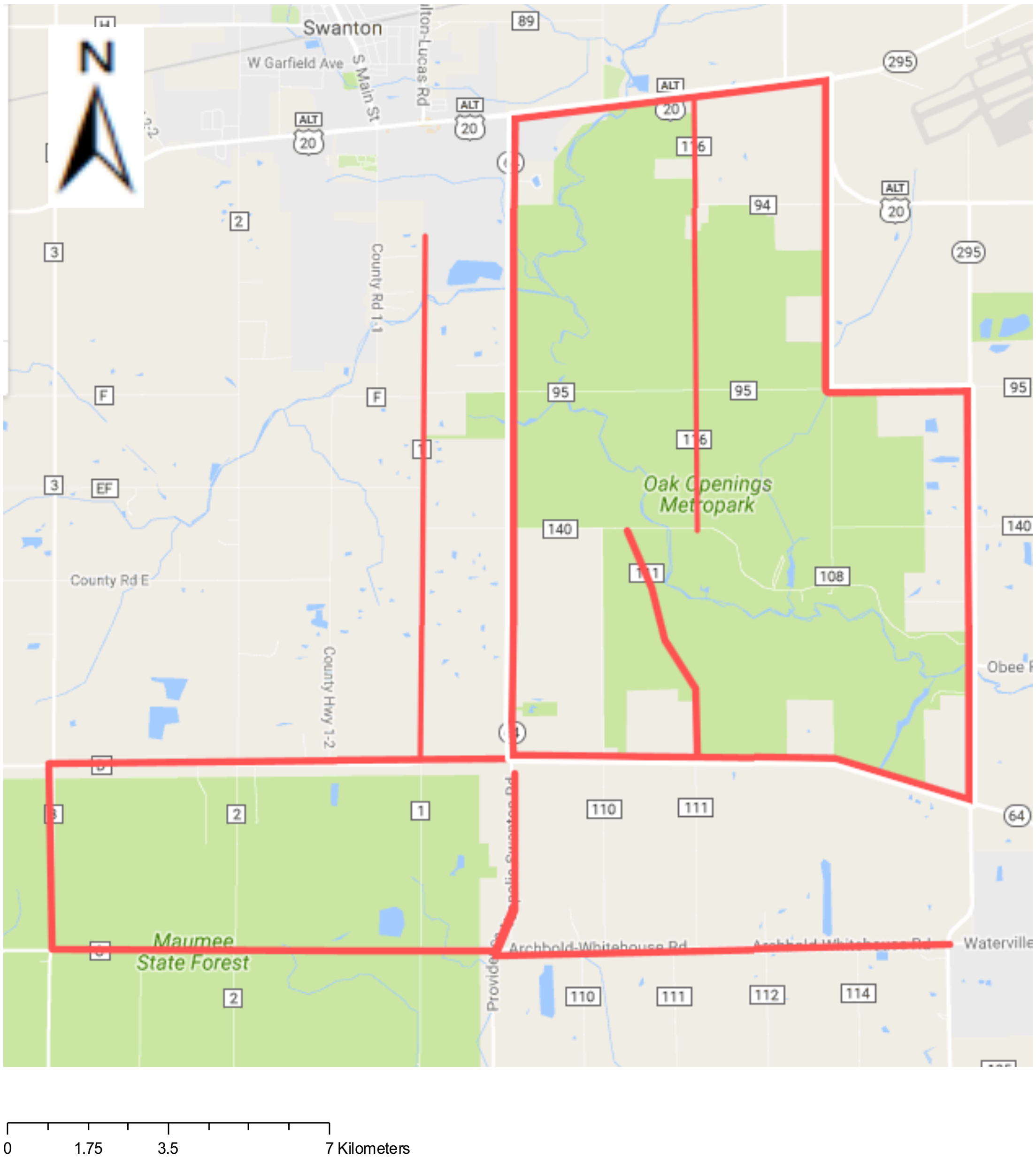

Figure 2. Shown is a map of the roads (highlighted in red) that were survyed in and near Oak Opening Preserve and Maumee Sate Forest in the Oak Openings Region of Northwest Ohio.

Figure 3. Photos of canopy cover from field surveys taken with a digital camera. Photo taken every 500 meters of trail and 6 meters from trail edge. Scored from 0-5. Canopy cover with a score of a 0 meant no canopy visible. Score of 1 top left. Score of 2 top middle. Score of 3 top right. Score of 4 bototom left. Score of 5 bottom right.

Figure 4. Vegetation density scores in relation to photographs taken with a digital camera. Photo taken every 500 meters along the trail and 6 meters from trail edge. Score of a 0 is no vegetation. From left to right is score 1-5 respectively.

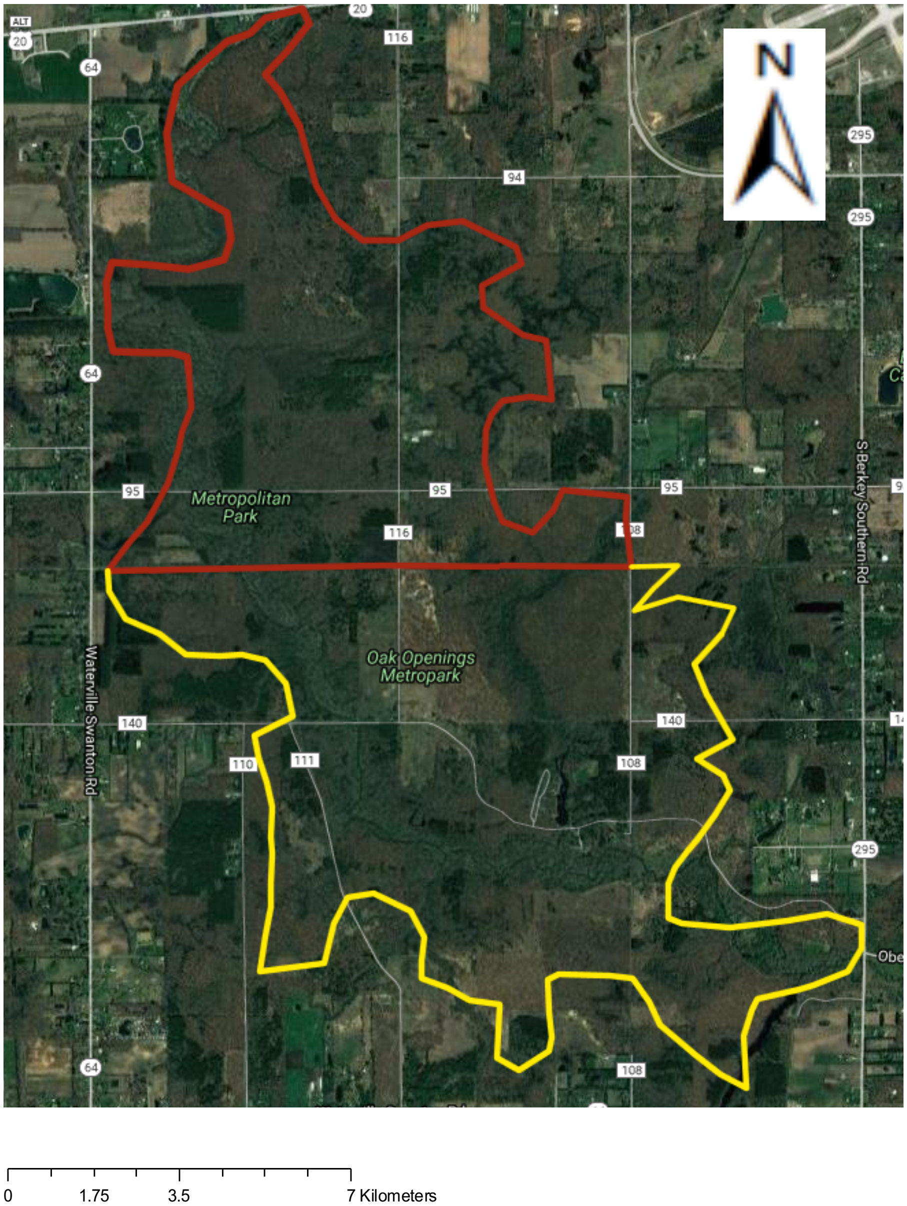

Figure 5. Map of Oak Openings Preserve, In Northwest Ohio, displaying the field survey path. Red line represents the trail of Boyscout #1 North. Yellow line represents the trail of Boyscout #2 South.

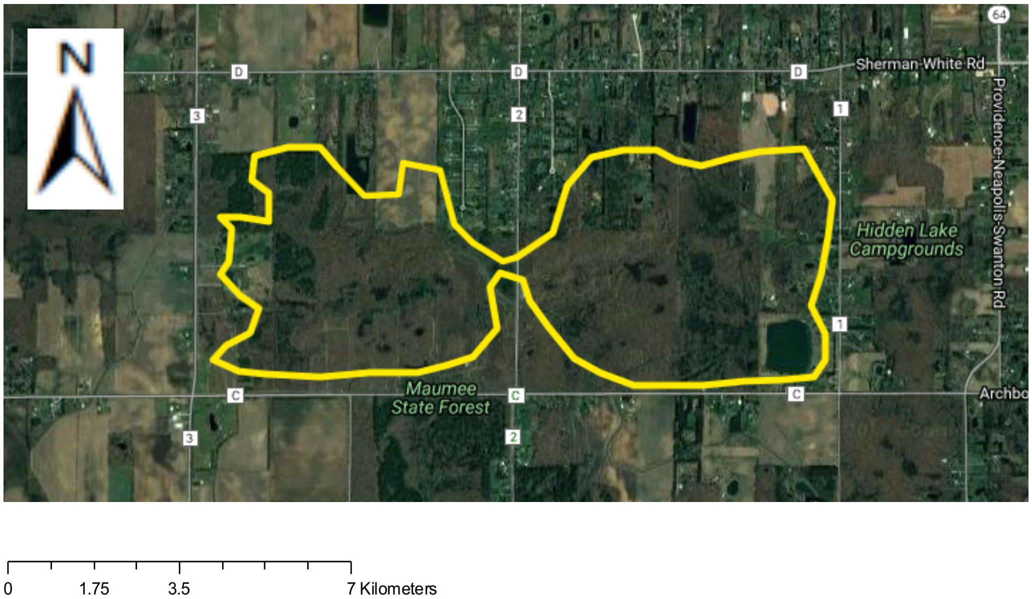

Figure 6. Map of ATV trail of Maumee State Forest in Northwest Ohio. Yellow line represent the trail that was surveyed for terrestrial vertebrates.

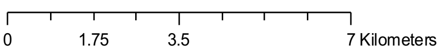

Figure 7. Example of average canopy and vegetation point locations with 250 m buffers on ArcGIS Software (version 11). Vegetation symbol represents average canopy and vegetion point locations with average scores labeled (1 and 2). Red circles represent 250 m buffer around average canopy and vegetation point locations. Deer crossing symbol represents roadkill with roadkill species labeled.

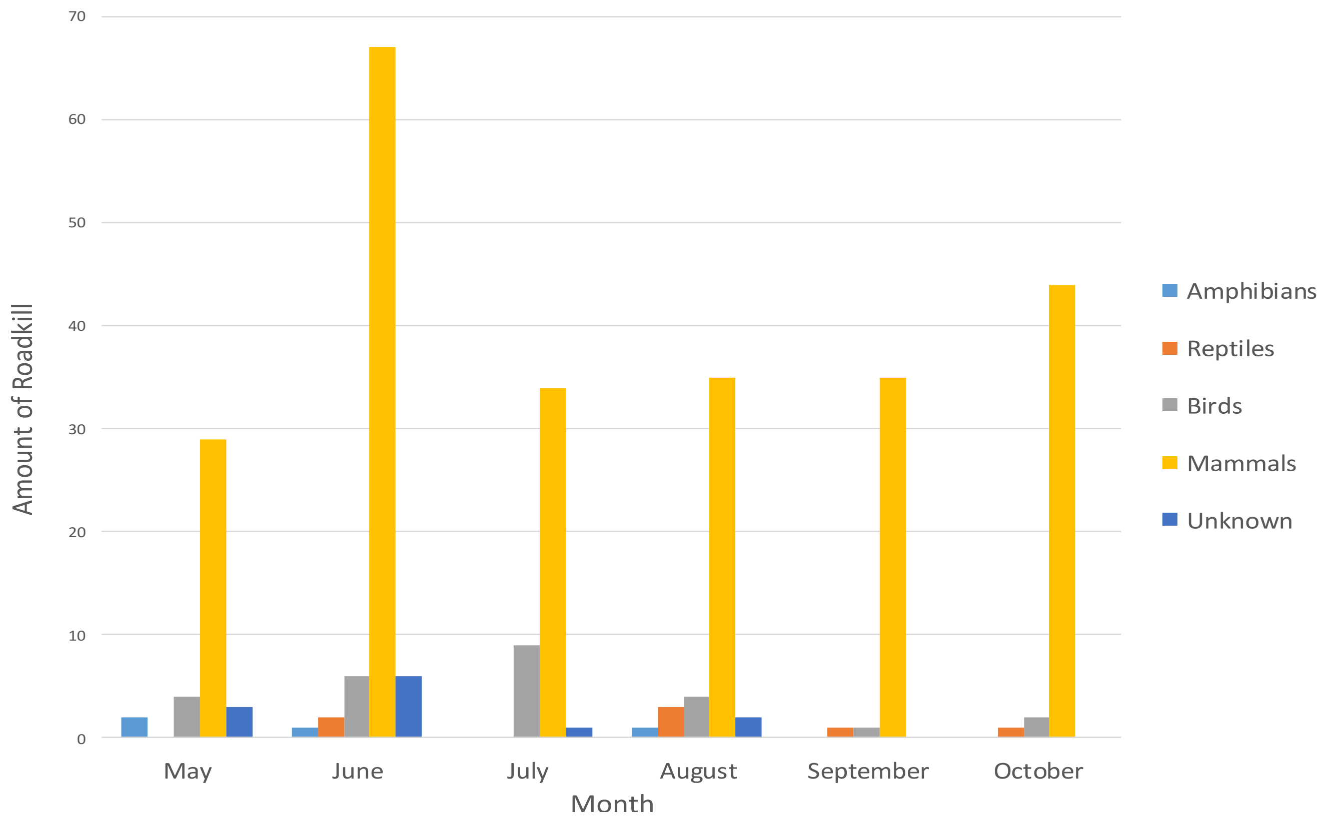

Figure 8. Roadkill per taxa per month on roads near Oak Openings Preserve and Maumee State Forest located in Northwest Ohio from May-October, 2016. Yellow bars represent roadkilled mammals. Light blue bars represent roadkilled amphibians. Orange bars represent roadkilled reptiles. Dark blue bars represent unknown roadkill.

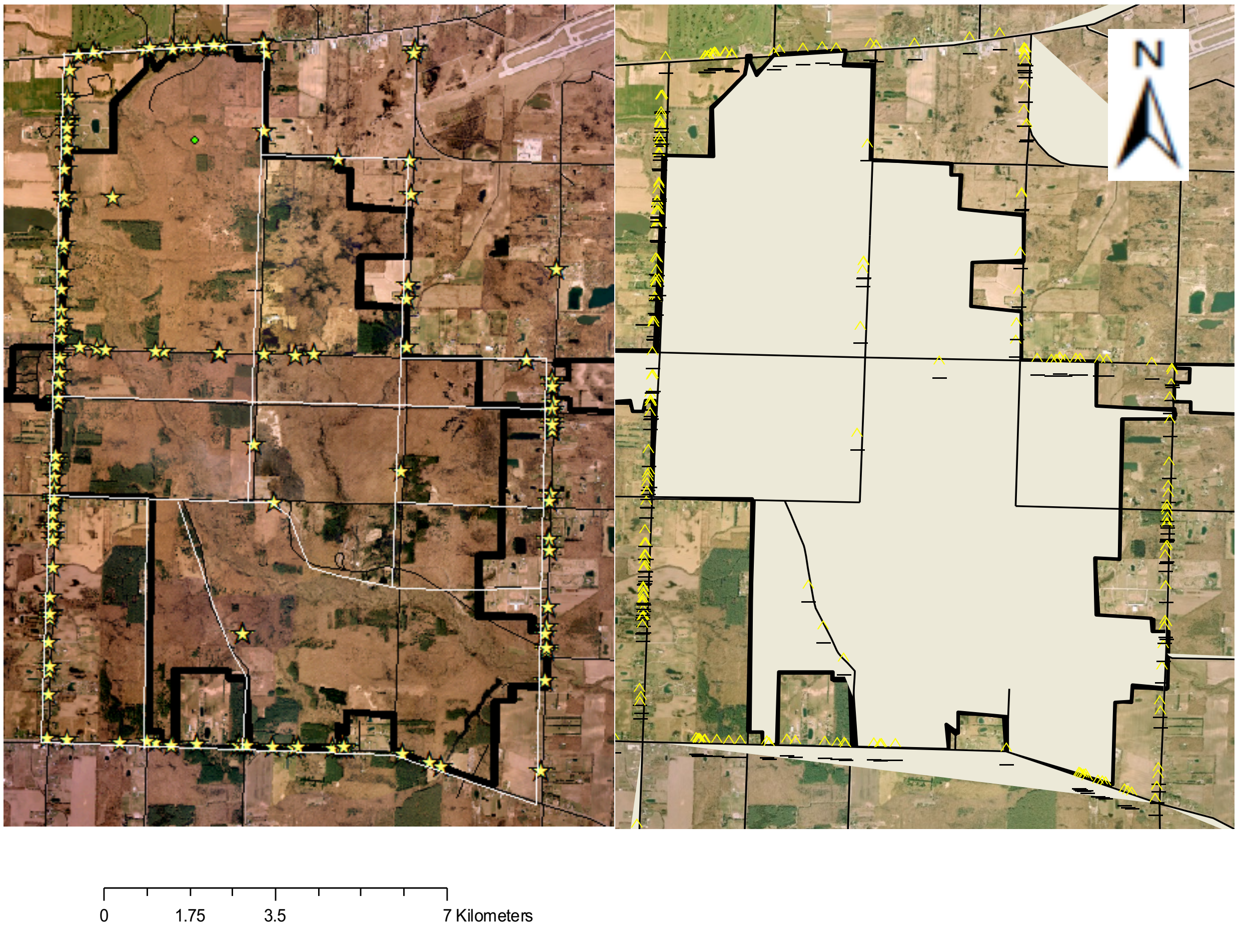

Figure 9. Shown are a comparison of vertebrates killed on roads (yellow stars) surrounding Oak Openings Preserve, in Northwest Ohio, from 2013 (Left, A. Kuntz, unpublished data) and 2016 (right, data from this study). Lines represent roads and bold lines represent the borders of the park.

Figure 10. Roadkill hot spot analysis performed on roads surrounding Oak Openings Preserve and Maumee State Forest in Northwest Ohio in ArcGis using a Getis-Ord G_i* statistic on each 250 m buffer where clusters of high and low values of roadkill were located on each road. Circles represent buffers. Circles that are yellow had no significance suggesting these areas are of low values of roadkill. Circles that are light orange have 90% confidence of significance suggesting potential roadkill hot spots. Circles that are orange have a 95% confidence of significance suggesting potential road kill hot spots. Circles that are red have a 99% confidence of significance suggesting a roadkill hot spot location and have high values of roadkill. Circles that are light blue have a 90% confidence of significant suggesting potential cold spots or areas of low values of roadkill.

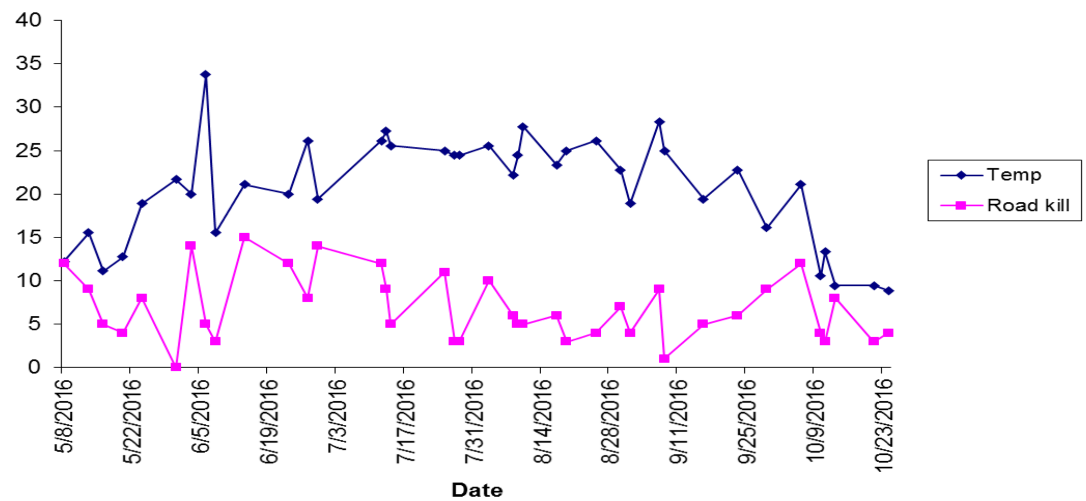

Figure 11. A comparison of the relationship between daily average temperature (C°) and number of roadkill on roads surround Oak Openings Preserve and Maumee State Forest located in Northwest Ohio from May-October 2016. Blue lines represent temperature. Pink lines represent roadkill.

Table 7. Eigenvectors from Principal Component Analysis of total roadkilled mammals detected within the fixed point location of the 250 m buffer and structural features within its borders along the roads surrounding and within the Oak Openings Preserve and Maumee State Forest of northwestern, Ohio, showing strength of influence of each landscape metric for first seven principal components.

	Prin1	Prin2	Prin3	Prin4	Prin5	Prin6	Prin7
Forest	0.27637	-0.09791	0.79647	0.00000	-0.21051	0.22106	0.43183
House	-0.46567	0.44639	0.21117	0.00000	-0.30367	0.52892	-0.40904
Ag. Field	-0.62382	0.08517	-0.09236	0.00000	0.33349	0.13496	0.68238
Grass Field	0.44040	0.25166	-0.53231	0.00000	-0.31335	0.48375	0.35661
Water	0.02974	0.72323	0.08133	0.00000	-0.24492	-0.61147	0.18856
Speed Sign	0.35041	0.44441	0.15013	0.00000	0.77076	0.21273	-0.13357
Wildlife Sign	0.00000	0.00000	0.00000	1.00000	0.00000	0.00000	0.00000

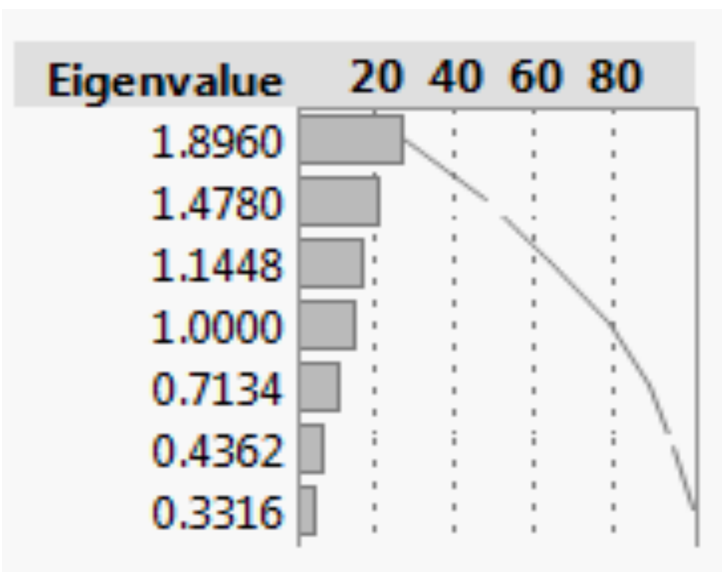

Figure 12. Eigenvalue/Principal component cumulative percentage table from Principal Component Analysis of total roadkilled mammals detected within the fixed point location of the 250 m buffer and structural features within its borders along the roads surrounding and within the Oak Openings Preserve and Maumee State Forest of northwestern, Ohio.

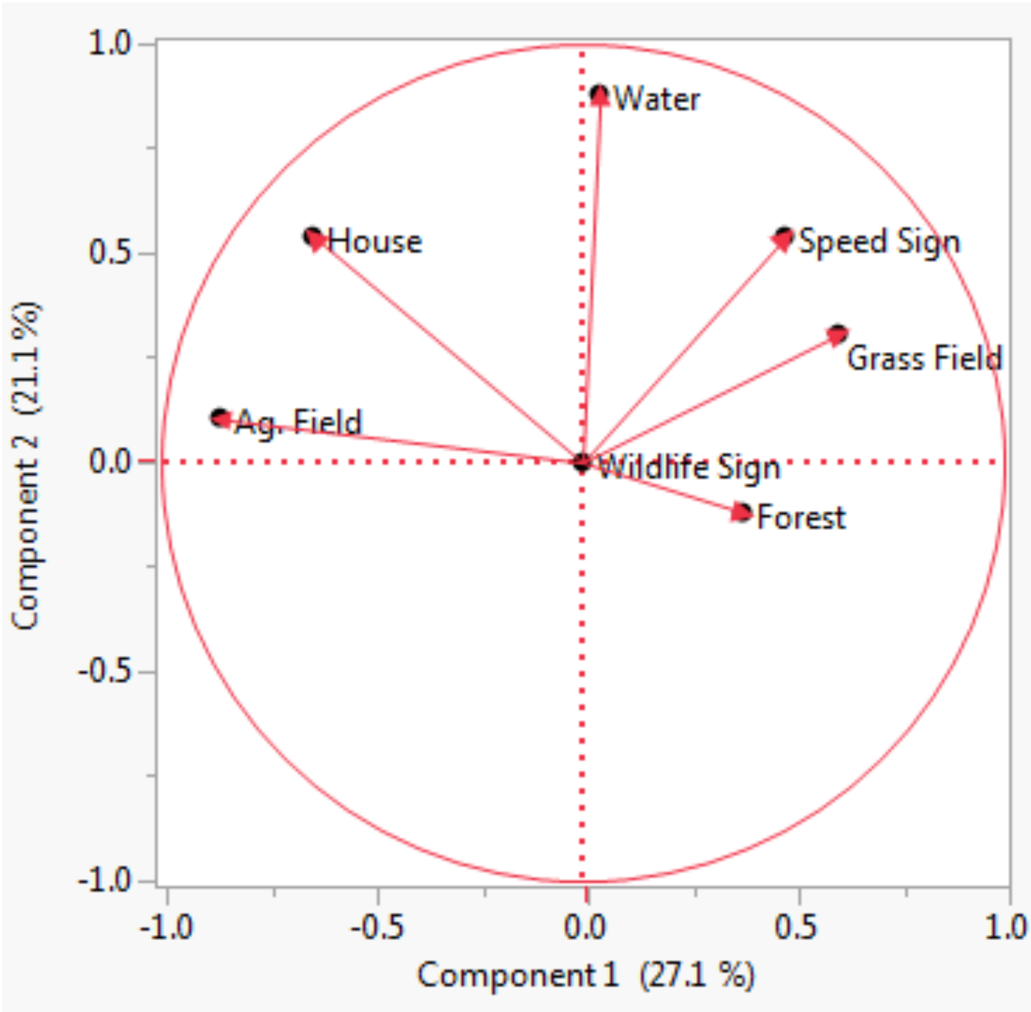

Figure 13. Principal Components Analysis shows vectors of total roadkilled mammals detected within the fixed point location of the 250 m buffer and structural features within its borders along the roads surrounding Oak Openings Preserve and Maumee State Forest in Northwest, Ohio. Association of each variable is represented by the orientation of the lines.

Table 8. Eigenvectors from Principal Component Analysis of total roadkilled birds detected within the fixed point location of the 250 m buffer and structural features within its borders along the roads surrounding and within the Oak Openings Preserve and Maumee State Forest of northwestern, Ohio, showing strength of influence of each landscape metric for first seven principal components.

	Prin1	Prin2	Prin3	Prin4	Prin5	Prin6	Prin7
Forest	0.16665	0.15400	-0.77331	-0.14308	-0.23328	0.20060	0.48514
House	-0.20104	0.55118	-0.25476	0.10348	0.73371	0.10837	-0.17347
Ag. Field	-0.52451	0.23008	0.39083	0.16973	-0.07054	0.17185	0.67520
Grass Field	0.50862	-0.31057	0.18948	-0.11923	0.58350	-0.06837	0.49959
Water	0.12413	0.53737	0.21575	-0.66653	-0.12442	-0.43212	0.05296
Speed Sign	0.47128	0.32687	0.31832	-0.00202	-0.14403	0.72948	-0.12974
Wildlife Sign	0.40062	0.35703	0.02366	0.69392	-0.15993	-0.44152	0.09708

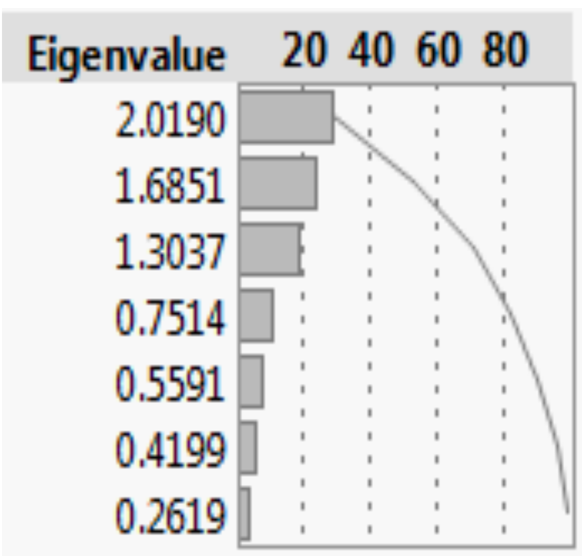

Figure 14. Eigenvalue/Principal component cumulative percentage table from Principal Component Analysis of total roadkilled mammals detected within the fixed point location of the 250 m buffer and structural features within its borders along the roads surrounding and within the Oak Openings Preserve and Maumee State Forest of northwestern, Ohio.

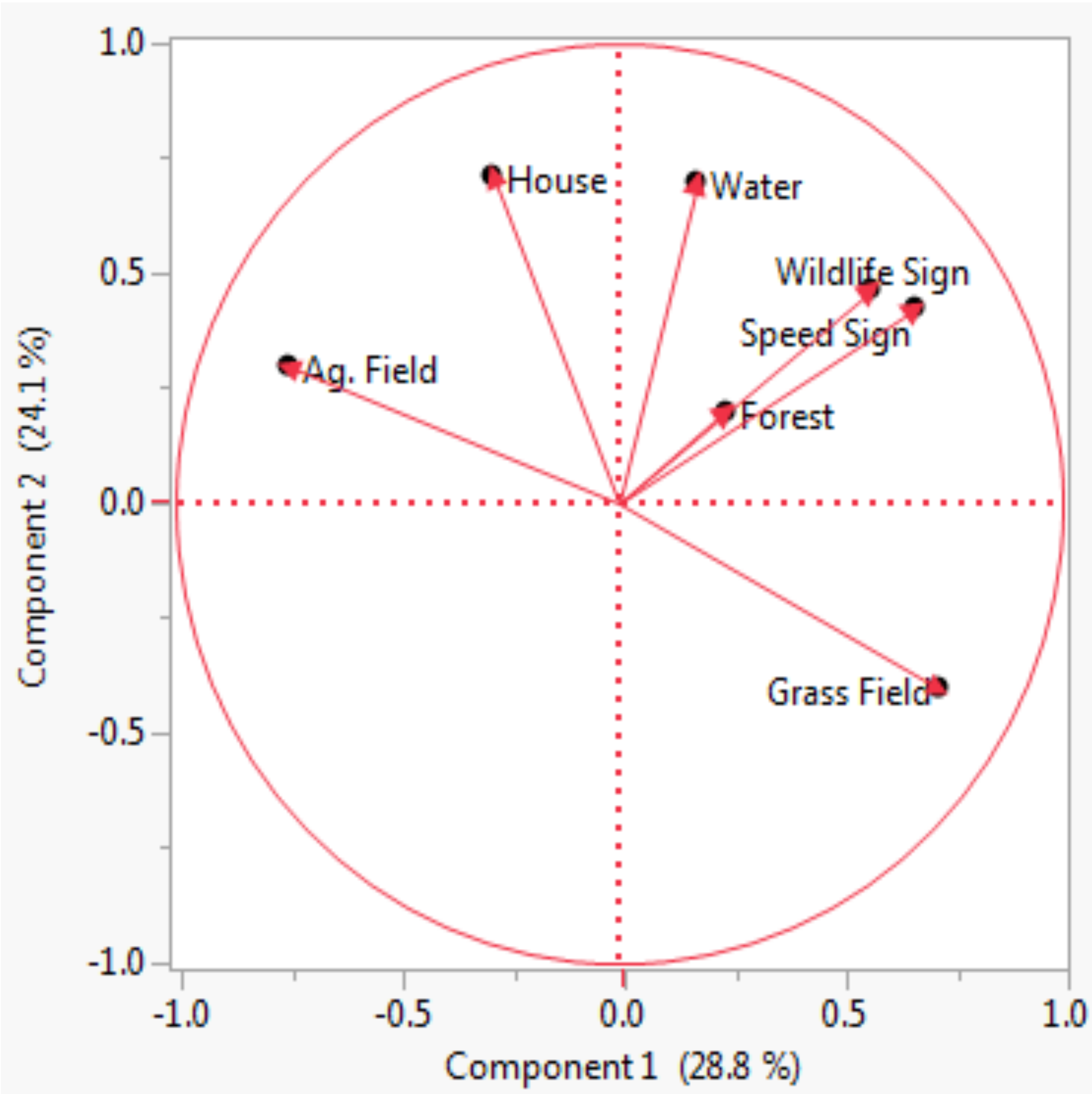

Figure 15. Principal Components Analysis shows vectors of total roadkilled birds detected within the fixed point location of the 250 m buffer and structural features within its borders along the roads surrounding Oak Openings Preserve and Maumee State Forest in Northwest, Ohio. Association of each variable is represented by the orientation of the lines.

Table 9. Eigenvectors from Principal Component Analysis of total roadkilled mammals detected within the fixed point location of the 250 m buffer and land cover types within its borders along the roads surrounding and within the Oak Openings Preserve and Maumee State Forest of northwestern, Ohio, showing strength of influence of each landscape metric for first twelve principal components.

	Prin1	Prin2	Prin3	Prin4	Prin5	Prin6	Prin7	Prin8	Prin9	Prin10	Prin11	Prin12	Prin13	Prin14	Prin15
1 Turf	0.02859	0.06461	0.39244	0.31444	-0.08992	-0.32121	0.59332	-0.23817	-0.24901	0.22211	-0.09775	-0.21745	0.04582	0.19010	0.12526
2 Wet Prairie	-0.09881	0.12331	0.05661	0.30273	0.71697	0.02862	-0.05649	0.22399	-0.03182	0.13240	-0.33806	0.31826	0.06724	-0.00101	0.27077
3 Residental	-0.01946	0.28076	0.39541	-0.34778	0.02775	0.25761	-0.21846	0.27993	-0.42130	0.09929	0.30402	-0.19785	0.12717	0.19668	0.27659
4 Asphult	-0.05695	-0.14883	0.22931	0.54687	-0.37634	0.09295	-0.40590	-0.01956	0.14342	0.26059	0.16416	0.05613	0.07262	-0.29055	0.31510
5 Pond	0.19069	-0.02698	0.28260	0.10958	-0.09829	0.61422	0.34137	0.20536	0.44035	-0.17090	-0.05896	0.07296	-0.16269	0.25848	0.01424
6 Savannah	-0.02784	0.36536	-0.45167	0.14325	-0.21549	0.21458	0.10913	-0.31751	-0.17822	-0.29126	0.04013	0.24677	0.22642	0.17813	0.41703
7 Shrub/Scrub	-0.14477	0.22418	-0.24885	0.04187	-0.27579	-0.17040	0.34515	0.71243	0.05504	0.16590	0.08768	0.09901	0.21317	-0.19215	-0.05401
8 Swamp Foest	0.49462	0.13485	0.03423	-0.03134	-0.02302	0.03071	0.12543	-0.02166	-0.32990	0.10982	0.16454	0.42942	-0.49951	-0.36747	-0.02035
9 Connifers	0.41294	0.06576	0.03493	-0.25357	0.05891	-0.23309	-0.03689	-0.15622	0.42307	0.38173	0.21772	0.30613	0.37824	0.24980	0.06921
10 Upland Forest	0.37306	0.31942	0.18846	0.25802	0.14366	0.02569	-0.06503	-0.02388	-0.01018	-0.38248	0.05261	-0.18414	0.46311	-0.31896	-0.36239
11 Floodplain	0.32860	0.14215	-0.38656	0.32566	-0.02342	0.15055	-0.22640	0.10987	-0.14310	0.39213	-0.11179	-0.31357	-0.13973	0.39467	-0.26350
12 Barrens	-0.23065	0.38588	0.13148	0.24591	0.05536	-0.35886	-0.16517	0.04660	0.20280	-0.25775	0.40401	0.12751	-0.38059	0.32596	-0.14385
13 Eurassian Meadow	-0.41373	0.24457	0.18337	-0.04367	-0.13521	0.28672	-0.01888	-0.24740	-0.10709	0.31601	-0.14008	0.37087	0.13242	0.00507	-0.53420
14 Prairie	-0.13985	0.46886	-0.11704	-0.13171	0.17784	0.14671	0.12714	-0.23816	0.36963	0.27951	0.06552	-0.41064	-0.21036	-0.37939	0.16632
15 Cropland	-0.14643	-0.34911	-0.19003	0.19377	0.35377	0.23988	0.26145	-0.07876	-0.11223	0.10745	0.68347	-0.01806	0.12467	-0.01180	-0.13595

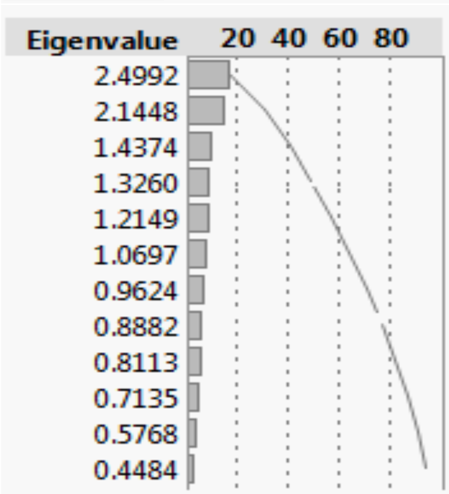

Figure 16. Eigenvalue/Principal component cumulative percentage table from Principal Component Analysis of total roadkilled mammals detected within the fixed point location of the 250 m buffer and land cover types within its borders along the roads surrounding and within the Oak Openings Preserve and Maumee State Forest of northwestern, Ohio.

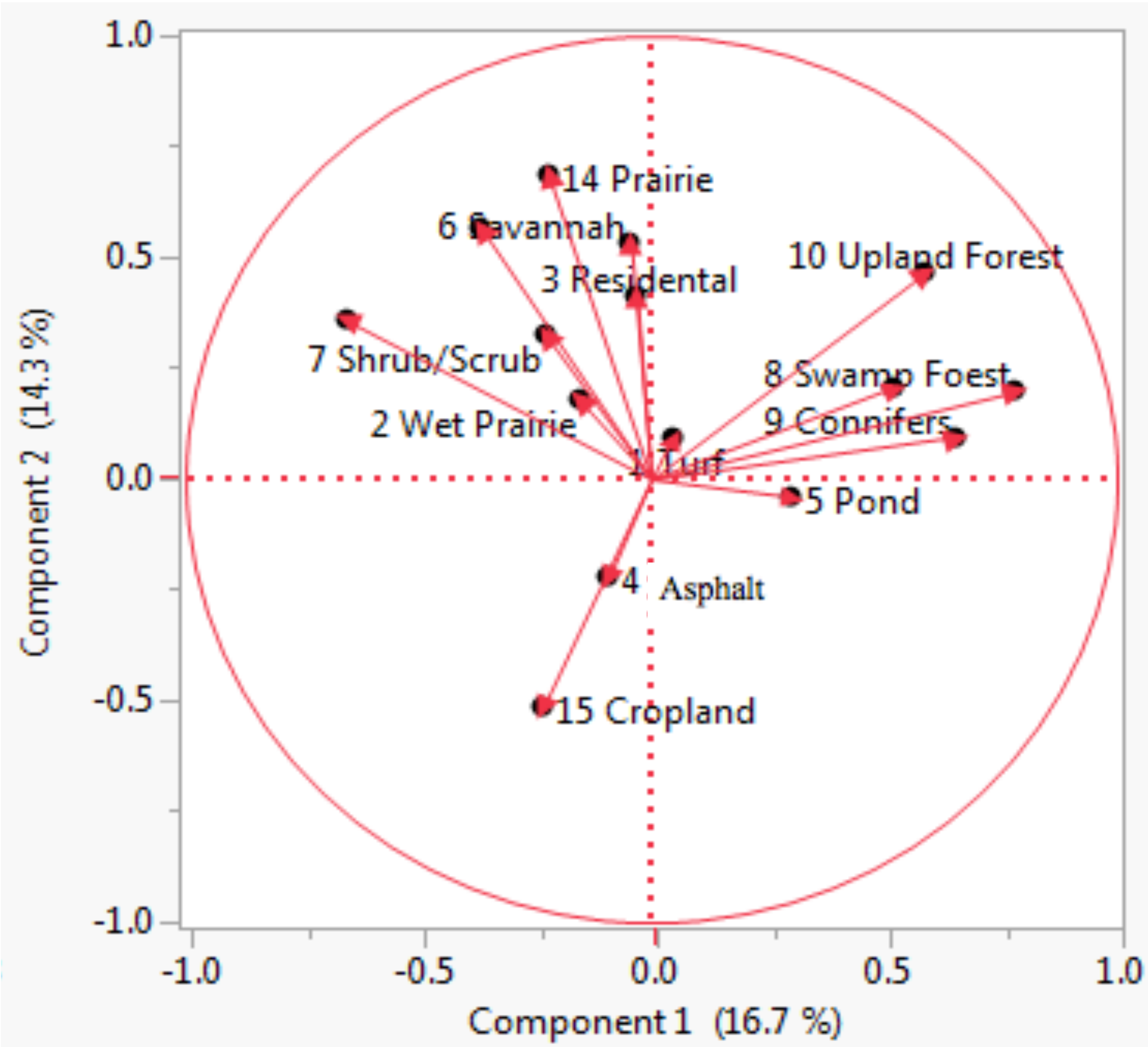

Figure 17. Principal Components Analysis shows vectors of total roadkilled mammals detected within the fixed point location of the 250 m buffer and land cover types within its borders along the roads surrounding Oak Openings Preserve and Maumee State Forest in Northwest, Ohio. Association of each variable is represented by the orientation of the lines.

Table 10. Eigenvectors from Principal Component Analysis of total roadkilled birds detected within the fixed point location of the 250 m buffer and land cover types within its borders along the roads surrounding and within the Oak Openings Preserve and Maumee State Forest of northwestern, Ohio, showing strength of influence of each landscape metric for first twelve principal components.

	Prin1	Prin2	Prin3	Prin4	Prin5	Prin6	Prin7	Prin8	Prin9	Prin10	Prin11	Prin12	Prin13	Prin14	Prin15
1 Turf	0.20608	-0.15932	0.15063	-0.16482	0.50587	0.00000	0.00000	0.53758	-0.04378	-0.50291	-0.15332	-0.09792	0.01916	0.15805	-0.15531
2 Wet Prairie	0.00000	0.00000	0.00000	0.00000	0.00000	1.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
3 Residental	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	1.00000	0.00000	-0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
4 Asphult	-0.20963	-0.22038	0.51315	0.24638	0.17281	0.00000	0.00000	0.01427	0.37464	0.06920	-0.16906	-0.23900	0.09583	-0.36662	0.42304
5 Pond	0.35517	-0.13692	-0.20054	0.25475	0.30502	0.00000	0.00000	-0.45403	-0.05688	-0.06284	-0.35685	0.24688	0.49844	-0.07680	-0.03169
6 Savannah	-0.07367	0.57300	-0.05401	-0.02604	0.10224	0.00000	0.00000	0.09217	0.08130	-0.12370	0.31782	-0.22410	0.47273	-0.43608	-0.23857
7 Shrub/Scrub	-0.19910	0.22495	0.06139	-0.24666	0.52569	0.00000	0.00000	-0.20670	-0.55766	0.10348	0.08047	0.08145	-0.18090	-0.10660	0.38364
8 Swamp Foest	0.46492	0.24386	0.07560	-0.13277	0.04141	0.00000	0.00000	0.02678	0.30894	0.00381	-0.06301	0.43236	-0.46029	-0.44744	-0.01339
9 Connifers	0.37359	0.14913	-0.12784	0.18395	0.08679	0.00000	0.00000	0.49953	-0.09632	0.65149	-0.05979	-0.11128	0.14606	0.11439	0.21647
10 Upland Forest	0.27105	0.28289	0.20992	0.48489	-0.17346	0.00000	0.00000	-0.02780	-0.11635	-0.42262	0.31647	0.08926	-0.01483	0.27062	0.40341
11 Floodplain	0.19922	0.23454	0.32074	-0.40857	0.16187	0.00000	0.00000	-0.33790	0.40227	0.17312	0.07723	-0.13778	0.12378	0.51289	-0.00003
12 Barrens	-0.31304	0.16065	0.39413	0.40960	0.21757	0.00000	0.00000	0.07112	-0.03294	0.22681	-0.00681	0.42295	-0.04442	0.18076	-0.48434
13 Eurassian Meadow	-0.39657	0.14999	-0.34163	-0.12967	0.04960	0.00000	0.00000	0.24572	0.37195	-0.10827	-0.09538	0.49156	0.21517	0.18136	0.37932
14 Prairie	-0.13154	0.37944	-0.34686	0.32286	0.19920	0.00000	0.00000	-0.12908	0.19027	-0.09345	-0.40197	-0.41010	-0.40338	0.13378	-0.05005
15 Cropland	0.03050	-0.34546	-0.32515	0.20825	0.42353	0.00000	0.00000	-0.07844	0.28494	0.08610	0.65475	-0.02253	-0.16149	0.01180	-0.03277

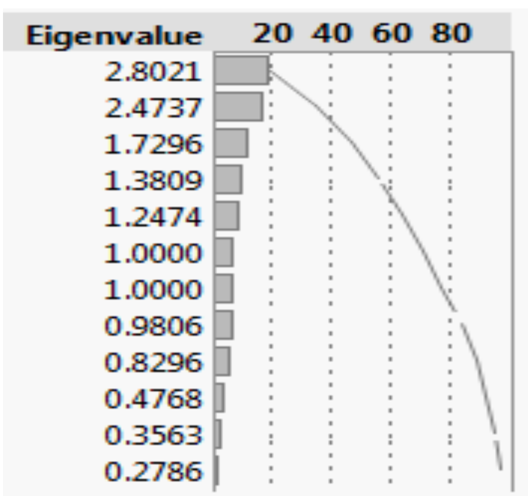

Figure 18. Eigenvalue/Principal component cumulative percentage table from Principal Component Analysis of total roadkilled birds detected within the fixed point location of the 250 m buffer and land cover types within its borders along the roads surrounding and within the Oak Openings Preserve and Maumee State Forest of northwestern, Ohio.

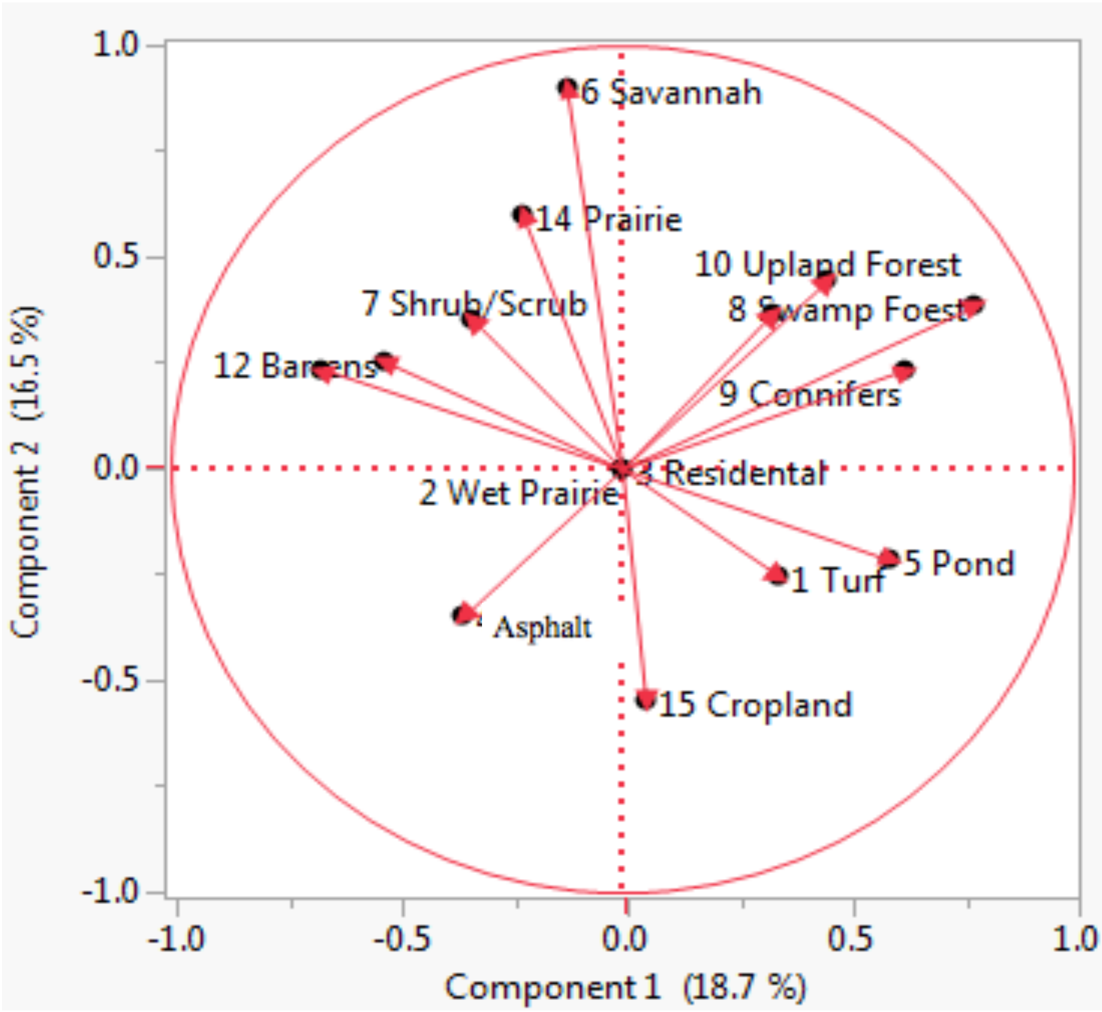

Figure 19. Principal Components Analysis shows vectors of total roadkilled birds detected within the fixed point location of the 250 m buffer and land cover types within its borders along the roads surrounding Oak Openings Preserve and Maumee State Forest in Northwest, Ohio. Association of each variable is represented by the orientation of the lines.

Table 11. Eigenvectors from Principal Component Analysis of total roadkilled mammals detected within the fixed point location of the 250 m by 100 m rectangular window and land cover types within its borders along the edge of roads surrounding Oak Openings Preserve and Maumee State Forest in Northwest, Ohio, showing strength of influence of each landscape metric for first fifteen principal components.

	Prin1	Prin2	Prin3	Prin4	Prin5	Prin6	Prin7	Prin8	Prin9	Prin10	Prin11	Prin12	Prin13	Prin14	Prin15
1 Turf	-0.21089	0.22267	-0.25509	-0.35072	0.00000	0.00000	0.00000	0.63757	0.13749	0.34938	-0.03832	0.15584	-0.07370	-0.02894	0.37200
2 Wet Prairie	0.00000	0.00000	0.00000	0.00000	1.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
3 Residental	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	1.00000	0.00000	0.00000	0.00000	0.00000	-0.00000	0.00000	0.00000	0.00000
4 Asphult	-0.16792	0.35234	0.30794	0.02527	0.00000	0.00000	0.00000	-0.08939	0.72921	-0.03336	0.00989	-0.36540	0.10640	-0.25770	-0.00095
5 Pond	0.02668	0.31679	0.50564	-0.20202	0.00000	0.00000	0.00000	0.10878	-0.36483	0.17937	0.61138	-0.10452	-0.12664	0.02679	-0.15451
6 Savannah	0.29171	0.31987	0.01902	-0.20158	0.00000	0.00000	0.00000	0.43562	-0.06566	-0.59021	-0.27633	-0.00594	0.11773	0.03750	-0.37083
7 Shrub/Scrub	0.00000	-0.00000	-0.00000	0.00000	0.00000	1.00000	0.00000	-0.00000	-0.00000	0.00000	-0.00000	0.00000	-0.00000	0.00000	0.00000
8 Swamp Foest	0.43569	-0.05407	0.22014	0.37882	0.00000	0.00000	0.00000	0.23203	-0.15071	-0.10687	-0.01295	-0.04575	-0.04706	-0.50767	0.51489
9 Connifers	0.36522	-0.23470	0.23765	-0.04017	0.00000	0.00000	0.00000	0.08507	0.43262	0.12083	-0.00314	0.41235	-0.56295	0.08381	-0.22463
10 Upland Forest	0.47972	-0.06760	0.02694	0.06827	0.00000	0.00000	0.00000	0.11936	0.17148	0.19127	0.06972	-0.33249	0.30794	0.65346	0.20598
11 Floodplain	0.39684	0.20181	-0.05116	-0.19888	0.00000	-0.00000	0.00000	-0.24256	-0.00463	0.43987	-0.10915	0.33343	0.49377	-0.32160	-0.19224
12 Barrens	-0.04098	0.22610	-0.34881	0.67895	0.00000	0.00000	0.00000	0.24836	0.12065	0.06108	0.38050	0.19203	0.09012	0.01362	-0.30761
13 Eurassian Meadow	0.04411	0.52955	0.01496	0.27358	0.00000	-0.00000	0.00000	-0.15731	-0.21766	0.30697	-0.51193	-0.14991	-0.40521	0.16461	-0.01943
14 Prairie	0.19797	0.42126	-0.29050	-0.15971	0.00000	-0.00000	0.00000	-0.40352	0.10332	-0.36131	0.30581	0.26218	-0.16783	0.13248	0.40178
15 Cropland	-0.29645	0.10629	0.51914	0.21036	0.00000	0.00000	0.00000	0.02257	-0.00558	-0.10818	-0.17597	0.55614	0.30838	0.30102	0.22101

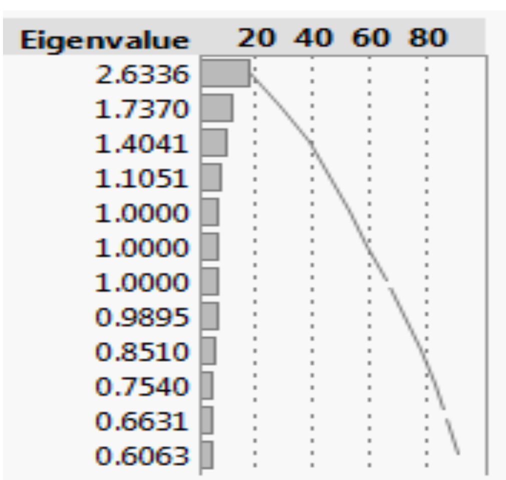

Figure 20. Eigenvalue/Principal component cumulative percentage table from Principal Component Analysis of total roadkilled mammals detected within the fixed point location of the 250 m by 100 m rectangular window and land cover types within its borders along the edge of roads surrounding Oak Openings Preserve and Maumee State Forest in Northwest, Ohio.

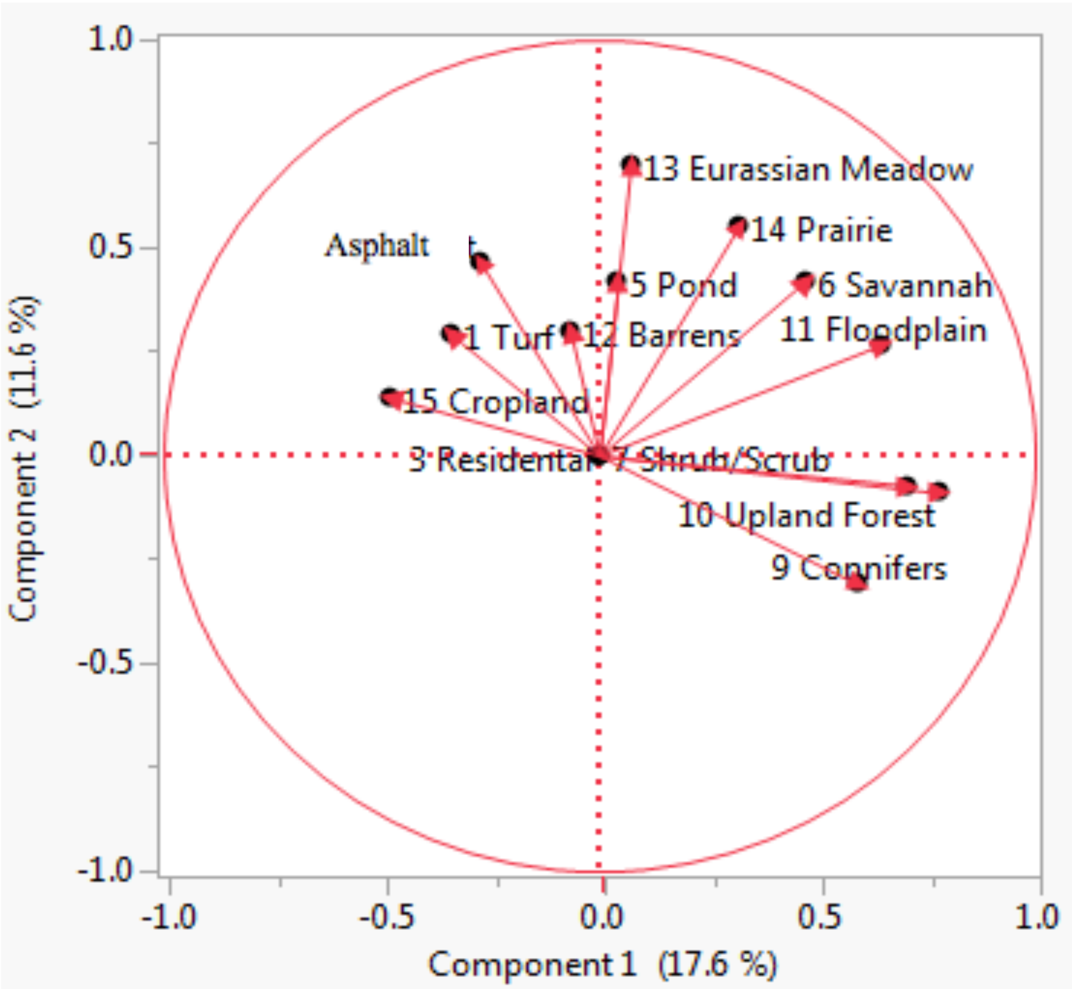

Figure 21. Principal Components Analysis shows vectors of total roadkilled mammals detected within the fixed point location of the 250 m by 100 m rectangular window and land cover types within its borders along the edge of roads surrounding Oak Openings Preserve and Maumee State Forest in Northwest, Ohio. Association of each variable is represented by the orientation of the lines.

Table 12. Eigenvectors from Principal Component Analysis of total roadkilled birds detected within the fixed point location of the 250 m by 100 m rectangular window and land cover types within its borders along the edge of roads surrounding Oak Openings Preserve and Maumee State Forest in Northwest, Ohio, showing strength of influence of each landscape metric for first fifteen principal components.

	Prin1	Prin2	Prin3	Prin4	Prin5	Prin6	Prin7	Prin8	Prin9	Prin10	Prin11	Prin12	Prin13	Prin14	Prin15
1 Turf	-0.18900	0.26299	-0.29432	-0.30606	0.37529	0.00000	0.00000	-0.00000	-0.55262	-0.17311	0.23085	0.22824	0.06793	0.29425	0.20502
2 Wet Prairie	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	1.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
3 Residental	0.00000	0.00000	0.00000	0.00000	0.00000	1.00000	0.00000	0.00000	-0.00000	-0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
4 Asphult	-0.35061	0.12252	0.04565	0.52686	0.17834	0.00000	0.00000	0.00000	0.26238	0.17202	0.31532	0.27674	0.46176	0.17917	-0.17555
5 Pond	-0.07636	0.27624	0.53289	-0.33564	-0.01154	0.00000	0.00000	-0.00000	-0.11691	-0.01757	-0.13963	-0.41682	0.53547	0.08175	-0.14311
6 Savannah	0.29761	0.38980	-0.22111	0.24306	-0.08142	0.00000	0.00000	-0.00000	-0.10397	-0.34781	0.45605	-0.35735	-0.02448	-0.27478	-0.31831
7 Shrub/Scrub	-0.00000	-0.00000	0.00000	-0.00000	0.00000	0.00000	0.00000	1.00000	0.00000	0.00000	0.00000	-0.00000	0.00000	0.00000	0.00000
8 Swamp Foest	0.32000	-0.23086	0.12361	0.27230	0.38685	0.00000	0.00000	-0.00000	0.15093	-0.59628	-0.20039	-0.11043	0.07398	0.38699	0.13896
9 Connifers	0.37715	0.13860	0.38325	0.18031	0.06253	0.00000	0.00000	0.00000	-0.36356	0.28003	-0.05645	0.27224	-0.31630	0.29396	-0.42533
10 Upland Forest	0.40357	-0.20537	-0.04264	0.20081	0.31762	0.00000	0.00000	0.00000	-0.27093	0.47410	0.09384	-0.20871	0.29821	-0.26791	0.37508
11 Floodplain	0.31880	-0.14992	0.18456	-0.47354	0.26435	0.00000	0.00000	-0.00000	0.30582	-0.11430	0.33232	0.43400	0.13505	-0.30512	-0.17603
12 Barrens	0.19845	0.21811	-0.42704	-0.25687	0.26665	0.00000	0.00000	0.00000	0.43654	0.36940	-0.09125	-0.27433	-0.05532	0.37308	-0.20186
13 Eurassian Meadow	0.04238	0.56619	-0.05183	0.13787	0.25068	0.00000	0.00000	0.00000	0.08814	-0.06587	-0.56721	0.27371	0.02814	-0.41835	0.09237
14 Prairie	0.32859	0.39612	0.15592	-0.01045	-0.42452	0.00000	0.00000	-0.00000	0.22201	0.03459	0.22497	0.16981	0.02433	0.26779	0.57293

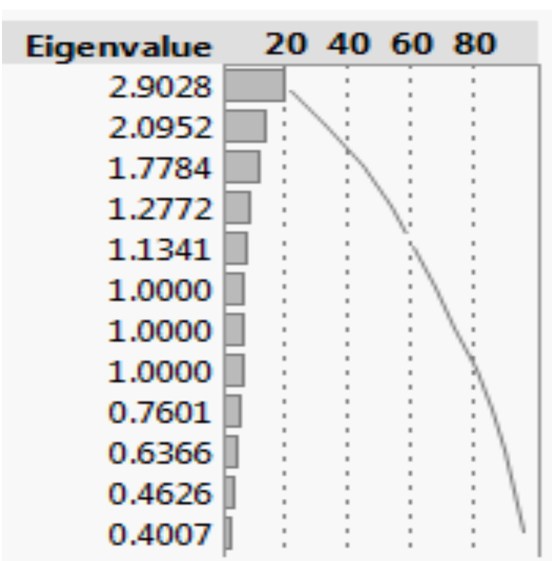

Figure 22. Eigenvalue/Principal component cumulative percentage table from Principal Component Analysis of total roadkilled birds detected within the fixed point location of the 250 m by 100 m rectangular window and land cover types within its borders along the edge of roads surrounding Oak Openings Preserve and Maumee State Forest in Northwest, Ohio.

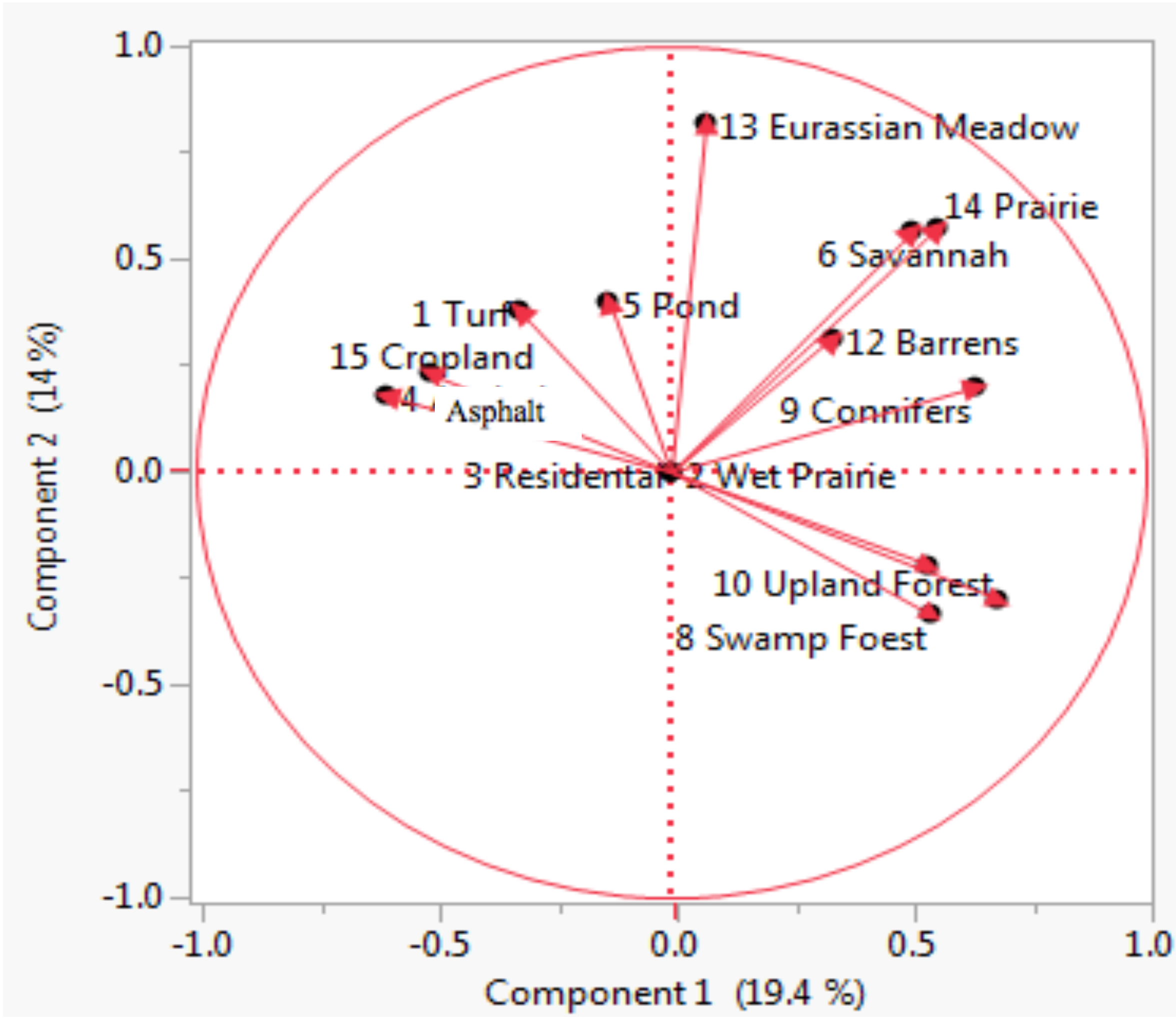

Figure 23. Principal Components Analysis shows vectors of total roadkilled birds detected within the fixed point location of the 250 m by 100 m rectangular window and land cover types within its borders along the edge of roads surrounding Oak Openings Preserve and Maumee State Forest in Northwest, Ohio. Association of each variable is represented by the orientation of the lines.

CHAPTER II. STRUCTURAL FEATURES OF ROADWAYS INFLUENCE MAMMAL MOVMENT AND ROAD MORTALITY

Abstract

Thousands of mammals each year are killed by animal vehicle collisions. Roadways provide a variety of resources for mammals, including roadside plants, insects, and other attractants along road edges such as different habitat types, residential areas or agricultural fields. It order to understand the impacts roads have on mammals, it is important to understand how structural features along roads (e.g. vegetation density, canopy cover, road characteristics, land cover types) spatial factors (e.g. traffic density, speed) and environmental variables (e.g. temperature, humidity, and rain) relate to animal life history, movement and road mortality. This study used roadkilled mammals as a lens to monitor animal movement and identify structural features of roads that could be managed to reduce road mortality in the Oak Openings Region, a biodiversity hotspot in Northwest Ohio. About four times a week, May-October, we surveyed roads surrounding and within Oak Openings Preserve and Maumee State Forest, the largest protected areas, for roadkilled mammals. We also performed daytime and nighttime surveys within the parks to account for sources of animal dispersal and dispersal corridors. We found 244 roadkilled mammals on roads surrounding protected areas. Almost 48% of road mortality was found on one roadway that appeared to be an area of frequent crossings and mortality from vehicle collisions. During visual surveys, 68 % of mammal movement was primarily in sections of one preserve, adjacent to the road that connects it to another. An increased number of roadkilled mammals were found in areas of the roads with low to intermediate levels or no canopy. Road structural features (e.g. presence of agricultural fields, vertical road signs, grass fields, road verges, forests and water) influenced mammal mortality but the effects varied among

species and months. Land cover types along the roads and edges influenced animal movement, abundance and road mortality for mammals during all surveyed months. Our approach can be used to predict animal movement and identify the features that may be managed to reduce road mortality, which is likely to be applicable to other reserves in human-dominated landscapes.

Introduction

Road networks increasingly impact remnant wildlife habitat and these impacts require careful study to aid conservation of the remaining biodiversity. Impacts can include landscape fragmentation, wildlife-vehicle collisions causing mortality and population decline, reduction in animal dispersal, barriers impeding animal movement and disturbance effects such as traffic, noise or volume (Seo et al., 2015; Forman, 2003, Grilo et al., 2009). Specifically, thousands of mammals each year are killed by animal vehicle collisions. Roadways provide a variety of resources for mammals, including roadside plants, insects, and other attractants along road edges such as different habitat types, residential areas or agricultural fields (Smith and Patten, 2008). In order to understand the impacts roads have on mammals, it is important to understand how structural features along roads (e.g. vegetation cover, canopy cover, road characteristics, land cover types) spatial factors (e.g. traffic density, speed) and environmental variables (e.g. temperature, humidity, and rain) relate to animal life history, movement and road mortality.

An example of a human-dominated landscape is the Oak Openings Region of northwest Ohio. This area is considered a biodiversity hotspot that contains one third of all of Ohio's rare plant communities and rare animals (Grigore, 2004). It also includes a set of unique habitats consisting of a mosaic of oak savanna, wet prairie, oak woodland, oak barrens, and floodplain forest (Brewer and Vankat, 2004). These habitats have historically been maintained by disturbances such as wild fires, which are routinely suppressed. Now, remaining remnants of natural early successional habitats are maintained by local land managers using prescribed fires and thinning that mimic historical disturbances (Albell et al., 2004) Unfortunately, this unique area is highly fragmented by increasing suburban development, agricultural fields, roads, railways and hiking trails (Abella, 2007). In order to preserve and restore such a special area, we need maintain viable populations, become more knowledgeable about mammal movement patterns and prevent mortality by animal-vehicle collisions.

We built our project on previous research, which demonstrated the effect that roadways have on mammal communities within the Oak Openings Preserve (Unpublished data, 2010). The previous research surveyed roadways surrounding and within Oak Openings Preserve for roadkill from May through November 2010, sampling four to five times per month. About 165 roadkill specimens were identified on the roadways surrounding and within the Oak Openings Preserve. One roadway on the border of Oak Openings Preserve had higher road mortality than the other roadways with 59% of road mortality discovered in that location (Unpublished, 2010). This road is likely an area for frequent mammal crossing because this roadway connects to another protected area known as Maumee State Forest. Such corridors could play a large role in how mammal communities disperse between the Oak Openings Preserve and Maumee State Forest and may have detrimental effects on local population sizes. Therefore, it is important to understand how structural features located on this and other roadways surrounding Oak Openings Preserve and Maumee State Forest influence mammal movement and road mortality.

Structural features, such as road edge or road characteristics should be considered because they can restrict or encourage animal movement and animal-vehicle collisions. Road edge is defined as the area that is in-between a road and a certain land cover type (e.g. forest).

Road edges can encourage animal movement by operating as corridors in which edge specialists like white-tailed deer or coyote can move more easily through an area. This can increase the chance of animal-vehicle collisions (Oxley, et al, 1974). Also, road edges can act as habitat for some species, like amphibians that are known to use roadside ditches when filled with water (Spellerberg, 1998; Gibbs, 1998). Road edge can restrict animal movement in which species may avoid edges to avoid predation from other edge specialists like red-tailed hawk. Also, species may avoid road edges due to traffic density, traffic noise pollution or other anthropogenic disturbances (Grilo et al., 2015; Clevenger et al., 2003; Forman, 1998). Road characteristics can also influence animal movement and road morality. For example, is a study conducted by D’Amico et al., (2015) in Doñana Natural Park looking at all vertebrate taxa, land cover types consisting of water bodies were more likely to increase roadkilled amphibians, but for snakes, lizards and small birds the opposite occurred. Also, the presence of vertical road signs along the road increased small bird and snake road mortality. Lastly, they discovered that the presence of culverts decreased the number of roadkilled snakes and small mammals by encouraging safe road crossing (D’Amico et al, 2015). Another study conducted by Jaeger et al, (2005) created models that analyzed the effects of roads on animal populations and found that small roads (one lane in opposite direction) or large roads (two or more lanes in opposite directions) with high traffic intensity resulted in higher road avoidance in animal populations despite road size differences (Jaeger et al., 2005). Therefore, it is important to understand how structural features of roads can influence animal movement and vehicle collisions.

To also understand the effect roadways have on mammals, temporal and spatial factors need to be considered. Movement changes across time. If we understand these variations across time, managers could anticipate when mammals may risk crossing a road or when road mortality is high. For example, temperature and rainfall can be linked to peaks in roadkill patterns (Clevenger, et al., 2003). Also, spatial variations in road structural features can also be associated with roadkill frequency in which the presence of certain road structural features (e.g. vertical road signs, roadside ditches) may increase or decrease mammal road mortality (D'Amico et al. 2015; Clevenger, et al., 2003). Specifically, in a study conducted by Barthelmess (2014) on mammals in northern New York State found that distance to cover (e.g. ground vegetation cover, canopy) or presence of road edge impacted the likelihood of mammal vehicle collisions, but varied between mammal species. Thus, it is important to incorporate all factors to better understand mammal movement and road mortality.

Understanding animal dispersal can help us better predict if, when, and where, an animal may cross a road. According to Greenwood (1980), innate animal dispersal has a major role in both population regulation and spatial distribution (Greenwood, 1980). Also, animals are known to disperse to invade areas that may have been depopulated due to disturbances or stochastic events (Howard, 1960). Since the Oak Openings Region is sporadically disturbed by prescribed fires and thinning to maintain historic oak savanna habitat, animals may disperse out of newly disturbed areas, which can increase the chances of road crossings and collisions with vehicles. Mammals may also be moving to take advantage of resources that may be temporally or spatially limited.

Mammals are an important taxon to focus on for several reasons. First, mammals have critical roles in ecosystems. For example, many herbivorous mammals can control plant or tree populations and a shift in abundance of certain plant species can be the result of too many herbivorous mammals being removed from a system. Also, predatory mammal species can control the abundance of prey species by limiting overgrowth and help sustain a balanced ecosystem (Roemer et al, 2009). Second, many mammal species are long-lived slow growing species, which can cause populations to become vulnerable if too many mammals are hit by vehicles. Lastly, mammals can modify the structure of a landscape by altering plant structure and species composition, and certain critical habitats (e.g. oak savanna) within the Oak Openings Region may depend on grazing mammal species (e.g. white-tailed deer). Therefore, mammals are important for overall health of the Oak Openings Region and it may be critical to understand their movement patterns and road mortality to maintain a healthy ecosystem.

To increase our understanding of mammal movement and mortality in a highly fragmented human-dominated landscape we combined roadkill surveys and visual surveys for mammals within two protected areas known as Oak Openings Preserve and Maumee State Forest that are within the Oak Openings Region. We hypothesized that mammals would cross most frequently on Waterville Swanton Road, as it is a potential corridor between the two protected areas. We also predicted that environmental variables (e.g. rainfall, humidity, and temperature), spatial factors (e.g. traffic density, speed) and structural features of roads (e.g. vegetation cover, canopy cover, road characteristics, and land cover types) would influence roadkill probability in these areas. Our field surveys within the parks were designed to identify which areas had a high abundance of animals and their proximity to roadways. By examining mammal road mortality, we expected to predict movement patterns and identify structural characteristics of roads that could be managed to reduce road mortality of this important taxa in human-dominated landscapes.

Methods

Study Location

Our research was conducted in Oak Openings Preserve and Maumee State Forest within the Oak Openings Region in northwest Ohio, Figure 25. The Oak Openings Preserve is roughly 1,214 hectares comprised of oak savanna, wet prairie, oak woodland, oak barrens, and floodplain forest and is managed by Metroparks of the Toledo Area. Maumee State Forest is roughly 1,292 hectares and is composed of hardwood forest, swamp forest, pine forest and wet prairie that is managed by Ohio Department of Natural Resources (ODNR) Division of Forestry.

Road Surveys

We surveyed roadways that surround Oak Opening Preserve and Maumee State Forest for mammals alive and dead. The roads that surround Oak Opening Reserve included: Airport Highway US HWY 20A, Wilkins Rd, Monclova Rd., Southern Berkley Rd., and Waterville Swanton Rd., which roughly constitute a road length of 20 kilometers (13 miles) around Oak Openings Preserve. The roads parallel to Waterville Swanton Rd. were also surveyed in this study; these roads were Fulton-Lucas Rd. and Archbold-Whitehouse Rd. The roads that were surveyed surrounding Maumee State Forest included: County Rd. D (which turns into Waterville Swanton Rd.), Providence Neapolis Swanton Rd., County Rd. 3, and County Rd. C., which roughly constituted 12.87 kilometers (8 miles) (Figure 26).

Three roads within the Oak Openings Preserve were surveyed, which included: Girdham Rd., Jeffers Rd., and Monclova Rd. (surveying of Monclova Rd. started in July). The paved bike trail was surveyed within the Oak Openings Preserve, which is known as the Wabash Cannonball Trail (Figure 26). This bike trail is a converted railway line that was similar in size and composition to a single land road with non-motorized traffic. There were no accessible roads within Maumee State Forest to survey.

Mammal road kill surveys were conducted at random times while allowing for logistical constraints, but at the same time within each week. Total number of roadkill for each species was recorded. We performed a vehicle survey driving 32-48 kph (20-30 mph) down each road 2-3 times a week. For each specimen found we recorded the location with a handheld GPS (Garmin Etrex), identified the species, noted what side of the road it was found on and took a photograph. We included animals found on the verges since they may have been injured by a vehicle, but later succumbed to the injured inflicted by vehicle collisions. Animals detected crossing the road were also identified to species, and we noted the direction or movement and what road it crossed. Lastly, a handheld GPS was used to track the movement of the observer on each road surrounding the parks.

Environmental Variables of Roads

For all roads we measured environmental variables, which included: season, rainfall, humidity and temperature. May-June 20nd 2016 was considered spring, June 21st-September 22nd was considered summer and September 23rd-November 1st was considered fall. Rainfall in centimeters for each survey day was obtained from the NOAA weather data (https://www.ncdc.noaa.gov/). Humidity, as a percentage, was measured and recorded at the start and end of each survey using a handheld weather station (Burton Atmosphere Pro). Air temperature, in degrees Celsius, was measured each survey day using NOAA weather data and the handheld weather station device throughout the survey until the completion of a survey.

Spatial Factors and Structural Features of Roads

Spatial factors and structural features of roads were estimated for all of the roads, which included: canopy cover, vegetation cover, road features, land cover types, traffic volume and speed. Canopy cover was measured at fixed sampling points, every 0.48 kilometers of the road and within 6.5 meters of either side of the road. The measurement of 6.5 meters was based on the average measurement of all verges on the roads. Moreover, 6.5 meters was a safe measurement since many verges merged onto private property. We created a canopy scale system using light meter estimates (Digital Lux Meter, Model: LX1330B). The scale system ranged from 0-5 with a score of a 0 representing no canopy cover and a score of a 5 representing dense canopy cover (Table 13; Figure 26). Canopy cover was measured for all roads in the months of April (spring), June (summer) and October (fall) to account for changes in canopy cover due to change of season. Vegetation cover at each sampling point was also scored on a scale from 0-5, with 0 representing bare ground vegetation and 5 equal to dense vegetation cover (Table 13; Figure 27).

Road features such as length and width of the road, verge (length and width) and verge slope were collected using a surveyor’s wheel (Meter Man, Komelon Series 45). Slope refers to the tangent of the angle of that surface, which exist on hills or ditches. Verge is the narrow strip of grass or plants located between the roadway, curb or boundary of the road. Usually verges will end where forest and vegetation begins. Also, roadside ditches were noted if they were filled with water and water depth i.e., shallow (2.5 cm), mid-deep (15 cm), and deep (30 cm) was recorded. Wildlife road crossing signs and bridges were marked on all roads using GPS. Speed limits were recorded for each road as well exact location of speed limit signs (latitude and longitude, GPS) to explore the relationship in amount of roadkill with speed limit sign location and road speed. At fixed sampling points, every 0.48 kilometers along each road, we calculated what proportion of houses, agricultural fields, forest patches, grass patches and water were found within a 250m buffer on ArcGIS Software (version 11). Lastly, land cover types (e.g. turf, wet praire, residential, asphalt, water, oak savanna, shrub/scrub, swamp forest, conifers, upland forest, floodplain forest, barrens, eurassian meadow, prairie, cropland) were noted for each sampling point, every 0.48 kilometers along roads and road edges using a land cover map in ArcGIS (Schetter and Root, 2013). Land cover types were defined as follows: Turf was characterized as a grassy area. Wet prairie was characterized as lowland grassland that occurs on saturated or seasonally flooded steam and river floodplains or lake boundaries (Hewes, 1951). Asphalt was characterized as a mixture of dark colored sand or gravel, used to surface roads. Oak savanna was characterized as a rare area that is lightly forested with oak trees and is historically maintained by wild fires or grazing animals. Shrub/scrub land cover was characterized by bush and shrub plant communities, but could also contain grasses and herbs. Swamp forests were characterized as forests that were flooded with freshwater permanently or seasonally and normally occurred around rivers or lakes. Conifers forests were characterized as containing predominantly coniferous trees. Upland forests were characterized by hardwood trees and had a distinct over story of shade-tolerant deciduous trees with an understory of woody shrubs (Host, et al., 1987). Floodplain forests were characterized as deciduous forest communities that occupied areas adjacent to rivers or streams and endured occasional flooding cycles. Barrens were characterized by thin soil and sand and, in general, had little vegetation cover. Eurasian meadows were characterized as “mesic to dry cool-season grassland and old fields dominated by Eurasian species” (Schetter & Root, 2013). Tall grasses and mixed or short grasses depending on soil quality and rainfall were characterized as prairies. Lastly, croplands were characterized as areas of lands used for crop production and harvest.

To measure traffic volume, we conducted random traffic counts for each road by choosing three time periods during the day to count the number of cars that pass in a half hour time span (as in Langen et al. 2009). For each road, we measured traffic during two weekdays and one weekend day. We also obtained the average 24-hour traffic data was obtained from the Ohio Department of Transportation website (http://www.dot.state.oh.us/pages/home.aspx, 2017).

Finally, we did not remove the carcasses, but we noted how many days a body remained on the road or the immediate vicinity. Carcasses were marked by GPS and were spray painted green to mark the animal.

Visual Surveys within the Protected Areas

Daytime and nighttime walking surveys were conducted in Oak Opening Preserve and Maumee State Forest. Daytime surveys from mid mornings to afternoons. Nighttime surveys were conducted from 5:00 pm (EST) onward. We used these surveys to estimate animal distribution and movement patterns within the parks and identify which areas had a high density of animals with proximity to roadways. We selected the Boyscout trail to survey the entire area to of the park in relatively close proximity to roadways. This trail was split into two sections by the Wabash Cannonball Trail. The north portion of the Boyscout trail we will refer to as the Boyscout #1 North and the south portion of the Boyscout trail will be referred to as the Boyscout #2 South (Figure 28). Boyscout #1 North was roughly 14.48 km (9 miles) and Boyscout #2 South was approximately 14.8 km (9.2 miles). In Maumee State Forest, the ATV trail was surveyed, which was approximately 11.3 km (7 miles) (Figure 29).

Daytime and nighttime walking surveys were conducted at the same time for each week. For example, if at the start of the week a daytime survey began at 12:00pm then the same time was used for the other surveys of that week. For each trail, we noted the amount of time it took to walk the specific trail and maintained the same speed each time so that we could take into account sampling effort. The Oak Openings trail halves took approximately five hours each to complete. In Maumee State Forest the ATV trail took approximately 2 ½ hours to complete. Trails were rotated each survey to guarantee that each trail was completed at different times of the day.

The visual surveys included all terrestrial vertebrate taxa identified to the species level. When an animal was seen, we recorded the species, what direction it was moving, what portion of the park it was located, if it was near a roadway, what kind of habitat it was found in, anthropogenic noise (categorized as mild, medium or severe) and canopy/vegetation scores 0-5 (Table 1; Figure 3; Figure 4). One day and one night survey were conducted for each month from mid-March to the end of October 2016.

Environmental Variables for Visual Surveys

Temporal factors were measured during each visual survey. Rainfall in centimeters for each survey date was obtained from NOAA weather data (https://www.ncdc.noaa.gov/). Humidity, as a percentage, was measured and recorded periodically throughout each survey using a handheld weather station (Brunton Atmosphere Pro). Air temperature, in degrees Celsius, was measured each survey day using NOAA weather data and periodically throughout surveys using a handheld weather station. Lastly, a GPS (Garmin Etrex) was used to track the movement of the observer on each trail during visual surveys within the parks.

Spatial Factors and Structural Features for Visual Surveys

Spatial factors were estimated for all trails, which included: canopy cover and vegetation cover. Canopy cover was measured at fixed sampling points, every 500 meters of the trail and within 6 meters of either side of the trail path. The trail width varied in size with an average width of 6 meters. We used the average trail width for sampling to include the trail and along its edge. The same canopy cover scale system was used for the trails as in the road surveys (Table 13; Figure 26). Similar to road survey canopy cover measurements, a scale system from 0-5 was used with a score of a 0 representing no canopy cover and a score of a five representing dense canopy cover (Table 13). Also, ground vegetation cover was scored similar to road vegetation coverage at the sampling points with a score of a 0 representing bare ground and a score of a 5 representing dense vegetation (Table 13; Figure 27).

Lastly, land cover types (i.e. turf, wet praire, residential, asphalt, water, oak savanna, shrub/scrub, swamp forest, conifers, upland forest, floodplain forest, barrens, eurassian meadow, prairie, cropland) were noted for each sampling point using a land cover map in ArcGIS (Schetter and Root, 2013).

<u>*Statistical Analysis*</u>

Road Surveys

We created 250 m buffers around each fixed sampling location along each road and summed the total number of roadkill found within each buffer for all surveyed months combined. We chose 250 m because it was the halfway point between each survey point location. We used the spatial statistics toolbox and performed a Getis-Ord G_i* statistic on each 250 m buffer. This statistical analysis identified where clusters of high and low values of roadkill were located on each road. The test is defined as:

$$\sum G_i{*}(d) = \sum W_{ij}\ (d)\ X_j / X_j \qquad \text{Equation 1}$$

$G_i{*}(d)$ is the statistic for point i where with distance d from i, $W_{ij}(d)$ is the spatial weight matrix at distance d in dimensions i and j and X_j is the total roadkill found throughout all surveyed months. The analysis summed the values of the target point with its neighboring points and divided it by the sum of all the features. A z score is calculated where significant clusters were present in each 250 m buffer. For a significant z score value, the score varied more than 1.96 away from zero, which is a confidence level of 95% (Mitchell 2005).

Environmental Variables of Roads

To test if the daily amount of roadkill discovered was linked to the environmental variables of rainfall, season, humidity and temperature, we used a nonparametric one-way analysis (Wilcoxon/Kruskal-Wallis Test) of the number of roadkill per survey day across all roads in relation to the average rainfall, humidity and temperature per survey day in JMP.

Spatial Factors and Structural Features of Roads

To look for monthly influence of canopy and vegetation cover, we averaged the scores for each month within the 250m buffer of each fixed sampling point location. We summed the roadkill found within each buffer and identified the average canopy and vegetation score associated with that buffer (Figure 30). To test for spatial auto correlation, we ran a Moran's I test on each 250 m buffer fixed sampling point for each surveyed month and noted if p-values were ≤ 0.05. We used a nonparametric one-way analysis (Wilcoxon Kruskal-Wallis test) in JMP to test for significant relationships. We analyzed each surveyed month separately (June-October 2016)

To examine the relationship between roadkill and the presence of road structural features such as vertical road signs, road verge slope (downhill or uphill), houses, agricultural fields, grassy fields, water and forests we used logistic regression in JMP. We analysed each surveyed season seperately (e.g. spring, summer, fall) and the number of roadkill within each season. We used a Spearman's rank correlation coefficient in JMP for a nonparametic measure of statistical dependence amoung all structural features and eliminated structural features that were highly correlated with one another (ρ=>0.7). Lastly, we used stepwise logistic regression to test the relationship between roadkill and combinations of structural features in JMP.

Within our 250 m buffers we identified structural features within its borders. We computed a principal components analysis for total number of roadkilled raccoons (*Procyon lotor*) across all surveyed months in JMP and examined their relationship with structural features (e.g. houses, water, agricultural fields, grass fields, forests, speed signs, wildlife road crossing signs) found within a 250 m buffers. Raccoons were chosen for this anaysis since they had the highest road mortality across all mammals species.

Next, we assessed the association amoung mammal species and the presence of the following land cover type within 250 m buffers of the fixed sampling points: turf, wet praire, residential, asphalt, water, savanna, shrub/scrub, swamp forest, connifers, upland forest, floodplain forest, barrens, eurassian meadow, prairie and cropland (Schetter and Root, 2013). We ran a spatial autocorrelation (Moran I) test on each 250 m buffer fixed for all seasons combined and noted if p-values were $\leq$0.05. We used a logistic regression comparing the relationship of each land cover type within each buffer to total roadkill within each buffer in JMP. Next, we used a Spearman's rank correlation coefficient in JMP for a nonparametric measure of statistical dependence among all land cover types and eliminated land cover types that were highly correlated with one another (ρ=>0.7). We used a stepwise logistic regression to test the relationship of roadkill with combination of land cover types in JMP. We analyzed all seasons combined (e.g. spring was May 1st to June 21st , summer was June 22nd to September 21st , fall was September 22nd to October 31st). Lastly, We computed a principal components analysis in JMP for total number of roadkilled raccoons and examined their relationship with land cover types within 250 m buffers of the fixed sampling pont locations we stopped at along each road. Raccoons had the highest roadkilled numbers.

We also assessed if certain land cover types along the edge of the road were related to roadkilled mammals. In ArcGIS we created a 250 by 100 m rectangular window within our 250 m buffers to identify land cover types within its borders. We used 100 m because edge effects often extend up to 50 m in fragmented landscapes and we wanted to account for both sides of the road (Murcia, 1995). Within our 250 by 100 m rectangular window, we evaluated what land cover types were present on road edges and the total number of roadkill per mamal species within each rectangular window. Using a logistic regression in JMP, we evaluated if land cover types along the edge were associated with certain roadkill species. Next, we used a Spearman's rank correlation coefficient in JMP for a nonparametric measure of statistical dependence among all land cover types along road edges and eliminated land cover types that were highly correlated with one another (ρ=>0.7). We used a stepwise logistic regression to test the relationship of roadkill associated with combinations of land cover types on road edges in JMP. We computed a principal components analysis in JMP for total number of roadkilled raccoons and examined their relationship with land cover types along the edge of the road within the 250 m by 100 m rectangular window.

Lastly, we anlayzed traffic on each road to see if they were correlated with one another. Next, we analysed traffic volume, speed, road width and length to evaluate the influence on the amount of mammal roadkill found daily across all surveyed months. To do so we calculated the number of vehicles per kilometer and roadkill per kilometer per surveyed road. We used a nonparametric one-way analysis (Wilcoxon Kruskal-Wallis test) in JMP to test for significant relationships.

Visual Surveys within the Protected Areas

From our visual survey data, we analyzed what direction each animal was moving by calculating what percentage of animals were moving ine ach cardinal and intercardinal direction (*i.e.*. N, E, S, W, NE, NW, SE, and SW) in Oak Openings Preserve and Maumee State Forest.

Environmental Variables for Visual Surveys

To test if animal movement within the parks was linked to the environmental variables of daily rainfall, humidity and temperature, we used a nonparametric one-way analysis (Wilcoxon/Kruskal-Wallis Test) of the total number of each species within both preserve per survey day across all trails in JMP. We were unable to test if animal movement was linked to monthly changes of environmental variables since we only surveyed twice a month.

Spatial Factors and Structural Features for Visual Surveys

Next, we created a 100 m buffer in ArcGIS around each canopy and vegetation fixed sampling point along each trail in Oak Openings Preserve and Maumee State Forest. We used a

100 m buffer around each canopy and vegetation point location because edge effects often extend up to 50 m in fragmented landscaped and we wanted to account for both sides of the trail (Murcia, 1995). Within each 100 m buffer we noted the presence of structural features (e.g. average canopy and vegetation score of all surveyed months combined), land cover types, and mammal species. Next, we used a logistic regression to test the relationship between structural features and land cover types to animal diversity along the trails, similar to the roadkill analysis. After, we used a Spearman's rank correlation coefficient in JMP for a nonparametric measure of statistical dependence among structural features and land cover types and eliminated those that were highly correlated (ρ=>0.7). Lastly, we used a stepwise logistic regression to assess whether mammal presence was associated with the various structural features and land cover types along the trails.

<u>Results</u>

Road Surveys

In total, 255 mammals were found dead on roads surrounding Oak Openings Preserve and Maumee State Forest (Table 14). This represented 6 dead mammals per 1 km of road. Specifically, 92 raccoons (*Procyon lotor*), 71 opossums (*Didelphimorphia*), 39 squirrels (*Sciurus niger*), 11 chipmunks (*Tamias striatus*), 14 ground hogs (*Marmota monax*), 11 skunks (*Mephitis mephitis*), 3 rabbits (*Sylvilagus floridanus*) and 14 white tailed deer (*Odocoileus virginianu*s) were found dead on roads. One roadway along the NW portion of Oak Openings Preserve, Waterville Swanton Rd., had 48% of roadkilled mammals, which was likely a corridor for mammal movement. Also, we identified 3 clusters of high values from the Getis-Ordinal Gį* test that were significant at the 99% confidence level ($z > 1.96$) and 2 clusters of high values from the Getis-Ordinal Gį* test at the 95% confidence level ($z> 1.96$) suggesting these clusters are areas of frequent terrestrial vertebrate road mortality. All 5 clusters were located on Waterville Swanton Rd. There were two clusters at the 90% confidence level that were located on Waterville Swanton Rd. and Berkley Southern Rd. (Figure 33). The number of roadkilled mammals on roadways surrounding Oak Openings preserve was significantly greater than near Maumee State Forest (Wilcoxon, $p<0.01$). Lastly, June had the highest number of roadkilled mammals (Figure 31).

Environmental Variables of Roads

The amount of roadkill mammals per day associated with rain, temperature and humidity per day was not significant. However, roadkill numbers appear to track temperature; decreasing, but not significantly, with decreasing temperatures (Figure 32). Daily rain, temperature and humidity did not influence road mortality for raccoon, opossum, ground hog, squirrel, chipmunk, rabbit, skunk or white-tailed deer.

Spatial Factors and Structural Features of Roads

We found that low to intermediate levels (average scores 1 and 2) or no canopy (average score 0) along roadways were associated with an increased mammal road mortality for all surveyed months (Wilcoxon, $p\leq0.01$).

For spring months, increased opossum road mortality was associated with low to intermediate levels of canopy (average scores of 1 and 2; Chi-Square, $p<0.03$; Table 15) along roadways, but there was no relationship between opossum road mortality and average vegetation cover along road verges. For spring months, increased squirrel road mortality was found with low to intermediate levels of canopy present along roadways (average scores of 1 and 2; Chi-Square, $p<0.01$; Table 16), but there was no significant relationship between squirrel road morality and average vegetation cover along road verges. The number of all other roadkilled mammal species was not significantly related to average canopy and vegetation cover scores in spring months (Table 16).

For summer months, there was no significant relationship between mammal roadkill and average canopy and vegetation scores (Table 16).

For fall months, increased opossum road mortality was associated with low to intermediate levels of canopy along the road (average canopy score of 1 and 2; Chi-square, $p<0.01$; Table 15), but average vegetation cover scores were not significantly related to mortality. The numbers of all other roadkilled mammal species had no significant relationship with average canopy and vegetation in fall months (Table 15).

In spring months, the presence of agricultural fields, vertical road signs and downhill verge slopes on both sides of the road were associated with increased raccoon road mortality (Chi-Square, $p\leq0.03$; Table 16). The presence of agricultural fields, grassy fields, water and downhills slopes on both sides of the road were associated with an increased opossum road mortality (Chi-Squared, $p\leq0.04$; Table 16). The presence of water was related to increased squirrel road mortality (Chi-Square, $p<0.01$; Table 16). No other species exhibited a significant relationship between road mortality and structural features for spring months (Table 16).

In summer months, the presence of houses and vertical road signs were related to an increased raccoon road mortality (Chi-square, $p\leq0.03$; Table 17). The presence of forest, water and downhill verge slopes along both sides of the road were related to an increased squirrel road mortality (Chi-square, $p\leq0.04$; Table 17). The presence of forests was associated with an increased chipmunk road mortality (Chi-square, $p<0.02$; Table 17). Lastly, the presence of forests, agricultural fields, downhill verges along both sides of the road and uphill verges along both sides of the road was related to an increased skunk road mortality (Chi-square, $p \leq 0.02$; Table 17). No other species number showed a significant relationship between mortality and these structural features for summer months (Table 17).

In fall months, the presence of houses and downhill verges on both sides of the road were related to increased roadkilled raccoons (Chi-square, $p \leq 0.03$; Table 18). For opossums, the presence of houses and vertical road signs were associated with an increased road mortality (Chi-square, $p \leq 0.03$; Table 18). Lastly, the presence of water was associated with an increased white-tailed deer road mortality (Chi-square, $p < 0.04$; Table 18). All other species exhibited no significant relationship between structural features and road mortality in fall months (Table 18).

The principal component analysis for total number of roadkilled raccoons associated with structural features (e.g. houses, agricultural fields, water, grassy fields, forests, speed signs, wildlife road crossing signs) for all surveyed months and within the 250 m buffer revealed a principal component 1 that explained 27.1% and was positively associated with grass fields, while principal component 2 explained 31.1% and was positively associated with houses (Figure 34; Figure 35; Table 22).

Next, we examined the influence of land cover on road mortality. The presence of cropland was related to an increased road mortality for raccoons (Chi-Square, $p \leq 0.01$; Table 19). For opossums, the presence of cropland was associated with an increased road mortality (Chi-squared, $p < 0.04$; Table 19). For squirrels, the presence of ponds was related to an increased road morality (Chi-Square, $p < 0.02$; Table 19). The presence of swamp forest and upland forest were associated with an increased chipmunk road mortality (Chi-square, $p < 0.02$; Table 19). Lastly, the presence of barrens was associated with increased skunk road mortality (Chi-Squire, $p < 0.01$;

Table 19). All other species exhibited no significant relationship between land cover types and the amount of road mortality (Table 19).

The principal component analysis for total number of roadkilled raccoons associated with land cover types for all surveyed months and within the 250 m buffer revealed a principal component 1 that explained 17.6% and was positively associated with floodplains, while principal component 2 explained 11.6% and was positively associated with croplands (Figure 36; Figure 37; Table 23).

We assessed the potential influence of land cover types along the edge of roads for mortality of individual mammal species. Raccoon road morality was associated with the presence of barrens along the road edge (Chi-Square, $p<0.02$; Table 20). The presence of ponds and conifers along the road edge was associated with squirrel road morality (Chi-Square, $p\leq0.04$; Table 8). All other species had no relationship with land cover types along the edge of the road and road mortality (Table 20).

The principal component analysis computed for total number of roadkilled raccoons associated with land cover types along the road edge for all surveyed months and within the 250 m by 100 m rectangular window revealed a principal component 1 that explained 17.5% and was positively associated with barrens and upland forests, while principal component 2 explained 15.1% and was positively associated with asphalt land cover types (Figure 38; Figure 39; Table 24).

Lastly, the number of roadkilled mammals per kilometers per road was not significantly related to road widths, vehicles per kilometer or road speeds for all surveyed months. Traffic between roads was also not significantly correlated.

Visual Surveys within the Protected Areas

In total, 271 mammals were seen on trails within both preserve (Table 14). This represents an average of 7 animals seen per 1 km of trail. During our visual surveys, 68% of animals were seen moving in the NW and SW direction within the Oak Openings Preserve. The NW and SW area of the preserve runs parallel to Waterville Swanton Rd., which had the highest amount of roadkill than any other surveyed road (44%). In Maumee State Forest, 58 % of animals were seen moving in the NW and SW direction. In the NW and SW area of Maumee State Forest is another large protected area. This area was not surveyed.

During our visual surveys of trails, squirrel presence along the trails was associated with low to intermediate levels of canopy (average scores of 1 and 2, Chi-Square, $p<0.04$). This was similar to spring months where there was a higher number of roadkilled squirrels associated with low to intermediate levels of canopy (average scores of 1 and 2). Presence of all other mammal species showed no significant relationship between activity on the trails to average vegetation, average canopy and land cover types along surveyed trails.

Lastly, mammal movement within the parks was not influenced by environmental variables of daily rainfall, humidity and temperature.

Our models that explored combinations of variables had very little explanatory power (i.e.. r^2 ranged from 0.07-0.47) and few or no significant relationships, which suggest a very complex response to factors across species or one that is not detectable with our data (Table 21).

<u>*Discussion*</u>

Road Surveys

By analyzing environmental variables, spatial factors and structural features of roads, our study assessed the effects roads and traffic had on mammal species in a highly fragmented human-dominated landscape. Roadkill provides a tool that combined with visual surveys within protected areas, helps us better understand mammal movement patterns. A total of 255 mammals were found dead on roads surrounding Oak Openings Preserve and Maumee State Forest, dominated by mesocarnivores (e.g. raccoons and opossums). These species are usually generalists, with broad dietary and habitat requirements. They are also very tolerant of fragmentation and human presence, so they are commonly found in suburban and urban areas (Prange and Gehrt, 2004). The Oak Openings Region is highly fragmented and consists of many urban and suburban areas, so mesocarnivores may take advantage of artificial food resources that area reliable and spatially fixed (Prange, et al., 2004). Therefore, animals may risk crossing the road to access different food resources. Mesocarnivores are known to live near roads, levees or other linear features as long as the surrounding habitat remains suitable (Frey and Conover, 2006). If animals live near roads, the chances of animal-vehicle collisions remain high. Moreover, roads provide many resources for generalist species, including: spilled grains, roadside plants, insects, basking vertebrates, roadkill, agricultural fields and road salts (Smith-Patten and Patten, 2008). Since mesocarnivores are generalist species, they may utilize the resources that roads provide, increasing the chance of road mortality.

Our results are similar to other mammal roadkill studies in which there are high roadkill numbers since they are easily detected and scavenging rates for larger mammals are lower. However, other studies tend to survey for longer periods of time and on more roadways. In a study conducted by Barthelmess (2014) in Northern New York State in the Adirondack foothills and Lawrence River valley lowlands, 309 roadkilled mammals were detected from March 2007-May 2008 on 7,094 km of road (Barthelmess, 2014). However, in Northern New York State there is less human activity and presence, which suggest that Oak Openings Preserve and Maumee State Forest may have higher roadkill numbers because of the artificial food resources that humans provide for generalist species. Also, the study area is highly fragmented and the resources available (human or natural) are in separate patches requiring movement across roads. Another example is a mammal roadkill study conducted by Kang et al., (2016) from 2006-2012 in protected areas of the Korean Peninsula, which found 1,070 roadkilled mammals (1.09 kills per kilometer of roadway) (Kang et al, 2016). Again, our results are much higher in that we found 6 kills per 1 km of roadway. Similar to the mammal roadkill study conducted in Northern New York State, this area of Korea does not have a lot of human activity and presence, suggesting artificial food resources could be an attractant for generalist species. Also, Oak Openings Preserve and Maumee State Forest are much more fragmented than the study areas in Northern New York State and Korea causing an animal to encounter a road much more often, risking animal vehicle collisions.

Waterville Swanton Rd. had the highest road mortality, with 48% of the roadkilled mammals. As we predicted, this area seemed to be a corridor for mammal movement since it bordered Oak Openings Preserve and provided a direct route to Maumee State Forest. Our visual surveys confirmed that a large proportion of the detected mammals were in the areas immediately adjacent to this road and would be, likely, be the source of the roadkill found. Management practices within Oak Openings Preserve are designed to mimic historic disturbances with prescribed fires, thinning or mowing, and animals may move in or out of these disturbed areas as resources change prompting movement between the two parks (Abella et al, 2004; Howard, 1960).

The number of roadkilled mammals around Oak Openings Preserve was significantly greater than roadkilled mammals found on roads surrounding Maumee State Forest. Oak Opening Preserve is more heterogeneous with a greater variety of habitats than Maumee State Forest. Maumee State Forest primarily consists of swamp forest, upland forest, conifers and floodplain forest. Therefore, more resources may be available in Oak Openings Preserve, which could attract more animals from Maumee State Forest while also providing a source of dispersing animals as densities increase.

The month of June had the highest amount of roadkilled mammals (Figure 8). Mammals in the northern region experience seasonal environments, with population growth in the summer resulting from reproduction and declines in the winter from the reduction of available resources and increased competition (Merritt et al, 2001). Therefore, the detected increase in roadkilled mammals in June may have been a result of the increase in population sizes. The month of June is a time when many juvenile mammals may be dispersing to new areas. According to Dobson (1982), juvenile mammals that remain in natal areas may have to compete for resources with related individuals. So, many juvenile mammals may disperse to new areas in search of territory, food, shelter and potential mates, and in doing so may risk crossing a road (Dobson, 1982). Also, June had an average temperature of 22 °C, while other months had high average temperatures of 28.6 °C (July), and low average temperatures of 11.6 °C (October). This suggests that June may have had temperatures that promoted mammal activity since mammals are known to be less active when temperatures are very low or very high (Garriga et al, 2017; Bennie et al, 2014).

Environmental Variables of Roads

The total number of roadkilled mammals per day and individual species per day was not significantly related to daily rain, temperature and humidity per day. This is in contrast, though, to previous studies. According to a roadkill study conducted by Garriga et al. (2017) in Catalonia, roadkill was highest in months with more precipitation and warmer temperatures, although it varies between species (Garriga et al., 2017). However, Catalonia has high climatic variability, with annual rainfall ranging from 400 -1,000 mm and temperatures ranging from 16°C to 26°C. Within our study, the total amount of rainfall across our surveys was 4.3 cm and daily average temperatures reached a maximum of 33 °C and a low of 8°C. Even though the relationship between roadkill and temperature was not significant in our study, roadkill seems to track temperatures with a higher number of roadkilled mammals associated with higher temperatures. These results suggest that temperature, humidity and rainfall may affect each species differently due to life history and these effects may vary by location. In the study conducted by Garriga et al, (2017), mammal roadkill was positively related to temperature, which appeared to be related to mammal activity (Garriga et al, 2017). Mammals are known to be less active when temperatures are very low or very high (Garriga et al, 2017; Bennie et al, 2014). Based on our data, June 6th 2016 had an average daily temperature of 33.7 °C and had the lowest numbers of roadkilled mammals detected only five animals, suggesting that temperatures were too extreme for high mammal activity. Also, October 24th had a low average temperature of 8 °C and only four mammals were found dead on roads suggesting temperatures were too cold for high mammal activity. However, June 3rd 2016 had an average daily temperature of 20 °C and had the highest number of dead mammals detected of 14 individuals, suggesting that temperatures were appropriate for increased mammal activity.

Spatial Factors and Structural Features of Roads

Low to Intermediate levels of ground vegetation cover (average score 1 and 2) or no canopy (average score 0) were characteristics associated with an increased mammal road mortality for all surveyed months. Low levels of ground vegetation may promote animal movement since it is less of a barrier and easier to move through. Thicker vegetation along the roads may decrease road kill probability because risk of predation. Certain mammal species may prefer thicker vegetation to remain hidden from predators. According to Camp et al. (2012), habitat and vegetation structure can influence predator prey interactions. Vegetation structure allows an animal to remain concealed, detect approaching predators, and provides refuge from predators (Camp et al., 2012). Therefore, denser vegetation along roads may decrease the risk of a mammal crossing the road since it provides protection against predators and refuge. No canopy along the roads corresponded with an increased road mortality for mammals in our study. One explanation for this effect is that denser canopy can provide for arboreal movements, allowing an animal to safely cross above the road (Chen and Koprowski, 2016). According to a study conducted by Chen and Koprowski (2016), red squirrels were less likely to cross roads with gaps in canopy cover compared to areas with many trees and dense canopy (Chen and Koprowski, 2016). Gaps in canopy can inhibit animal movement or increase road mortality, so forest management such as thinning that opens forest canopy can increase barrier effects and habitat fragmentation (Forman and Alexander, 1998).

We found a variety of seasonal effects of average ground vegetation and canopy scores for individual mammal found roadkilled. In spring and fall months, both opossum and squirrel road mortality was associated with low to intermediate levels of canopy along roadways. Both opossums and squirrels use trees and canopy for arboreal movements, so fewer trees and less canopy along the roads may increase road mortality since they may cross the road instead of using tree canopy as a bridge. Both of these species are strongly associated with forests, and increased road mortality rates are known to be associated with forest dwelling species that have large distances to move between forest margins when crossing a roadway (Oxley, 1974). This relationship did not hold for summer months for opossums and squirrels. Perhaps in the summer months there was adequate canopy and ground vegetation along the roads to allow for successful arboreal crossing or provided refuge and protection from predators. We did not find a significant relationship between canopy and vegetation cover with the number of roadkilled animals of other mammal species. However, many of the other forest dwelling mammal species found dead were in low numbers and further research may be needed to detect a relationship, if one exists.

Structural features along the road were associated with roadkilled mammals, but varied between species and months. Specifically, the presence of agricultural fields (in spring), vertical road signs (spring and summer), houses (summer and fall) and downhill verges on both sides of the road (spring and fall) were associated with raccoon road mortality. Again, raccoons are a generalist species that can thrive in many different environments. According to a study conducted by Bozek et al, (2007), human use areas are used more frequently by raccoons than other environments, like forests, aquatic or grassy habitats. Also, home ranges of raccoons living in rural areas were significantly larger than raccoons living in suburban areas since they may be searching for resources (Bozek et al, 2007). This is because raccoons take advantage of the artificial food resources than humans provide. Therefore, raccoons may be drawn to houses and agricultural fields in search of resources and may risk crossing a road, resulting in animal vehicle collisions. The association of roadkilled raccoons with vertical road signs is not clear. However, vertical road signs could change human driving behavior, which can make a vehicle a less predictable “predator” to an animal, although further analysis is needed. Downhill verges on both side of the road may be associated with roadkilled raccoons because they may reduce animal visibility. According to Camp et al., (2012), animal visibility is “the property that provides sightlines to allow a prey animals to visually detect oncoming predators” (Camp et al., 2012). If a vehicle is considered a “predator”, downhill verges may not provide adequate sightlines to see when a vehicle is coming down the road, therefore causing an animal to be hit by a vehicle.

The presence of agricultural fields (spring), grassy fields (spring), water (spring), houses (fall), vertical road signs (fall) and downhill verges on both sides of the road (spring) were also associated with opossum road mortality. Similar to raccoons, opossums are generalist species that can live in many different habitats and may be attracted to the resources agricultural fields and houses provide. Therefore, an opossum my risk crossing a road to access artificial food resources. Also, downhill verges on both sides of the road may reduce road visibility for opossums, increasing the chance of animal vehicle collisions. Grassy fields may provide adequate habitat for opossums in which it may provide shelter and refuge from predators. Lastly, the presence of vertical road signs could change human behavior, which can make a vehicle a less predictable “predator”, but this relationship needs further analysis.

The presence of water (spring and summer), forests (summer) and downhill verges along both sides of the road (summer) was associated with squirrel road mortality. For obvious reasons, water provides many different resources that squirrels may be attracted to, and therefore may risk crossing roads to access water sources. Also, squirrels are forest dependent species, so the presence of forests along the road may increase the chances of squirrels crossing the road (Chen and Koprowski, 2016). Again, downhill verges along both sides of the road may limit a squirrel’s visibility to see an oncoming vehicle.

Similar to squirrels, the presence of forests was associated with increased chipmunk road mortality in summer months. According to a study conducted by Bider, (1968), eastern chipmunk tend to be more abundant in mature forests and near field-forest ecotones (Bider, 1968). Since the Oak Openings Region is highly fragmented, there are many areas that have patches of forests near agricultural or grassy fields. Therefore, there may be more roadkilled chipmunks with the presence of forests near the road. Also, similar to squirrels, chipmunks are forest dependent species.

The presence of forests, agricultural fields, and downhill verges along both sides of the road and uphill verges along both sides of the road was related to an increase in skunk road morality in summer months. Again, skunks are forest dwelling animals, and use forests for many different resources. Interestingly, skunks have impressive arboreal agility, hunt in trees and use trees as escape routes from predators (Brashear et al., 2010). Therefore, if forests are present along roads, skunks may be more likely to cross the road and risk road mortality. Also, the presence of agricultural fields may result in skunk road mortality for several reasons. Agricultural fields are known to act as food resources and breeding habitat for migrating birds, and skunks are known to prey on the eggs of migrating birds. Also, skunks are known to prefer habitats associated with cropland and farm seeds since these habitats provide resources (Crimmins et al., 2015). Similar to other species in our study, downhill verges along both sides of the road and uphill verges along both sides of the road may be associated with skunk road mortality because it could limit the animal’s visibility to see an oncoming vehicle.

In fall, the presence of water along the side of the road was associated with white-tailed deer road mortality. Water sources are used by deer are common during mornings and evening hours, and deer are known to return to the same water source areas (Boroski and Mossman, 1997). Since deer drink water often and return to the same water resource areas, this may increase their chances of crossing roads with water nearby, risking road mortality. Spring and summer did not have many roadkilled deer, so this relationship of road mortality associated with water present near roads was not significant.

Next, the presence of land cover types was related to species road mortality. The presence of cropland was related to an increased road mortality for raccoons. Again, raccoons are a generalist species and may take advantage of the resources croplands provide. Prey species like young migratory birds or small mammals are associated with croplands and raccoons may be able to easily prey upon these species in agricultural areas (Crimmins et al., 2015). Opossum road morality was also associated with cropland areas along the road and similar to raccoons, may utlize croplands for finding prey or other resources. Squirrel road mortality was associated with the presence of ponds, which not only acts as a water source but may provide other food resources squirrels forage for. The presence of swamp forest and upland forest was associated with an increased chipmunk road mortality. Since both of these land cover types provide suitable habitat for forest dependent species like chipmunks, it is not suprising that an increased road mortality in chipmunks was associated with these land cover types. Lastly, the presence of skunk road mortality was associated with the presence of barrens near the road. Barrens are important habitat for many species including the lark sparrow (*Chondestes grammacus*), green salamander (*Aneides aeneus*), piping plover (*Charadrius melodus*), easatern pheoebe (*Sayornis phoebe*), and eastern box turtle (*Terrapene carolina*). Therefore, skunks may be attracted to this habiat in search of eastern-box turtle eggs, bird eggs, or juvenile species they can prey upon, and may encounter roads in which can increase the chance of vehical collisions. No other species exhibited a relationship with the presence of land cover types near the road and road mortality.

Land cover types along the edge of the road were related to some roadkilled mammal species. Raccoon road mortality was associated with the presence of barrens along the edge of the road. Raccoons forage for many resources, including eggs. As previously stated, barrens are a habitat in which birds or turtles are known to nest and lay eggs in which raccoons may forage for. Similar to our other results, the presence of ponds along the edge of the road was associated with squirrel road mortality. Ponds act as a water sources for many different species and provide other resources in which squirrels may take advantage of and risk crossing a road. Therefore, if these land cover types are located along road edges, these species may cross roads more often, increasing the chance animal vehichal collisions.

We did not find a significant relationship between mammals and road widths, vehicles per kilometer or road speeds for all surveyed months. However, other studies have found that roadkill is associated with speed and traffic. For example, in a study conducted by Jaeger et al, (2005) in which they analyzed the effects of roads and traffic on animal populations by creating models, it was discovered that small roads (one lane in each direction) or large roads (two or more lanes in each direction) with high traffic intensity resulted in higher road avoidance in animal populations. So, roads that have less traffic, with less noise pollution may have increased animal vehicle collisions (Jaeger et al, 2005). Also, according to Trombulak and Frissell (2000) in a review of the ecological effects of roads, high speed roads (96 -120 kph) usually are associated with less roadkill than on medium-speed roads (72-88 kph) because high speed roads have cleared vegetation along the edge, which creates less attractive habitat and greater visibility for both animals and drivers (Trombulak and Frissell, 2000). In our study area, road speed limit was between 80 kph (50 mph) and 88 kph (55 mph) for all roads, with as low as 12 to as high as 1,092 vehicles per hour. Roads with high traffic intensity (1,092 per hour) had low total roadkill numbers (26 dead mammals) whereas roads with medium traffic intensity (250 per hour) had the highest total roadkill numbers (110 dead mammals). Our results are similar to the results of Jaeger et al, (2005) and Trombulak and Frissell (2000) since high traffic intensity had less roadkill suggesting higher road avoidance and medium speed roads had higher roadkill numbers than high speed roads (Jaeger et al, 2005; Trombulak and Frissell, 2000).

Visual Surveys within the Protected Areas

During our visual surveys, 68% of mammal movement was primarily seen in the NW and SW portion of the Oak Openings Preserve, which highlighted a potential corridor for mammal movement and an area that warrants potential management attention to reduce risks. For example, our results suggest that canopy structure was a large influence on squirrel presence and management actions that targeted canopy cover could mitigate squirrel road mortality. Also, our results indicate that forest patterns matters. If the forests are fragmented as in the Oak Opening Region, there is likely to be more mortality as animals move from one patch to another, especially if roads separate patches. Also, dense forests characterize Maumee State Forest, and many forest dwelling species may be attracted to this area. Therefore, forest dwelling species may be at high risk for animal vehicle collisions since they may leave Oak Openings Preserve to access the thick forests of Maumee State Forest.

Lastly, animal movement within the parks was not influenced by environmental variables of daily rainfall, humidity and temperature. This may be because we did not survey enough to see daily or monthly relationships of animal movement with environmental variables. However, other studies suggest that animal movement is related to environmental variables. For example, in a study conducted by Webb et al. (2009) in Oklahoma found that monthly rainfall was linked to an increase in movement of white-tailed deer because of increased abundance of forage (Webb et al., 2009). Another study conducted by Bennie et al, (2014) used mammals as a case study and found that mammals have thermal limits, and that activity is greatly reduced and there are high physiological costs when temperatures are too high or too low. Therefore, we may need to survey more often or throughout the year to get a range of temperatures to understand if animal movement and activity within our study area is related to environmental variables.

The stepwise models with combinations of variables had very little explanatory power and suggested a very complex response to factors and across species, which could be caused by several reasons. First, there could be high variation of structural features and land cover types between roads and trails that we did not successfully capture within the study areas. Second, we may have needed to sample more often along roads and trails for certain variables, like canopy or ground vegetation cover. Third, the scale at which we sampled may have not been able to accurately capture a combination of variables to understand mammal movement and road mortality. Lastly, sample sizes of certain variables or species may have been too small to accurately assess mammal movement and road mortality across the landscape. Therefore, while some significant relationships were detected, caution is warranted as there is a temporal and spatial complexity within these two protected areas, which is typical of human-dominated landscapes.

Conclusions & Management Implications

Our results indicate that in order to understand mammal movement and road mortality it is important to incorporate road and visual surveys as well as consider environmental variables, spatial factors and structural features of roads. In this study, we used roadkill as a tool to understand mammal movement and mortality in a highly-fragmented human dominated landscape. Based on our results, there is no single variable that influences mammal movement and mortality, and the influence of variables varied over time and space and differ across mammal species. However, management can alter structural features of roads to reduce road morality and animal movement. For example, allowing dense vegetation or canopy along roads may reduce animal-vehicle collisions for a number of mammal species discussed (e.g. raccoons, opossums, squirrels, chipmunks). Denser vegetation along roads may provide a more suitable habitat and protection against predators, therefore decreasing animal movement and road crossing events. Denser canopy along roads has also been linked to a decrease in roadkill because denser canopy allows arboreal movements to cross the road (Chen and Koprowski, 2016). Also, canopy levels seemed to be associated with animal presence within the parks and on the roads, so canopy structure can be managed to reduce road mortality. Likewise, mangers can identify structural features that may deter species presence or road crossings to prevent animal-vehicle collisions. Lastly, we identified certain land cover types along the edge of the road and within the reserves that animals find attractive and increase the change of crossing the road. Managers can identify the locations of these land cover types and can create wildlife crossing structures, (e.g. culverts, land bridges) or wildlife road crossing signs to allow for successful animal road crossing and increase human awareness. To finish, our approach can predict animal movement and identify structural features that may be managed to reduce road mortality, which is likely to be applicable to other protected areas in human-dominated landscapes.

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Literature Cited

Albella, S. R., Jaeger, J. F., & Brewer, L. G. (2004). Fifteen years of plant community dynamics during a northwest Ohio oak savanna restoration. *The Michigan Botanist*, *43*(2).

Abella, S. R., Jaeger, J. F., & Schetter, T. A. (2007). Public land acquisition and ecological restoration: an example from northwest Ohio's Oak Openings region. *Natural Areas Journal*, *27*(1), 92-97.

Barthelmess, E. L. (2014). Spatial distribution of road-kills and factors influencing road mortality for mammals in Northern New York State. *Biodiversity and conservation*, *23*(10), 2491-2514.

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.

Bider, J. R. (1968). Animal activity in uncontrolled terrestrial communities as determined by a sand transect technique. *Ecological Monographs*, *38*(4), 269-308.

Brashear, W. A., Dowler, R. C., & Ceballos, G. (2010). Climbing as an escape behavior in the American hog-nosed skunk, *Conepatus leuconotus*. *Western North American Naturalist*, *70*(2), 258-260.

Boroski, B. B., & Mossman, A. S. (1998). Water use patterns of mule deer (*Odocoileus hemionus*) and the effects of human disturbance. *Journal of Arid Environments*, *38*(4), 561-569.

Bozek, C. K., Prange, S., & Gehrt, S. D. (2007). The influence of anthropogenic resources on multi-scale habitat selection by raccoons. *Urban Ecosystems*, *10*(4), 413-425.

Brewer, L. G., & Vankat, J. L. (2004). Description of Vegetation of the Oak Openings of Northwestern Ohio at the Time of Euro-American Settlement1. *The Ohio Journal of Science*, *104*(4), 76.

Camp, M. J., Rachlow, J. L., Woods, B. A., Johnson, T. R., & Shipley, L. A. (2012). When to run and when to hide: the influence of concealment, visibility, and proximity to refugia on perceptions of risk. *Ethology*, *118*(10), 1010-1017.

Chen, H. L., & Koprowski, J. L. (2016). Barrier effects of roads on an endangered forest obligate: influences of traffic, road edges, and gaps. *Biological Conservation*, *199*, 33 40.

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.

Crimmins, S. M., Walleser, L. R., Hertel, D. R., McKann, P. C., Rohweder, J. J., & Thogmartin, W.E. (2015). Relating mesocarnivore relative abundance to anthropogenic land-use with a hierarchical spatial count model. *Ecography*.

D'Amico, M., Román, J., de los Reyes, L., & Revilla, E. (2015). Vertebrate road-kill patterns in Mediterranean habitats: who, when and where. *Biological Conservation*, *191*, 234 242.

Dobson, F. S. (1982). Competition for mates and predominant juvenile male dispersal in mammals. *Animal behaviour*, *30*(4), 1183-1192.

Forman, R. T. (2003). *Road ecology: science and solutions*. Island Press.

Forman, R. T., & Alexander, L. E. (1998). Roads and their major ecological effects. *Annual Review of Ecology and Systematics*, *29*(1), 207-231.

Frey, S. N., & Conover, M. R. (2006). Habitat use by meso-predators in a corridorenvironment. *Journal of Wildlife Management*, *70*(4), 1111-1118.

Greenwood, P. J. (1980). Mating systems, philopatry and dispersal in birds and mammals. *Animal Behaviour*, *28*(4), 1140-1162.

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.

Gibbs, J. P. (1998). Amphibian movements in response to forest edges, roads, and streambeds in southern New England. *The Journal of Wildlife Management*, 584-589.

Goheen, J. R., & Swihart, R. K. (2005). Resource selection and predation of North American red squirrels in deciduous forest fragments. *Journal of Mammalogy*, *86*(1), 22-28.

Grigore, M. T. (2004). *Living in the Oak Openings: a homeowner's guide to one of the world's last great places*. Homewood Press.

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.

Hewes, L. (1951). The northern wet prairie of the United States: Nature, sources of information, and extent. *Annals of the Association of American Geographers*, *41*(4), 307-323.

Host, G. E., Pregitzer, K. S., Ramm, C. W., Hart, J. B., & Cleland, D. T. (1987). Landform mediated differences in successional pathways among upland forest ecosystems in northwestern Lower Michigan. *Forest Science*, *33*(2), 445-457.

Howard, W. E. (1960). Innate and environmental dispersal of individual vertebrates. *American Midland Naturalist*, 152-161.

Jaeger, J. A., Bowman, J., Brennan, J., Fahrig, L., Bert, D., Bouchard, J., & von Toschanowitz, K. T. (2005). Predicting when animal populations are at risk from roads: an interactive model of road avoidance behavior. *Ecological modelling*, *185*(2), 329348.

Kang, W., Minor, E. S., Woo, D., Lee, D., & Park, C. R. (2016). Forest mammal roadkills as related to habitat connectivity in protected areas. *Biodiversity and Conservation*, *25*(13), 2673-2686.

Lettow, M. C., Brudvig, L. A., Bahlai, C. A., & Landis, D. A. (2014). Oak savanna management strategies and their differential effects on vegetative structure, understory light, and flowering forbs. *Forest Ecology and Management*, *329*, 89-98.

Murcia, C. (1995). Edge effects in fragmented forests: implications for conservation. *Trends in ecology & evolution*, *10*(2), 58-62.

Merritt, J. F., Lima, M., & Bozinovic, F. (2001). Seasonal regulation in fluctuating small mammal populations: feedback structure and climate. *Oikos*, *94*(3), 505-514.

Oxley, D., Fenton, M. B., & Carmody, G. R. (1974). The effects of roads on populations of small mammals. *Journal of Applied Ecology*, 51-59.

Prange, S., & Gehrt, S. D. (2004). Changes in mesopredator-community structure in response to urbanization. *Canadian Journal of Zoology*, *82*(11), 1804-1817.

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.

Roemer, G. W., Gompper, M. E., & Van Valkengurgh, B. (2009). The ecological role of the mammalian mesocarnivore. *BioScience*, *59*(2), 165-173.

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 and ecological engineering*, *11*(1), 87-99.

Schetter, T. A., T. Walters and Root, K.V. 2013. A multi-scale spatial analysis of native and exotic plant species richness within a mixed-disturbance oak savanna landscape. *Environmental Management* 52(3): 581-594

Schetter, T. A., & Root, K. V. (2013). Assessing an imperiled oak savanna landscape in northwestern Ohio using landsat data. *Natural Areas Journal*, *31*(2), 118-130.

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.

Spellerberg, I. A. N. (1998). Ecological effects of roads and traffic: a literature review. *Global Ecology and Biogeography*, *7*(5), 317-333.

Trombulak, S. C., & Frissell, C. A. (2000). Review of ecological effects of roads on terrestrial and aquatic communities. *Conservation biology*, *14*(1), 18-30.

Webb, S. L., Riffell, S. K., Gee, K. L., & Demarais, S. (2009). Using fractal analyses to characterize movement paths of white-tailed deer and response to spatial scale. *Journal of Mammalogy*, *90*(5), 1210-1217.

Table 13. Canopy cover scores as it relates to light meter estimates. Canopy cover was scored from 0-5 with 0 representing no canopy and 5 representing dense canopy.

Canopy Score	**Light Meter (LUX, lumen/m^2)**
0	1450 or higher
1	1250 - 1449
2	950 - 1249
3	640 - 949
4	400 - 639
5	399 or below

Table 14. Displaying the number of mammals observed per season killed on roads surrounding and within Oak Openings Preserve and Maumee State Forest in Northwest Ohio. Also, the number of mammals per season observed on trails during visual surveys within both preserves.

	Mammals Killed on Roads		Mammal Visual Surveys	
	Oak Openings	Maumee	Oak Openings	Maumee
Spring	55	21	84	2
Summer	69	41	103	29
Fall	44	25	42	10

Table 15. Significant logistic regression values of average canopy and vegetation scores associated with the observed roadkilled mammal species per season based on the Chi-square test. Significant values displayed in bold.

	Raccoon	*Opossum*	*Ground hog*	*Squirrel*	*Chipmunk*	*Rabbit*	*Skunk*	*White-Tailed Deer*
Spring	N/S	**Vegetation Score: 1, 2 p<0.03**	N/S	**Canopy Score: 1, 2 p<0.01**	N/S	N/S	N/S	N/S
Summer	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S
Fall	N/S	**Canopy Score: 1, 2 p<0.01**	N/S	N/S	N/S	N/S	N/S	N/S

Table 16. Significant logistic regression values of structural features associated with observed roadkilled mammal species for spring months based on the Chi-square test. Significant values displayed in bold. Roadkill species that had no significance with structural features were eliminated.

	Raccoon	*Opossum*	*Squirrel*
Vertical road signs	**$X^{2=}<0.05$ $p\leq0.03$**	N/S	N/S
House	N/S	N/S	N/S
Agricultural Field	**$X^{2=}<0.05$ $p\leq0.03$**	**$X^{2=}<0.05$ $p\leq0.01$**	N/S
Grassy Field	N/S	N/S	N/S
Forests	N/S	N/S	N/S
Water	N/S	N/S	**$X^{2=}<0.05$ $p<0.01$**
Verge Slope: Downhill Both Sides of Road	**$X^{2=}<0.05$ $p\leq0.03$**	**$X^{2=}<0.05$ $p\leq0.01$**	N/S

Table 17. Significant logistic regression values of structural features associated with observed roadkilled mammal species for summer months based on the Chi-square test. Significant values displayed in bold. Roadkill species that had no significance with structural features were eliminated.

	Raccoon	*Opossum*	*Squirrel*	*Chipmunk*	*Skunk*
Vertical road signs	**$X^{2=}<0.05$** **$p\leq0.03$**	N/S	N/S	N/S	N/S
House	**$X^{2=}<0.05$** **$p\leq0.03$**	N/S	N/S	N/S	N/S
Agricultural Field	N/S	**$X^{2=}<0.05$** **$p\leq0.01$**	N/S	N/S	**$X^{2=}<0.05$** **$p\leq0.02$**
Grassy Field	N/S	N/S	N/S	N/S	N/S
Forests	N/S	N/S	**$X^{2=}<0.05$** **$p\leq0.04$**	**$X^{2=}<0.05$** **$p<0.02$**	**$X^{2=}<0.05$** **$p\leq0.02$**
Water	N/S	N/S	**$X^{2=}<0.05$** **$p\leq0.04$**	N/S	N/S
Verge Slope: Downhill Both Sides of Road	N/S	**$X^{2=}<0.05$** **$p\leq0.01$**	**$X^{2=}<0.05$** **$p\leq0.04$**	N/S	**$X^{2=}<0.05$** **$p\leq0.02$**
Verge Slope: Uphill on Both Sides of Road	N/S	N/S	N/S	N/S	**$X^{2=}<0.05$** **$p\leq0.02$**

Table 18. Significant logistic regression values of structural features associated with observed roadkilled mammal species for fall months based on the Chi-square test. Significant values displayed in bold. Roadkill species that had no significance with structural features were eliminated.

	Raccoon	*Opossum*	*Squirrel*	*Chipmunk*	*Skunk*	*White-Tailed Deer*
Vertical road signs	N/S	**$X^{2=}<0.05$** **$p\leq 0.03$**	N/S	N/S	N/S	N/S
House	**$X^{2=}<0.05$** **$p\leq 0.03$**	**$X^{2=}<0.05$** **$p\leq 0.03$**	N/S	N/S	N/S	N/S
Agricultural Field	N/S	N/S	N/S	N/S	**$X^{2=}<0.05$** **$p\leq 0.02$**	N/S
Grassy Field	N/S	N/S	N/S	N/S	N/S	N/S
Forests	N/S	N/S	**$X^{2=}<0.05$** **$p\leq 0.04$**	**$X^{2=}<0.05$** **$p<0.02$**	**$X^{2=}<0.05$** **$p\leq 0.02$**	N/S
Water	N/S	N/S	**$X^{2=}<0.05$** **$p\leq 0.04$**	N/S	N/S	**$X^{2=}<0.05$** **$p<0.04$**
Verge Slope: Downhill Both Sides of Road	**$X^{2=}<0.05$** **$p\leq 0.03$**	N/S	**$X^{2=}<0.05$** **$p\leq 0.04$**	N/S	**$X^{2=}<0.05$** **$p\leq 0.02$**	N/S
Verge Slope: Uphill on Both Sides of Road	N/S	N/S	N/S	N/S	**$X^{2=}<0.05$** **$p\leq 0.02$**	N/S

Table 19. Significant logistic regression values of land cover types within a 250 m buffer associated with observed roadkilled mammal species for all surveyed months based on the Chi-square test. Significant values displayed in bold. Land cover types with no significant relationship to any roadkilled species were excluded. Species that had no significance to the presence of land cover types were eliminated.

	Raccoon	*Opossum*	*Squirrel*	*Chipmunk*
Cropland	**$X^{2=}$<0.05** **$p\leq0.01$**	**$X^{2=}$<0.05** **$p<0.04$**	N/S	N/S
Ponds	N/S	N/S	**$X^{2=}$<0.05** **$p<0.02$**	N/S
Swamp Forest	N/S	N/S	N/S	**$X^{2=}$<0.05** **$p<0.02$**
Upland Forest	N/S	N/S	N/S	**$X^{2=}$<0.05** **$p<0.02$**

Table 20. Significant logistic regression values of land cover types along the edge of the road associated with observed roadkilled mammal species for all surveyed months based on the Chi-square test. Significant values displayed in bold. Land cover types with no significant relationship to any roadkilled species were excluded. Species with no significant relationship to land cover types along the edge of the road were eliminated.

Landcover	*Raccoon*	*Squirrel*
Ponds	N/S	**$X^{2=}<0.05$** **$p<0.04$**
Barrens	**$X^{2=}<0.05$** **$p<0.02$**	N/S

Table 21. Model results, including degrees of freedom (DF), chi-square (X^2), significance (p-value), correlation (R^2) and Akaike's Information Criterion (AICc) for each model that examined all structural features and land cover types affecting presence of observed roadkilled mammal species in the Oak Openings Preserve and Maumee State Forest of Northwest Ohio. Species that did not have a large sample size were eliminated.

	Model	DF	X^2	P <0.05	R^2	AICc
All Mammals	Turf + Savanna + Swamp Forest + Floodplain + Cropland	5	32.2	<0.0001	0.47	49.07
Raccoon	Vertical Road Sign + Savanna	2	12.8	<0.0017	0.13	91.9
Opossum	Water + Cropland	2	12.2	<0.0022	0.13	82.7
Squirrel	Pond	1	5.1	<0.0236	0.06	80.0
Chipmunk	Upland Forest	1	13.2	<0.0003	0.24	45.2

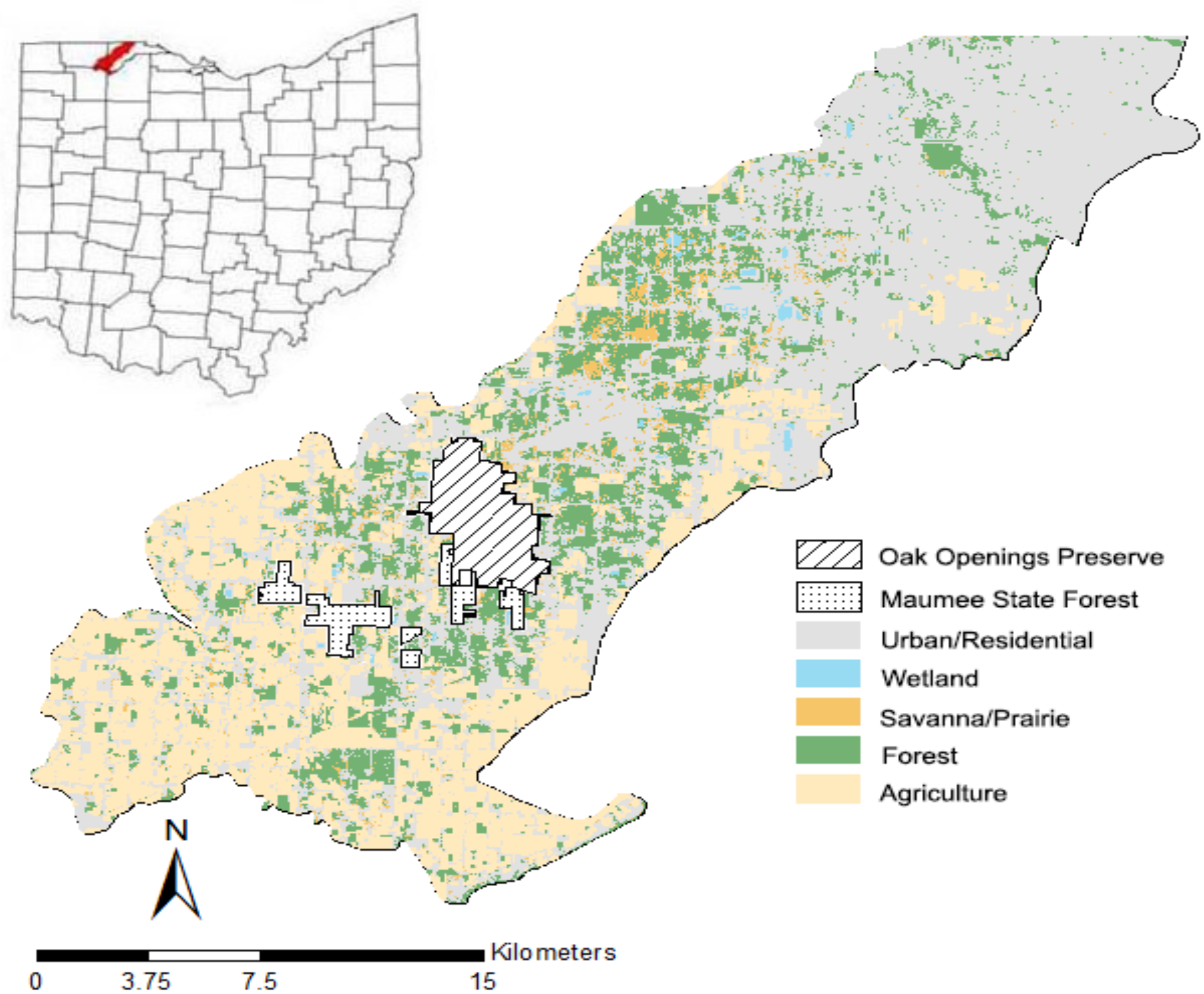

Figure 24. Oak Openings Region (red) in Northwest Ohio is a mosaic of remnant forest, savannas, and wetlands within an agricultural and urban/residential matrix (Schetter and Root, 2011). Oak Openings Preserve and Maumee State Forest are shown in hatched and dot patterns, respectively.

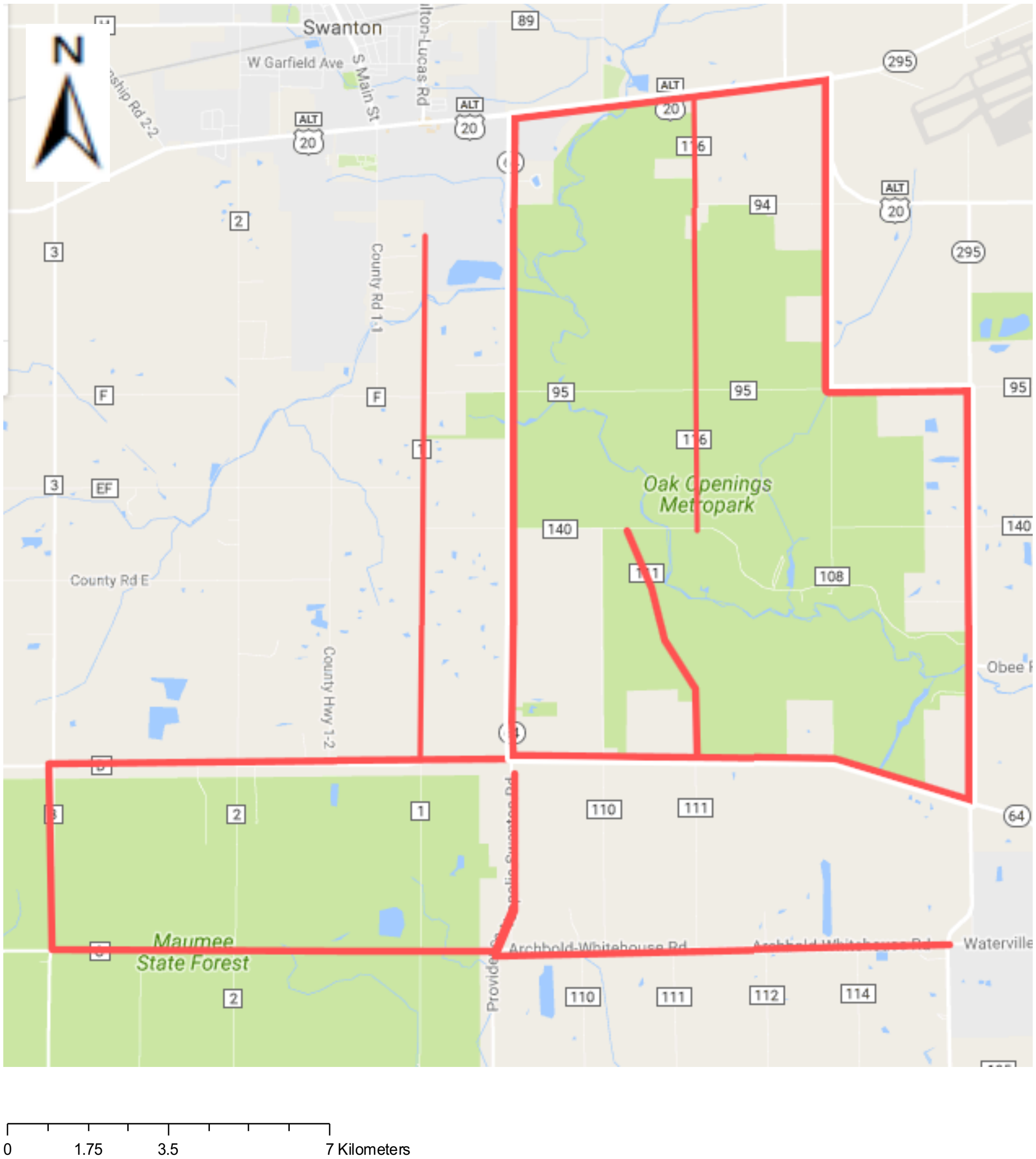

Figure 25. Shown is a map of the roads (highlighted in red) that were survyed in and near Oak Opening Preserve and Maumee Sate Forest in the Oak Openings Region of Northwest Ohio.

Figure 26. Photos of canopy cover from field surveys taken with a digital camera. Photo taken every 500 meters of trail and 6 meters from trail edge. Scored from 0-5. Canopy cover with a score of a 0 meant no canopy visible. Score of 1 top left. Score of 2 top middle. Score of 3 top right. Score of 4 bototom left. Score of 5 bottom right.

Figure 27. Vegetation density scores in relation to photographs taken with a digital camera. Photo taken every 500 meters along the trail and 6 meters from trail edge. Score of a 0 is no vegetation. From left to right is score 1-5 respectively.

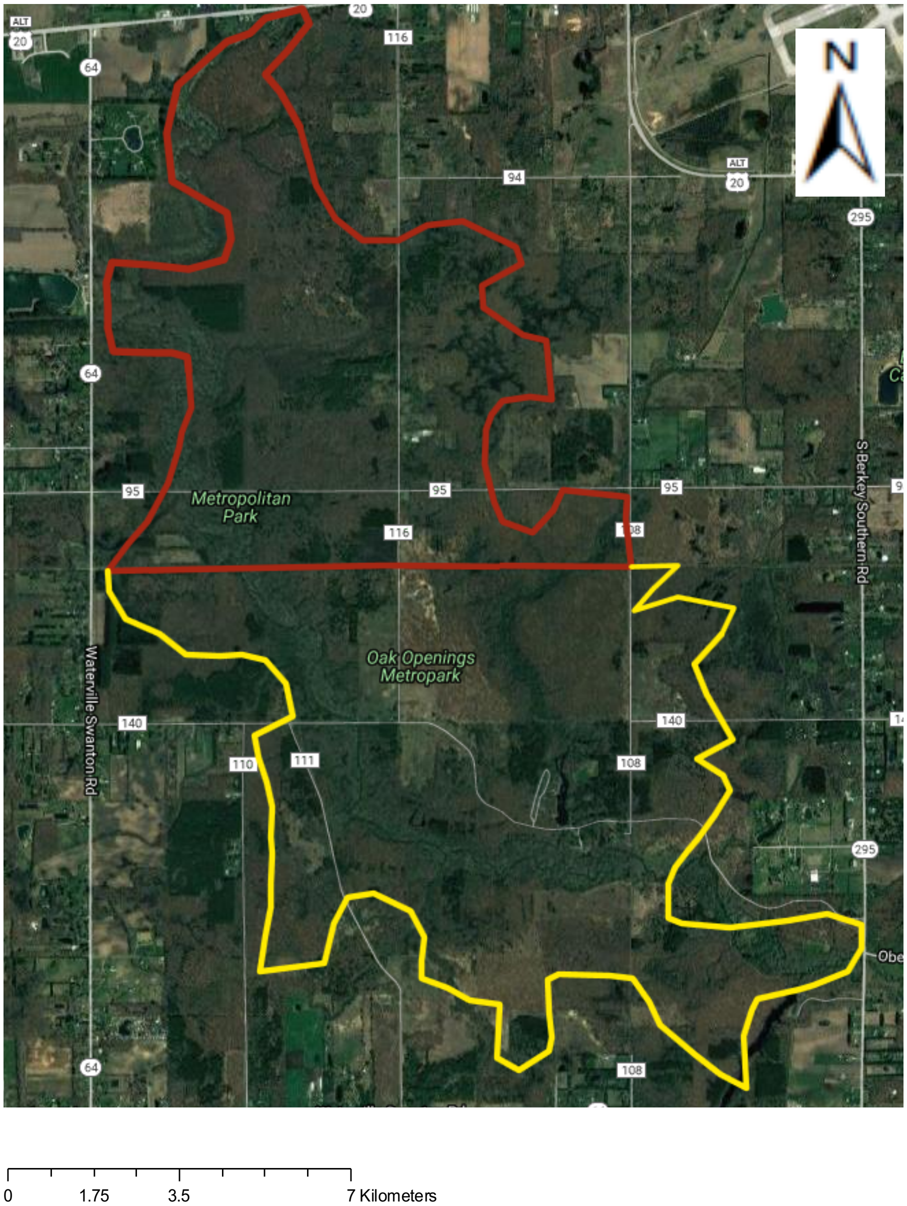

Figure 28. Map of Oak Openings Preserve, in Northwest Ohio, displaying the field survey path. Red line represents the trail of Boyscout #1 North. Yellow line represent the trail of Boyscout #2 South.

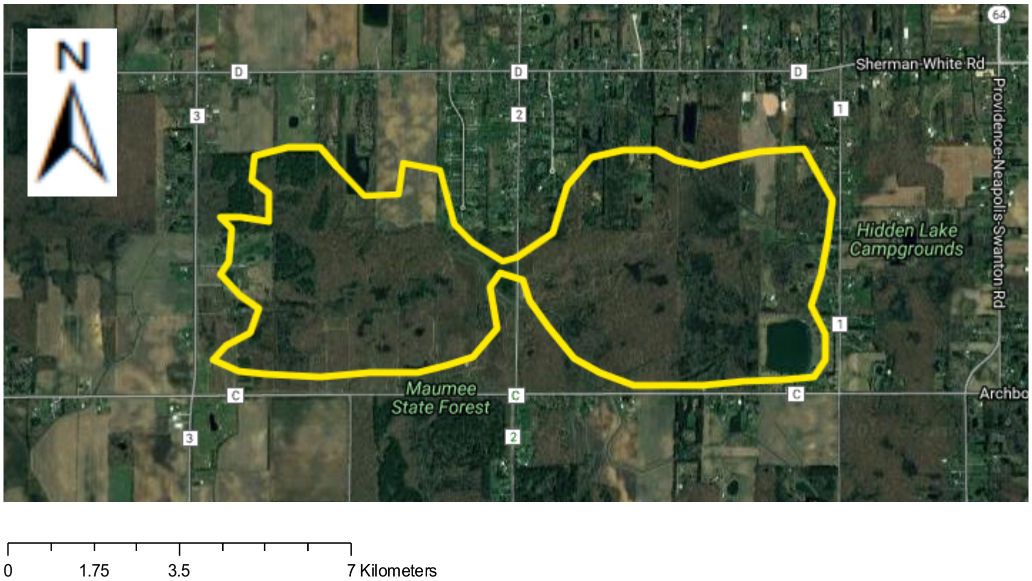

Figure 29. Map of ATV trail of Maumee State Forest in Northwest Ohio. Yellow line represent the trail that was surveyed for vertebrates.

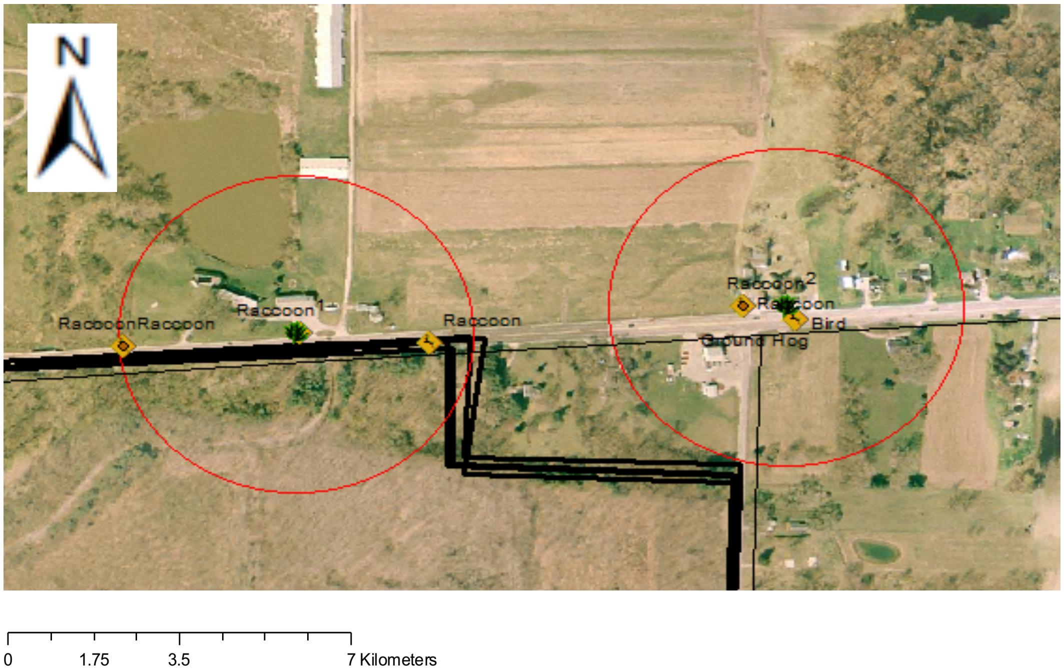

Figure 30. Example of average canopy and vegetation point locations with 250 m buffers on ArcGIS Software (version 11). Vegetation symbol represents average canopy and vegetion point locations with average scores labeled (1 and 2). Red circles represent 250 m buffer around average canopy and vegetation point locations. Deer crossing symbols represents roadkill with roadkill species labeled.

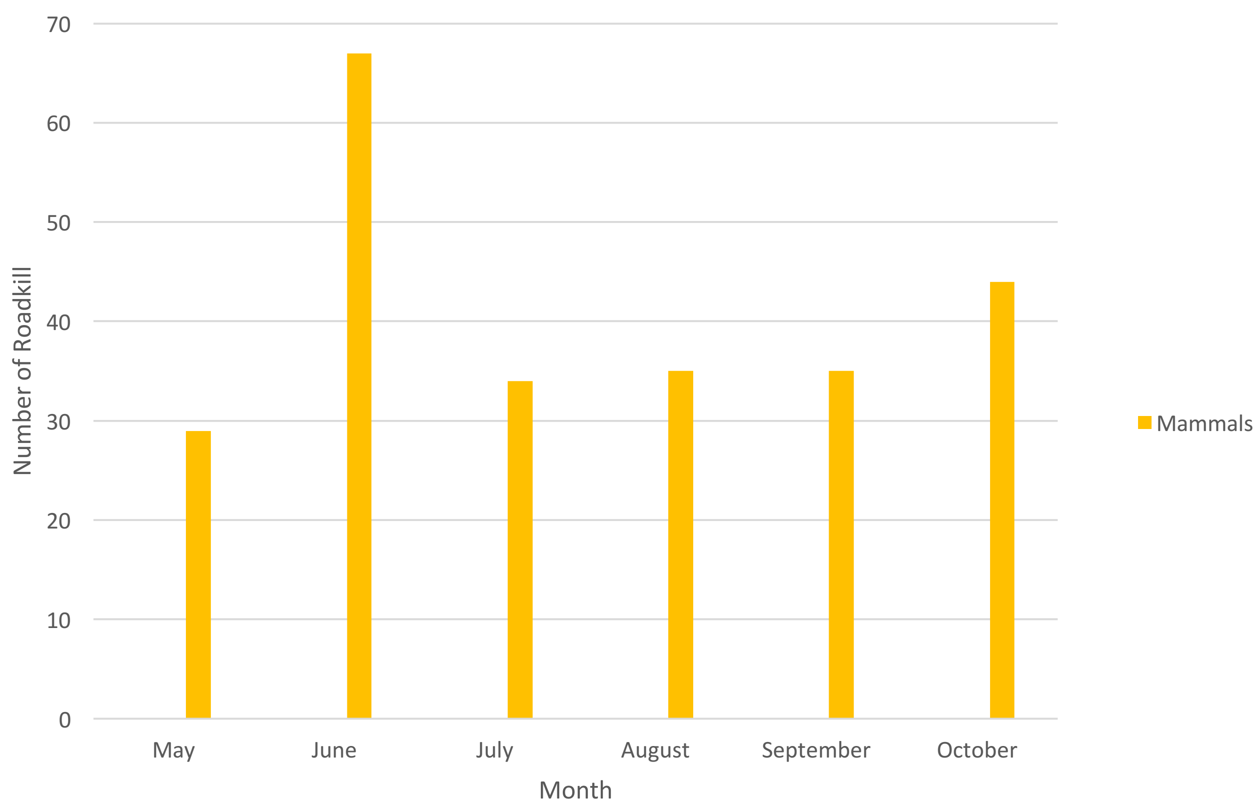

Figure 31. Roadkilled mammals per month detected on roads near Oak Openings Preserve and Maumee State Forest located in Northwest Ohio from May-October, 2016. Yellow bars represent roadkilled mammals.

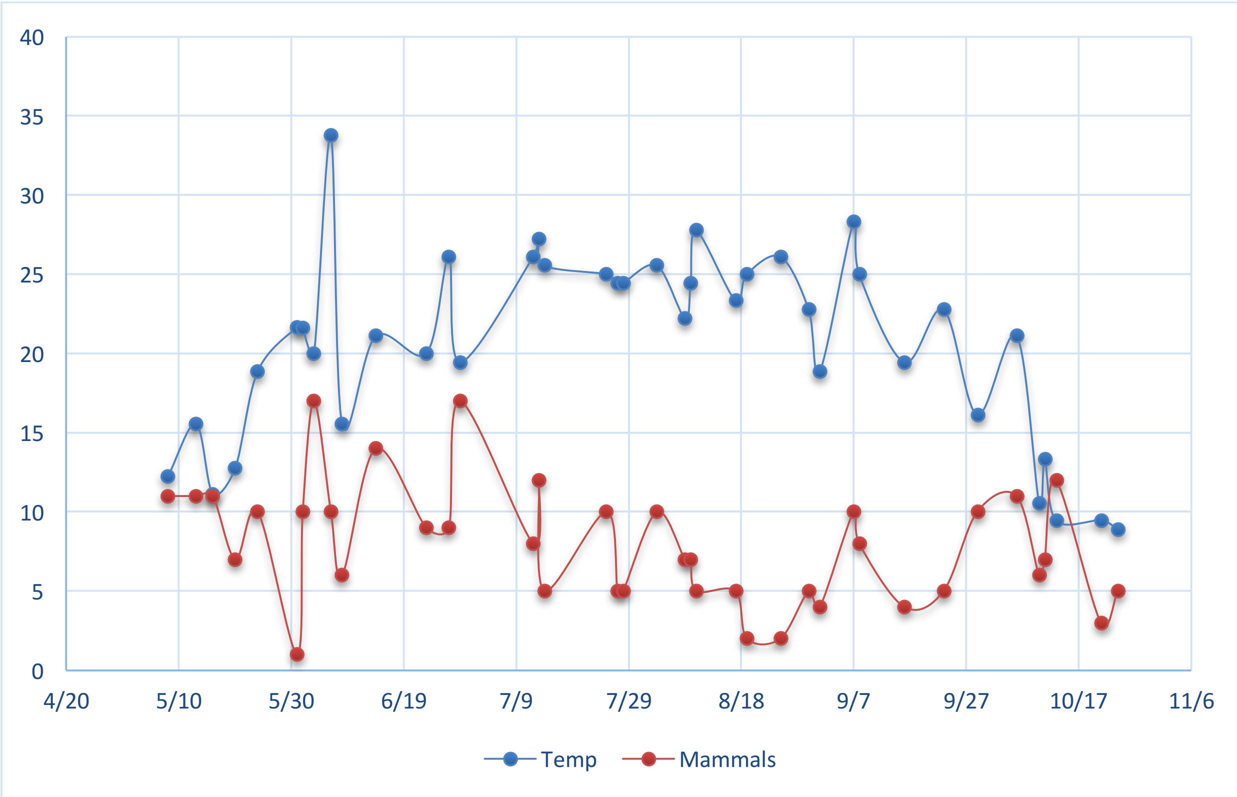

Figure 32. A comparison of the relationship between daily average temperature (C°) and number of roadkilled mammals on roads surround Oak Openings Preserve and Maumee State Forest located in Northwest Ohio from May-October 2016. Blue lines represent temperature. Red lines represent roadkilled mammals.

Figure 33. Roadkill hot spot analysis performed on roads surrounding Oak Openings Preserve and Maumee State Forest in Northwest Ohio in ArcGis using a Getis-Ord G_i* statistic on each 250 m buffer where clusters of high and low values of roadkill were located on each road. Circles represent buffers. Circles that are yellow had no significance suggesting these areas are of low values of roadkill. Circles that are light orange have 90% confidence of significance suggesting potential roadkill hot spots. Circles that are orange have a 95% confidence of significance suggesting potential road kill hot spots. Circles that are red have a 99% confidence of significance suggesting a roadkill hot spot location and have high values of roadkill. Circles that are light blue have a 90% confidence of significant suggesting potential cold spots or areas of low values of roadkill.

Table 22. Eigenvectors from Principal Component Analysis of total roadkilled raccoons detected within the fixed point location of the 250 m buffer and structural features within its borders along the roads surrounding and within the Oak Openings Preserve and Maumee State Forest of northwestern, Ohio, showing strength of influence of each landscape metric for first seven principal components.

	Prin1	Prin2	Prin3	Prin4	Prin5	Prin6	Prin7
Forest	0.27637	-0.09791	0.79647	0.00000	-0.21051	0.22106	0.43183
House	-0.46567	0.44639	0.21117	0.00000	-0.30367	0.52892	-0.40904
Ag. Field	-0.62382	0.08517	-0.09236	0.00000	0.33349	0.13496	0.68238
Grass Field	0.44040	0.25166	-0.53231	0.00000	-0.31335	0.48375	0.35661
Water	0.02974	0.72323	0.08133	0.00000	-0.24492	-0.61147	0.18856
Speed Sign	0.35041	0.44441	0.15013	0.00000	0.77076	0.21273	-0.13357
Wildlife Sign	0.00000	0.00000	0.00000	1.00000	0.00000	0.00000	0.00000

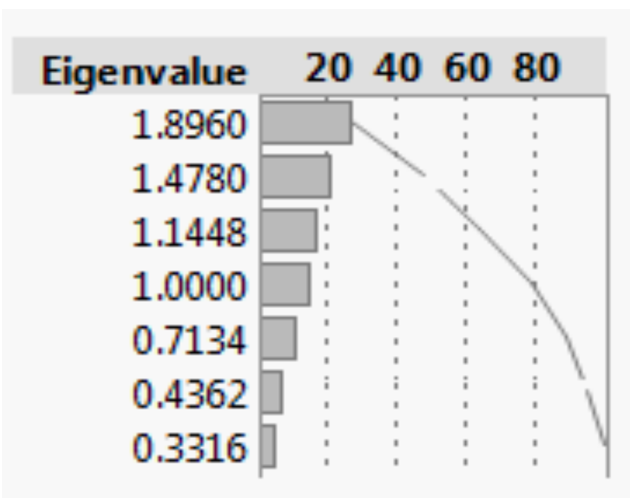

Figure 34. Eigenvalue/Principal component cumulative percentage table from Principal Component Analysis of total roadkilled raccoons detected within the fixed point location of the 250 m buffer and structural features within its borders along the roads surrounding and within the Oak Openings Preserve and Maumee State Forest of northwestern, Ohio.

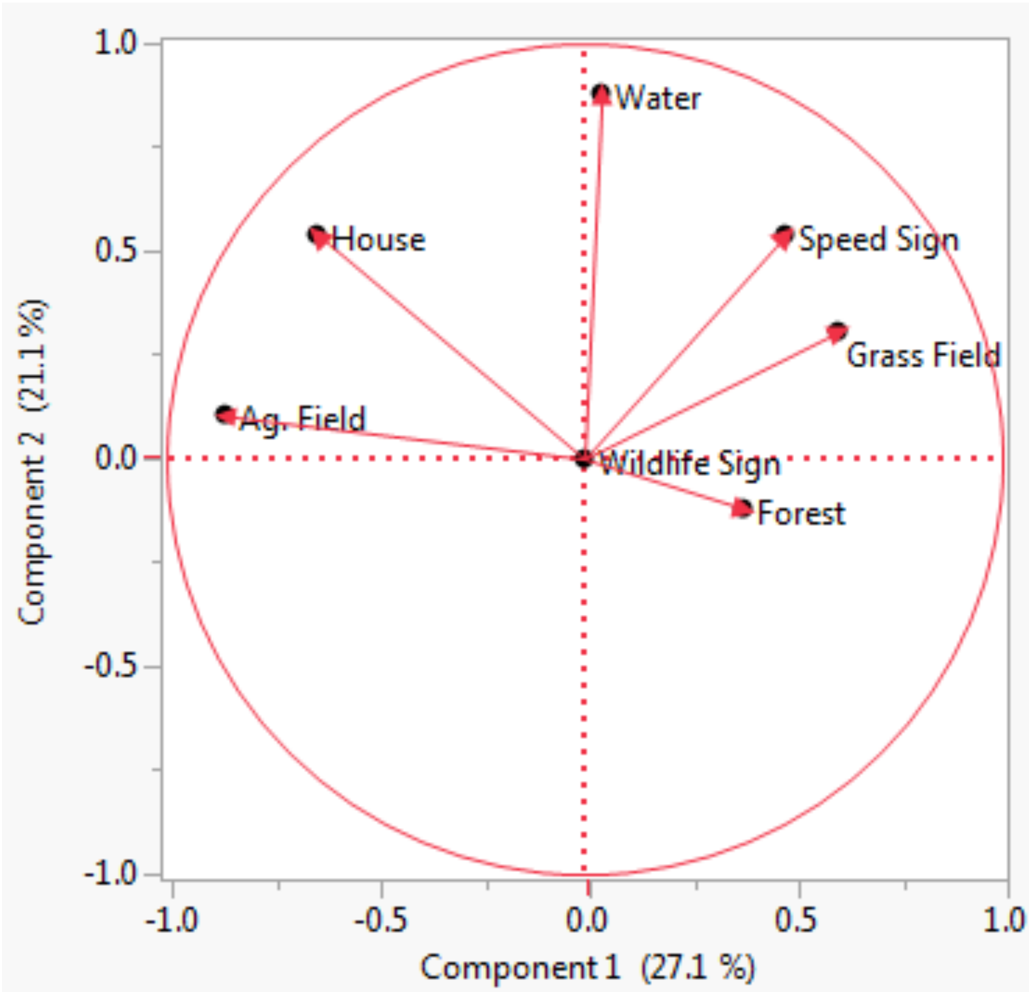

Figure 35. Principal Components Analysis shows vectors of total roadkilled raccoons detected within the fixed point location of the 250 m buffer and structural features within its borders along the roads surrounding Oak Openings Preserve and Maumee State Forest in Northwest, Ohio. Association of each variable is represented by the orientation of the lines.

Table 23. Eigenvectors from Principal Component Analysis of total roadkilled raccoons detected within the fixed point location of the 250 m buffer and land cover types within its borders along the roads surrounding and within the Oak Openings Preserve and Maumee State Forest of northwestern, Ohio, showing strength of influence of each landscape metric for first fifteen principal components.

	Prin1	Prin2	Prin3	Prin4	Prin5	Prin6	Prin7	Prin8	Prin9	Prin10	Prin11	Prin12	Prin13	Prin14	Prin15
1 Turf	-0.21089	0.22267	-0.25509	-0.35072	0.00000	0.00000	0.00000	0.63757	0.13749	0.34938	-0.03832	0.15584	-0.07370	-0.02894	0.37200
2 Wet Prairie	0.00000	0.00000	0.00000	0.00000	1.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
3 Residental	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	1.00000	0.00000	0.00000	0.00000	0.00000	-0.00000	0.00000	0.00000	0.00000
4 Asphult	-0.16792	0.35234	0.30794	0.02527	0.00000	0.00000	0.00000	-0.08939	0.72921	-0.03336	0.00989	-0.36540	0.10640	-0.25770	-0.00095
5 Pond	0.02668	0.31679	0.50564	-0.20202	0.00000	0.00000	0.00000	0.10878	-0.36483	0.17937	0.61138	-0.10452	-0.12664	0.02679	-0.15451
6 Savannah	0.29171	0.31987	0.01902	-0.20158	0.00000	0.00000	0.00000	0.43562	-0.06566	-0.59021	-0.27633	-0.00594	0.11773	0.03750	-0.37083
7 Shrub/Scrub	0.00000	-0.00000	-0.00000	0.00000	0.00000	1.00000	0.00000	-0.00000	-0.00000	0.00000	-0.00000	0.00000	-0.00000	0.00000	0.00000
8 Swamp Foest	0.43569	-0.05407	0.22014	0.37882	0.00000	0.00000	0.00000	0.23203	-0.15071	-0.10687	-0.01295	-0.04575	-0.04706	-0.50767	0.51489
9 Connifers	0.36522	-0.23470	0.23765	-0.04017	0.00000	0.00000	0.00000	0.08507	0.43262	0.12083	-0.00314	0.41235	-0.56295	0.08381	-0.22463
10 Upland Forest	0.47972	-0.06760	0.02694	0.06827	0.00000	0.00000	0.00000	0.11936	0.17148	0.19127	0.06972	-0.33249	0.30794	0.65346	0.20598
11 Floodplain	0.39684	0.20181	-0.05116	-0.19888	0.00000	-0.00000	0.00000	-0.24256	-0.00463	0.43987	-0.10915	0.33343	0.49377	-0.32160	-0.19224
12 Barrens	-0.04098	0.22610	-0.34881	0.67895	0.00000	0.00000	0.00000	0.24836	0.12065	0.06108	0.38050	0.19203	0.09012	0.01362	-0.30761
13 Eurassian Meadow	0.04411	0.52955	0.01496	0.27358	0.00000	-0.00000	0.00000	-0.15731	-0.21766	0.30697	-0.51193	-0.14991	-0.40521	0.16461	-0.01943
14 Prairie	0.19797	0.42126	-0.29050	-0.15971	0.00000	-0.00000	0.00000	-0.40352	0.10332	-0.36131	0.30581	0.26218	-0.16783	0.13248	0.40178
15 Cropland	-0.29645	0.10629	0.51914	0.21036	0.00000	0.00000	0.00000	0.02257	-0.00558	-0.10818	-0.17597	0.55614	0.30838	0.30102	0.22101

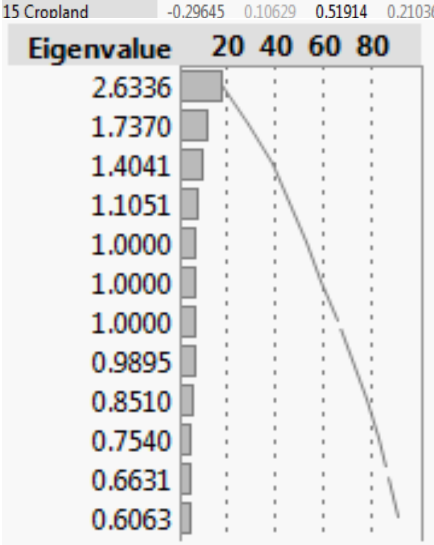

Figure 36. Eigenvalue/Principal component cumulative percentage table from Principal Component Analysis of total roadkilled raccoons detected within the fixed point location of the 250 m buffer and land cover types within its borders along the roads surrounding and within the Oak Openings Preserve and Maumee State Forest of northwestern, Ohio.

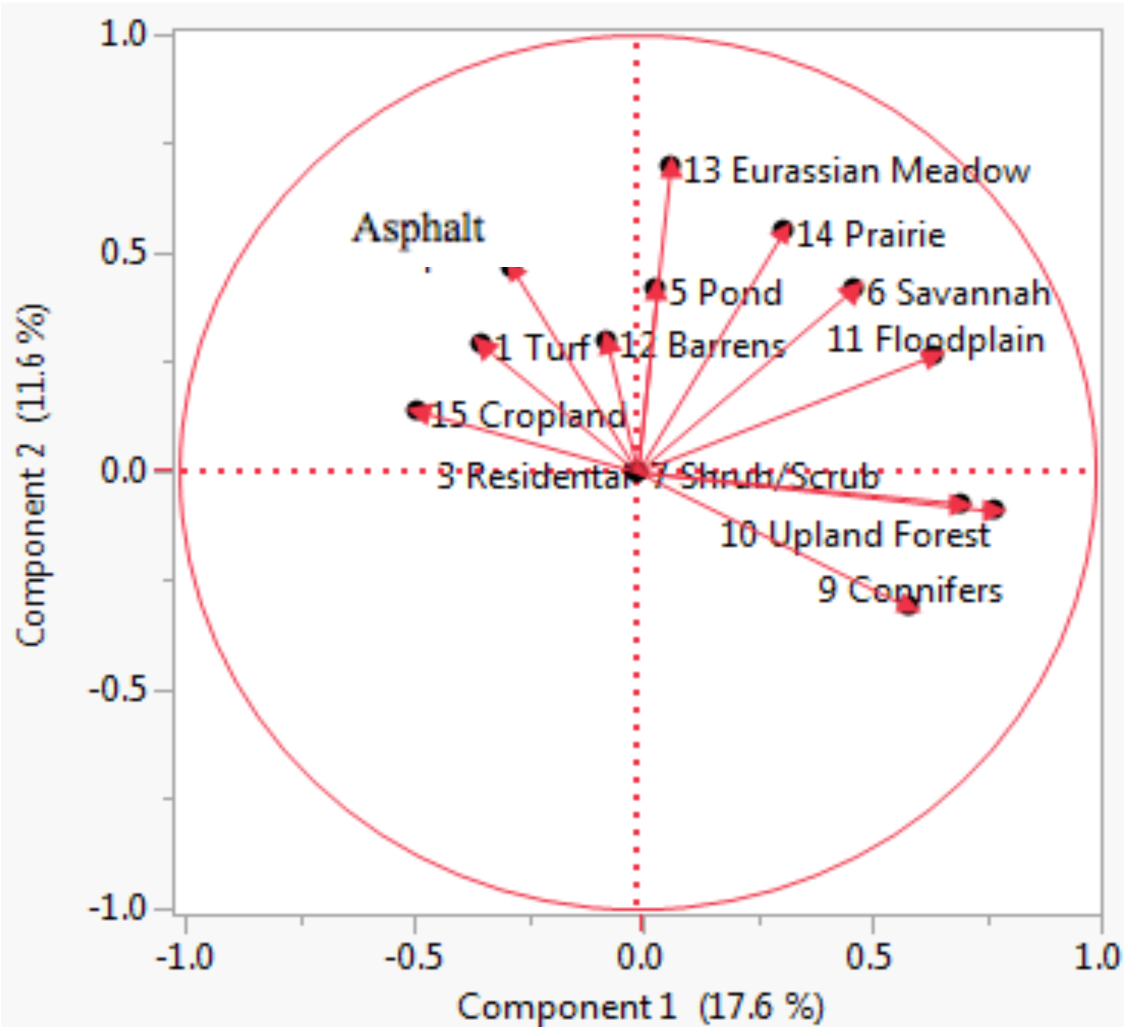

Figure 37. Principal Components Analysis shows vectors of total roadkilled raccoons detected within the fixed point location of the 250 m buffer and land cover types within its borders along the roads surrounding Oak Openings Preserve and Maumee State Forest in Northwest, Ohio. Association of each variable is represented by the orientation of the lines.

Table 24. Eigenvectors from Principal Component Analysis of total roadkilled raccoons detected within the 250 m by 100 m rectangular window and land cover types within its borders along road edges on roads surrounding Oak Openings Preserve and Maumee State Forest in Northwest, Ohio, showing strength of influence of each landscape metric for first fourteen principal components.

	Prin1	Prin2	Prin3	Prin4	Prin5	Prin6	Prin7	Prin8	Prin9	Prin10	Prin11	Prin12	Prin13	Prin14
2 Wet Prairie	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	1.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
3 Residental	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	1.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
4 Asphult	-0.29330	0.29842	-0.02821	0.55475	0.34418	-0.00000	0.00000	0.00000	0.14024	0.14683	-0.05053	0.34594	-0.08918	0.47593
5 Pond	-0.10351	0.08895	0.67947	-0.37919	-0.03918	-0.00000	0.00000	0.00000	0.01789	0.08277	0.49120	0.29265	0.00511	0.20062
6 Savannah	0.09106	0.26227	-0.06950	0.29551	-0.72887	-0.00000	0.00000	0.00000	0.32442	0.12372	0.29939	-0.25614	-0.05387	0.14428
7 Shrub/Scrub	0.00000	0.00000	0.00000	-0.00000	0.00000	1.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
8 Swamp Foest	0.39093	0.00907	-0.18052	-0.32208	0.21728	0.00000	0.00000	0.00000	0.57048	0.25791	-0.03604	0.12281	-0.50513	0.00741
9 Connifers	0.33875	-0.20889	0.46930	0.19209	0.17650	0.00000	0.00000	0.00000	0.19584	0.33632	-0.28289	-0.39627	0.36352	0.18067
10 Upland Forest	0.52676	0.08641	-0.02561	0.32584	0.03091	-0.00000	0.00000	0.00000	-0.07267	0.10927	0.24073	0.50662	0.29728	-0.43251
11 Floodplain	0.32781	0.21320	0.21857	0.03715	-0.02133	0.00000	0.00000	0.00000	0.20288	-0.81944	-0.22611	0.07233	-0.00652	0.16863
12 Barrens	0.18370	0.44320	-0.26798	-0.15583	0.43269	0.00000	0.00000	0.00000	-0.11682	-0.06450	0.45340	-0.43316	0.23104	0.14877
13 Eurassian Meadow	-0.04175	0.50633	-0.12308	-0.41095	-0.21759	0.00000	0.00000	0.00000	0.01923	0.23595	-0.47237	0.22194	0.42013	0.07464
14 Prairie	0.04450	0.51772	0.36350	0.13291	0.04604	0.00000	0.00000	0.00000	-0.31744	0.14208	-0.20898	-0.21538	-0.47994	-0.36973
15 Cropland	-0.45275	0.12954	0.11103	0.04611	0.17974	0.00000	0.00000	0.00000	0.59139	-0.11496	0.06122	-0.10857	0.23057	-0.54756

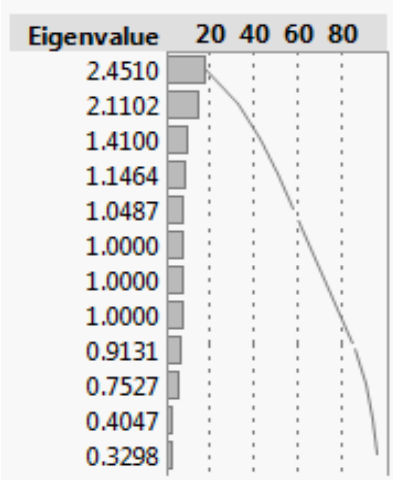

Figure 38. Eigenvalue/Principal component cumulative percentage table from Principal Component Analysis of total roadkilled raccoons detected within the 250 m by 100 m rectangular window and land cover types within its borders along road edges on roads surrounding Oak Openings Preserve and Maumee State Forest in Northwest, Ohio.

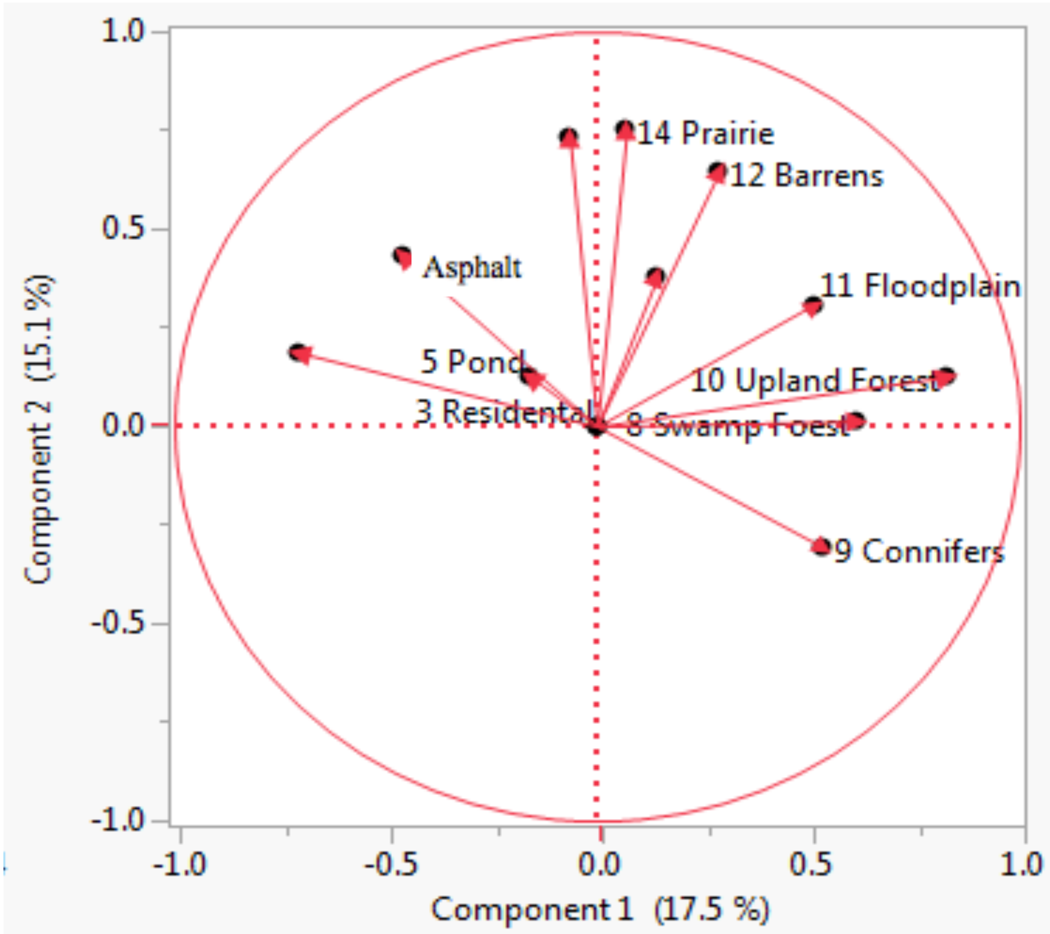

Figure 39. Principal Components Analysis shows vectors of total roadkilled raccoons detected within the fixed point location of the 250 m by 100 m rectangular window and land cover types within its borders along road edges on roads surrounding Oak Openings Preserve and Maumee State Forest in Northwest, Ohio. Association of each variable is represented by the orientation of the lines.

APPENDIX A. INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE APPROVAL

IACUC Inquiry
Sent: Monday, February 29, 2016 7:46 AM
To: Lauren A Jonaitis
Subject: RE: IACUC Approval

Lauren,
Thank you for contacting me.
Since your study will be observational with no environmental manipulation, you do not need IACUC approval to conduct this study. I encourage you to check back in with me if your proposed methods change.
Good luck with your research!

Hillary

Hillary Snyder, Ph.D.
Research Compliance Officer
Office of Research Compliance
280 Hayes Hall
Bowling Green State University
Bowling Green, OH 43403
Phone: 419-372-7722