ASSESSING PERMEABILITY THROUGH A MIXED DISTURBANCE LANDSCAPE FOR VERTEBRATES

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ABSTRACT

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Conflict with wildlife continues to escalate as human population increases and development expands. Understanding how vertebrates interact with the environment is a critical component to conservation ecology. Movement patterns reflect spatial and temporal changes associated with resource availability, life history stages, and habitat use. This study explored how vertebrate mortality could be used to understand the critical factors impacting the consequences of permeability, i.e., ability to move between patches on the landscape, in a mixed disturbance landscape. We assessed how spatial and temporal heterogeneity influenced terrestrial vertebrate mortality. In the biodiversity hotspot of the Oak Openings Region (OOR) of northwest Ohio, we surveyed repeatedly, across three years, approximately 50 kilometers of road segments. Vertebrate mortality locations (N=654) were related to road (e.g., traffic, road width), structural (e.g., canopy cover, soil average water capacity), compositional (e.g., landcover) and productivity (e.g., NDVI) measurements. We found vertebrate mortality locations were positively related to traffic, road width, canopy cover, and normalized difference vegetation index (NDVI) but negatively related to landcover as it becomes more altered (i.e., natural to agricultural). Our consistent findings across years suggest that the spatial components were influencing mortality differences more than temporal differences, and intra-year differences do not impact mortality in a way that would steer long term mitigation of permeability issues. We developed spatially explicit models for predicting current vertebrate mortality probabilities across the entire OOR. Proportion of residential/mixed landcover area was the most influential variables of mortality occurrence probability. We found mortality was well predicted and the results of the same key variables were robust across taxa and years. The models developed can serve as an assessment tool for evaluating conservation and management to improve landscape permeability. Our research demonstrates the validity of employing road surveys as a reliable method for gaining insights into locations of roadway vertebrate mortalities and the spatial factors influencing the corresponding lack of permeability. Our methodology is not limited to the OOR; it can be applied anywhere with sufficient mortality and environmental data to address ecological questions of interest. It is an accessible approach to address a wide variety of conservation challenges.

I dedicate this to my family, my friends, and all the people who have touched my life to get me through this experience.

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INTRODUCTION

Conflict with wildlife continues to escalate as the human population increases and development expands. One of the major threats to plants and animals is anthropogenically caused habitat destruction (Kautz et al., 2006). Habitat loss or destruction not only causes mortality but also forces organisms to disperse to new areas to find resources. As organisms disperse, they are likely to encounter roads. Roads fragment landscapes and increase the opportunity for human-wildlife conflict. Few studies, though, have examined these road effects across all vertebrates and at regional scales with a focus on permeability (i.e., ability to move from patch to patch) and productivity. Rather, studies typically focus on a single taxon (da Rosa & Bager, 2012; Philcox et al., 1999; Steen & Gibbs, 2004; Sutherland et al., 2010), small spatial scales (Grace et al., 2017; Mumme et al., 2000), small temporal scales (Assis et al., 2019; Bautista et al., 2004), or homogeneous environments (Brehme et al., 2013; Develey & Stouffer, 2001). Our research will evaluate the effects of roads and their context on the distribution of vertebrates in the Oak Openings Region (OOR) in northwestern Ohio.

Road ecology is a relatively new field and arose from the focus on the adverse impact of roads on nature (Forman et al., 2003). Roads have physical, biological, and ecological effects. The focus of road ecology is to avoid, mitigate, or offset these negative effects of roads (van der Ree et al., 2015). Roads cause direct mortality, which has detrimental effects on wildlife populations (Benítez-López, 2010). Roads also can act as barriers, prevent or alter dispersal, produce changes in population composition, affect competition or predation, and have effects on other ecological processes (Coffin, 2007). Chen et al. (2019) investigated how proximity to roads affects seed dispersal effectiveness by rodents and found that seed dispersal distance and effectiveness decreased closer to the road.

Roads can also have positive effects on wildlife. These linear features can act as corridors and provide quicker access to resources. Large predator species have been found to select anthropogenic linear features such as roads to quickly move between habitat patches (Bennett, 1991; Dickie et al., 2020). Moving along roads can be more energetically efficient due to the decreased resistance from dense vegetation and clutter (Getz et al., 1978). In particular, the movement of generalist species that are able to exploit more variable habitats can be facilitated by road corridors (Forman & Alexander, 1998). Carrion feeders move along roads and have benefited from the resources provided, not only in terms of food (Lambertucci et al., 2009), but turkey vultures, for example, use thermal drafts from roads to reduce their energy expenditure (Mandel & Bildstein, 2007). Richness of birds with an omnivorous diet was positively affected by the presence of roads (Kroeger et al., 2022). A similar positive effect can be found in bats, depending on their foraging habitat preference (i.e., open versus forested) (Myczko et al., 2017). Roadsides can also provide favorable habitat for certain species to thrive (Oxley et al., 1974; Getz et al., 1978). In addition, roadside ditches act as a temporary water source that can facilitate amphibian movement (Garriga et al., 2012; Sillero et al., 2019).

Roads cut through natural habitat, causing habitat fragmentation which can reduce connectivity between populations (Riley et al., 2006). The type, quantity, and quality of resources in the resulting fragments cause organisms to move around in order to find and obtain appropriate resources. There is an associated cost with this movement to resources (Matsumura et al., 2010); energy expenditure is increased the farther an organism has to travel. In response to fragmentation and habitat loss, increasing connectivity is the goal of many conservation biologists. For example, Braaker et al. (2014) found that habitat connectivity was an essential component to ground-dwelling animal movement. Connectivity can mitigate the effects fragmentation has had on impeding processes such as seasonal migration, gene flow, and nutrient flow (Braaker et al., 2014; Clark et al., 2010; Cushman & Lewis, 2010). However, corridors that increase connectivity may also increase the dispersal of invasive or other unwanted species or disease or increase the spread of unwanted disturbances (Haddad et al., 2014). Although, some research indicates that other facets of landscape structure, such as habitat amount, quality, or temporal changes in habitat, may be more important than connectivity (Fahrig, 1992; Fahrig, 1998; Franzén & Nilsson, 2010). For example, Lindell and Maurer (2010) demonstrated that patch quality had much greater positive effects on the patch population size than immigration.

In addition to fragmentation, roads subdivide natural areas into islands and create edges where the biotic (i.e., species composition, predation, competition, seed dispersal) and abiotic (i.e., light, temperature, moisture, wind) conditions differ from those found toward the interior of a habitat patch (Laurance et al., 2001; Ries et al., 2004). Edges tend to be dominated by disturbance tolerant species (Forman & Alexander, 1998). Island biogeography theory (1967), when applied to the terrestrial landscape, suggests that the effect of fragmentation will be to isolate and reduce in size the remaining patches of habitat, which should result in loss of species and reduce dispersal within the fragmented landscape matrix. However, Gardiner et al. (2018) found that habitat amount within a 1 km buffer in a fragmented landscape was a better predictor of occupancy and dispersal among patches for the eastern bettong *Bettongia gaimardi*, a keystone woodland specialist, than either patch size or isolation, providing support for the habitat amount hypothesis.

The quality of these patches, or the distribution of resources, has an effect as well. Understanding how vertebrates interact with the environment is a critical component to conservation ecology (Maskey Jr & Sweitzer, 2020; Howze & Smith 2015; Roe & Georges, 2008). Considering all terrestrial vertebrates, provides a way of investigating across varied ecology, rather than focusing on a specific taxon. Movement patterns depict spatial and temporal changes associated with resource availability and accessibility, life history stages, and habitat use (Kidd-Weaver et al., 2020; Roshier et al., 2008; Johnson et al., 1992). In turn, resource distribution and abundance can affect the movements and spatial distribution of a variety of animals (McIntyre & Wiens, 1999; Wilmshurst et al., 1999; Patterson & Messier, 2001; Reed, 2018). Local structural components such as vegetative cover (Clevenger et al., 2003), and regional components, such as landscape matrix (Jaberg & Guisan, 2001; Sillero et al., 2019), can greatly impact the way wildlife use space.

The makeup and arrangement of ecosystems directs the processes occurring within them (Wenbo et al., 2002). The availability and the pattern of resources affect the responses of biota, and, therefore, impact the composition of organisms contained within a habitat. The spatial arrangement of the landscape controls the ecological processes which operate within it. Current studies focus on the diversity of single trophic groups rather than the functional effects that may occur from the interactions and dependency between groups (Balvanera, 2014). Ecologists have demonstrated that the productivity of an ecosystem is related to the number of species that comprise the food web within it (Cardinale et al., 2006; Naeem et al., 1994).

Biodiversity, specifically species richness, has been found to be higher within protected areas (Gray et al., 2016). Due to the increased resources in these natural protected areas, these areas act as a source for road mortality (Garriga et al., 2012). Therefore, as organisms are using these natural areas for resources, roads fragmenting these areas are more likely to be associated with hot spots in roadkill, as vertebrates are likely to be crossing at higher numbers between protected areas, especially given the small size of many of these. These protected areas may also be the destination of vertebrates in surrounding resource deficient areas who are risking mortality to access the resources they require. Therefore, there will also be roadkill associated with areas where roads form an edge to protected areas.

Our research is focused on how the environmental and structural characteristics of the landscape influence resource availability for vertebrates within the Oak Openings Region (OOR). Our objective is to assess how spatial and temporal heterogeneity influences distribution patterns of vertebrates and the ease in which they can move from patch to patch. Overall, our goal is to understand and contribute to spatial ecology, as well as facilitate conservation and management within the OOR. Anthropogenic activities alter the abundance and distribution of resources, which affect ecosystem processes (e.g., nutrient cycling). In our study area, land cover changes caused by the increase in human activities threaten the long-term viability of OOR natural areas and the vertebrate species that depend upon them. Research is typically concerned with viability or the living whereas we are looking through the lens of the dead. Vertebrate mortality is a possible consequence of low permeability. The greater the mortality, the less successful the movement between patches of habitat to access resources has been. In addition, working with all terrestrial vertebrate taxa provides better representation at a landscape scale of what is going on in the area. Many vertebrates are imperiled by human activities and may need larger or more diverse areas than those that are currently protected.

We used vertebrate mortality and the environmental context, as well as bat activity, to understand resource availability and how the environmental, landscape, and biotic (i.e., other taxa) may play a role in the movement of organisms in a mixed disturbance landscape, areas subject to both natural and anthropogenic disturbances (Grossmann & Mladenoff, 2007; Schetter et al., 2013). While there is a direct relationship between road mortalities and the density living in the surrounding landscape, the relationship is more complicated than a 1:1 (Gehrt, 2002; Baker et al., 2004; George et al., 2011). We used vertebrate mortality as a lens to understand animal movement and the context in which it occurs. In this way we can examine the factors that influence this movement to address the question of how the distribution of vertebrates is affected by a mixed disturbance environment. This approach also highlights what are the critical factors impacting permeability, or the ability to move between patches of habitat on the landscape.

Study Area

The current study occurred in the Oak Openings Region (OOR) in northwest Ohio (Figure 0.1), although this region also extends into southeast Michigan. The work was conducted in and around the OOR natural areas of the Kitty Todd Nature Preserve, Oak Openings Preserve Metropark, and Maumee State Forest. These three areas within OOR were chosen because of the differences in the surrounding habitats (i.e., different landscape context). Kitty Todd Nature Preserve area is approximately 567 hectares and dominated by non-forested natural areas, such as upland savannah and wet prairie, and is near a more residential/urban matrix compared to the other natural areas of interest (Figure 0.2). The Oak Openings Preserve area is 2,023 hectares and is dominated by forested areas, with a large amount of non-forested natural areas as well, while the Maumee State Forest area, which is approximately 1,250 hectares, is surrounded by mostly agricultural land. These three natural areas are the largest protected areas of the region (Figure 0.2). This region is considered a mixed disturbance landscape because natural disturbances occur on the landscape as well as anthropogenic disturbances (Schetter & Root, 2011).

OOR is considered a biodiversity hotspot due to its unique and complex collection of ecosystems, including globally rare oak savanna, and native species (Brewer & Vankat 2004; Thieme, 2016). There are over 150 rare species within this region, more than any other region in

Ohio with a similar area (ODNR, 2020). Despite the numerous protected areas, there is a large amount of fragmentation from roads running throughout this region. This area is similar to other mixed disturbance regions where residential and agricultural lands are included in the matrix around protected areas. In the last 15 years, there have been many changes, including the conversion of natural areas to early successional habitat and loss of woodland cover in the OOR (Martin & Root, 2020). These changes can greatly impact the distribution of organisms and resource use across the OOR. This area provides a unique opportunity to investigate a small model of a complex world with various natural habitats juxtaposed with residential and agricultural areas within a relatively compact region, making it ideal for investigating potential patterns and impacts.

Overview

This study is divided into four chapters:

1. How spatial variation affects the distributions of vertebrate mortality
2. How temporal variation affects the distributions of vertebrate mortality
3. Modeling hotspots of mortality for vertebrates
4. Predicting probability of mortality for vertebrates across the landscape

The purpose of chapter 1 is to examine the spatial factors, such as the structural components of roads themselves, land use classification, landscape matrix, and proximity to protected areas, associated with vertebrate mortality patterns. Chapter 2 is focused on the temporal factors, e.g., annual changes in precipitation, temperature, and green vegetation availability, associated with vertebrate mortality patterns across the study area. In Chapter 3 we develop models of vertebrate mortality hot spot locations across the study area. The purpose of Chapter 4 is to develop spatially explicit models for predicting current vertebrate mortality probabilities across the entire Oak Openings Region. The models developed in Chapter 4 will serve as an assessment tool for evaluating conservation and management to affect landscape permeability for terrestrial vertebrates.

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CHAPTER I. HOW SPATIAL VARIATION AFFECTS PERMEABILITY PATTERNS OF VERTEBRATES WITHIN THE OAK OPENINGS REGION

Introduction

In complex environments, the spatial variation in biotic and abiotic factors can shape the distribution of species on the landscape. These effects can differ depending on the spatial scale considered, especially across taxa that vary in their feeding, breeding, and shelter needs. Previous research on permeability across roads suggests that species traits and foraging preferences contribute to the effects of roads (Brehme et al., 2013). Duffett et al. (2020) demonstrated that smaller solitary species of ungulates with non-grazing food habits are more likely to flee from roads, confirming that traits, body mass and foraging habits, are predictors of both initial distance to roads and approach tolerance distances. Baladrón et al. (2017) demonstrated that species have varying responses to land cover types, including whether or not expansion of urban or agricultural areas would pose a threat.

Structural components of roads themselves, such as traffic volume and speed and topography, affect the distribution and abundance of organisms as well as roadkill (Brehme et al., 2013; Esperandio et al., 2019; Cooke et al., 2020). Cooke et al. (2020) found the abundances of bird species were strongly significantly related to road exposure; generally, exposure to minor roads was associated with increased abundance and exposure to major roads was associated with lower abundance. Sillero et al. (2019) determined the number of amphibian roadkill hotspots depended exclusively on traffic volume.

The orientation of the road (i.e., whether it runs east-west or north-south) can impact the effect of roads by affecting visibility, the surrounding microclimate, as well as use by vertebrates. Drivers are exposed to sun glare at certain times of the day, and drivers on east-west oriented roads are more susceptible to the effects of reduced visibility due to sun glare (Sun et al., 2018). In addition, the exposure to sunlight could affect road surface conditions, particularly in winter when sunlight can reduce the amount of time the roads remain icy (Cao & Meng, 2015; Zaki et al., 2020). Vertebrate use of roads has been found to be affected by road orientation, where ungulates, in particular, travel along east-west roads more than north-south roads (Mulero-Pázmány et al., 2022).

At a broader scale, landscape factors can influence how animals move through the area. For medium sized mammals, the amount of urban area in the landscape and forestry cover were positively associated with increased roadkill, while water bodies area and distance to the nearest river also contributed to roadkill (de Freitas et al., 2015). Thus, spatial components of the landscape play a role in movement and vulnerability. Proximity to water, proximity to forested area, and presence of wetland areas are the most important variables associated with the number of roadkill, confirming habitat conditions and the presence of water bodies are the main factors influencing roadkill occurrence (Sillero et al., 2019). The matrix surrounding a habitat patch plays a vital role in the functioning of the patch and can determine the degree of "effective isolation" of the patch compared with isolation determined purely by distance (Ricketts, 2001). Whether or not the area is protected also contributes to the impact of roads. Garriga et al. (2012) demonstrated that highly protected areas are associated with greater amounts of total roadkill, particularly for amphibians and reptiles. Therefore, land cover changes caused by the increase in human activities or their intensities, in particular, can threaten the long-term viability of natural areas and the vertebrate species that depend upon them (Newbold, 2018; Gómez-Ruiz et al., 2021; Jaureguiberry et al., 2022).

In this study we identify vertebrate road mortality locations across the study area and evaluate how they are influenced by landscape features, such as protection status, land cover type, road characteristics, and environmental variables, as well as the effects of biotic factors, such as taxon composition. We are interested in the structural, environmental, and landscape factors that could influence the diversity, abundance, and distribution of terrestrial vertebrate mortality in the OOR. In the context of this study permeability is the ease at which vertebrates can move from patch to patch through the mixed disturbance landscape; since the study area is so fragmented by roads, vertebrates will need to cross the roads to accomplish this movement between patches. Therefore, we are considering vertebrate mortality, specifically from roads, as a tool for evaluating permeability. Vertebrate mortality, as well as acoustic monitoring of bat activity, will illuminate how animals are moving through the area in relation to each other and their environment, as it is important to understand the real tradeoffs that animals confront as they move through altered ecosystems. We are using bat activity as an independent, complementary line of investigation to the mortality data, providing an alternate view of resource availability and helping build a better picture of where organisms are concentrated.

We will identify the patterns in how these pieces function on the landscape as habitat for the organisms. It is important to consider the individual characters that occupy a habitat; therefore, our study is not just focused on one species, taxon, or guild, but is aimed at determining the overall multi-species, community wide impacts. A mixed-disturbance landscape, with a variety of active management and anthropogenic activities, provides an opportunity to examine species habitat associations in a variety of contexts.

Research Questions

1. What factors that vary spatially are associated with decreased permeability within the study area (factors influencing mortality)?
2. How do these influencing factors differ among taxa?
3. How do the influencing factors of decreased permeability differ based on spatial scale?

Methods

Road Surveys

We surveyed a total of 23 roads, covering 49.0 kilometers (km). The roads were situated near one of three natural areas, Kitty Todd Nature Preserve, Maumee State Forest, or Oak Openings Preserve. We surveyed a total of 8 roads (15 transects), covering 19.57 km, near Kitty Todd Nature Preserve; a total of 7 roads (9 transects), covering 13.71 km near Maumee State Forest; and a total of 9 roads (10 transects), covering 15.77 km, near Oak Openings Preserve. The roads were divided into transects at designated intersections; therefore, the resulting 34 road transect lengths ranged from 0.8 km – 2.0 km. All roads were two lane, paved roads, with speed limits that vary between 56.3 kph (35 mph) and 88.5 kph (55 mph).

Roads within the study area were surveyed for all dead terrestrial vertebrates, e.g., amphibians, birds, mammals, and reptiles. We surveyed all transects every other week from May to October in 2020, 2021, and 2022, totaling 36 surveys (12 per year). We conducted the surveys by bicycle, riding 16.1 - 24.9 kph (10.0 - 15.5 mph), similar to Garrah et al., (2015). Walking the transects was not feasible, so bicycling was used as car surveys tend to underestimate roadkill, especially reptiles and amphibians (Langen et al., 2007). However, car surveys were conducted when weather was unsafe to complete the survey by bicycle (approximately 10% of the total number of surveys). When car surveys were employed, we evaluated all transects by car to keep the data consistent across transects for that survey.

For each vertebrate mortality found, we recorded the location with a Garmin Etrex GPS, identified the organism to at least taxonomic class (and species, if possible), took photographs of the organism (Figure 1.2) and surrounding areas, noted where and in what condition the organism was, as well as recorded a variety of environmental and spatial variables (Table 1.1). Due to the quality of the carcass, most identifications were only to class. Animal carcasses were not removed, duplicate entries of persistent carcasses were avoided by referring to prior data before recording new entries.

Spatial, Structural, and Productivity Features of Road Areas

Along each transect sampling points were placed approximately every 400 meters to evaluate the heterogeneity along the road segment. We created 200-meter buffers (radius from fixed sampling point) around each sampling point, resulting in a 160,000 square meter area to provide an evaluation of landscape context. Spatial and structural features were determined at each mortality point, as well as for all buffers at these fixed points. The features we assessed included microhabitat data, such as canopy cover, adjacent vegetation height, understory presence, and water presence. These features were measured at each mortality point and during point surveys in June 2020, May-October 2021, and June and October 2022 (Table 1.1). For all continuous variables, the minimum, maximum, and average values were investigated.

The height of vegetation directly adjacent to the road was measured on both sides in centimeters at each sampling point. The presence of an understory within 10 m of the road was recorded for each side of the road, and the sampling point was classified as no presence, present on one side, or present on both sides.

Canopy cover data was collected at taken on each side of the road and was estimated by taking photographs and analyzing them in Canopeo, which measures canopy cover by calculating fractional green canopy cover, turning images black and white and measuring the shade values as the amount of white pixels vs. black pixels, where the white pixels are areas covered by canopy (Figure 1.3) (Patrignani & Ochsner, 2015). The minimum, maximum, and average percent canopy cover values from each side were recorded.

We also collected macrohabitat data, such as land use type, landcover, protected area, available water capacity, available water storage, water table depth, normalized difference vegetation index (NDVI), moisture stress index (MSI), normalized difference water index (NDWI), enhanced vegetation index (EVI_2) (Table 1.2).

Land use type on each side of the transects was delineated as natural, developed, or agricultural using GoogleEarth Pro and was verified through field surveys. The information was then used to categorize the land use on a scale from 1 – 6 based on both sides of the road at each sampling point. Category 1 indicated that both sides of the road were considered natural (i.e., areas not altered by humans measuring at least 50 m x 50 m); category 2 denoted one side of the road was natural while the other was developed; category 3 indicated that one side of the road was natural and the other was agricultural; category 4 indicated that both sides of the road were developed; category 5 denoted that one side of the road was developed and the other side was agricultural; and category 6 indicated that both sides of the road are considered agricultural. Land use categories were utilized as proportions for buffer and road analyses. Road segments within each buffer (400 m in length) and corresponding land use category types for the buffer were combined to obtain a proportion for each land use category for each buffer. For the overall road, these lengths were combined, and proportions were calculated to get the totals for each road.

Landcover types were identified using the Oak Openings Region land cover map (Martin & Root, 2020). There were 14 landcover classes: upland prairie, upland savanna, sand barrens, upland deciduous forest, upland coniferous forest, wet prairie, swamp forest, floodplain forest, wet shrubland, perennial pond, Eurasian meadow, and cropland (the 15th class of turf/pasture was not found within the study area). We estimated patch size and area of landcover classes in FRAGSTATS ver. 4.2.598 (McGarigal & Marks, 1995). Average, minimum, and maximum patch areas were calculated for the buffers and for the roads. Proportions of area of each landcover class were also calculated for the buffers and for the roads.

Protected area was characterized on each side of the road based on the mapping provided by MetroParks Toledo (Joshua Brenwell, personal communication, April 7, 2020). The information was then used to categorize the areas from 1 – 3 based on both sides of the road at each sampling point. Category 1 indicated that both sides of the road were considered protected; category 2 denoted one side of the road was protected land while the other was unprotected; category 3 indicated that both sides of the road were unprotected land. This information was used to calculate the proportion of road within each buffer as well as the proportion of the total buffer area that fell within each protected area category. The level of protection for each road was estimated as the total length of road in each protected area category individually divided by the total road length.

Available water capacity, available water storage and water table depth information was acquired from the Web Soil Survey database from United States Department of Agriculture Natural Resources Conservation Service (USDA NRCS, 2019). Available water capacity is measured in centimeters of water per centimeter of soil and refers to the quantity of water that the soil is capable of storing for use. Available water storage is the water capacity times the thickness of the soil (Figure 1.4). Water table depth is the distance between the soil surface and the saturated zone, known as the water table.

NDVI (Figure 1.5), EVI_2, MSI, and NDWI indices were calculated from Landsat-8 images for our study area (Path 20, Row 31) acquired from the U.S. Geological Survey from early spring (March or April), summer (June), and fall (September or October) and then combined across all three study years (2020-2022). The indices were then calculated from the aggregate image in ESRI ArcGIS version 10.8.1. NDVI and EVI_2 use the satellite data to evaluate biomass patterns, while MSI and NDWI use the satellite data to evaluate water content (Valerio et al., 2022).

Road features we measured included traffic volume (AADT), road speed limit (kph), width of road (m), road topography, road markings, road quality, shoulder presence, shoulder material, ditch presence, telephone line presence, and presence of potential obstructive human structures (e.g., fences, signs, culverts, etc.) (see Table 1.1).

The annual average daily traffic volume (AADT) was acquired from the Ohio Department of Transportation Data Management System (https://odot.public.ms2soft.com/tcds/tsearch.asp?loc=Odot&mod=) once a year, following the year of sampling. Road speed limits, in mi/h, were obtained for each road during the study from either Fulton County Auditor's Office, Henry County Sheriff's Office, or Lucas County Engineer's Office. The speed limits were then converted to kilometers per hour (kph).

Road topography at the cross section of the road was categorized using Clevenger et al. (2003) methodology. The category 'buried' referred to when both verges (areas directly adjacent to the road) were higher than and sloped down to the road. Roads with level topography had roadways and both verges at the same heights. Raised referred to when both verges sat lower than the road. Roads with buried-raised topography had roadways with verges sloping opposite each other and neither even with the road. Part-buried or part-raised referred to when one verge was level and the other verge was either sloped toward the road or away from the road, respectively.

The presence of road markings was noted while surveying, as well as whether passing or edge lines were present. Width of road, in m, was measured in Google Maps and confirmed in the field with a Meter Man, Komelon Series 45 surveyor's wheel. Road quality was determined based on the rideability of the road and was noted as poor (very bumpy, unpleasant to ride at higher speeds), fair (not a smooth ride, but can maintain speed), or good (smooth ride). Shoulder presence, as well as shoulder material was recorded. The presence of ditches, telephone lines, or potentially obstructive human structures (e.g., fences, signs, culverts, guardrails etc.) was recorded and we noted if present, whether on one or both sides.

Cross-taxa Effects

We investigated the impact that specific taxon occurrence had on mortalities. We obtained a proportion of total mortality occurrence for each taxon. We totaled mortalities for each specific taxon (either mammal, bird, reptile, or amphibian) and divided by the total of all mortalities.

Additionally, we investigated the relationship or mortalities with living vertebrate activity through acoustic monitoring of bats. We used the same 34 road transects for acoustic surveys of bat activity. The bat call surveys were conducted during the peak activity timeframe, which starts approximately 30 minutes after sunset and ends approximately 3 hours after sunset (Hayes, 1997;

Sewald, 2012). We completed the surveys by extending an Anabat SD2 acoustic monitor with an attached GPS unit out the car window while driving along the transect at approximately 32 km/hr (Fisher-Phelps et al., 2017). The calls were continuously recorded while their geolocations were marked. All transects were surveyed within the same day once a month in 2021 either on or shortly after the new moon, which is the time in the lunar cycle when bats are most active (Lang, 2006). Acoustic surveys were conducted similarly in 2022, but twice a month, once on or shortly after the new moon and either two weeks before or two weeks after the new moon, depending on the occurrence of the new moon within the month. Acoustic surveys were only conducted when conditions were appropriate, which includes when wind speeds were less than 15 mph and chance of rain was less than 50% during the survey time .

All recorded calls were investigated by manually screening the sonogram output files that were recorded from the Anabat SD2 in AnalookW (Titley Scientific, version 20.21.3.5). Each sonogram output file was considered a call when it had at least 3 distinct pulses (Buckman-Sewald et al., 2014; Schimpp et al., 2018; Figure 1.6). Calls were further verified by analysis of frequency and other species-specific call characteristics in BCID version 2.7c (Bat Call Identification, Inc.) and comparison to existing call libraries from the OOR area (Buckman-Sewald et al., 2014). While this additional analysis provided species identification, we were focused on total activity; therefore, number of calls (i.e., count) was used as a measure of relative bat activity. Bat call counts were totaled for each buffer and also for each road.

Analysis

Three scales (individual mortality points, buffers, and road segments) were used to assess the variables associated with vertebrate mortality (Figure 1.1).

Mortality point scale. We investigated the effects of the above variables on the total mortality count recorded across all road surveys using the data gathered at each mortality point. We also considered the effects of variables on each taxon by examining each taxon mortality counts separately. The variables associated with vertebrate mortalities were assessed by examining the frequencies. In addition, chi square goodness of fit tests were conducted on protected area and land use, comparing the amount of expected mortalities based on the areas in each category to the mortalities that were observed. Standardized residuals (R) were used to determine which groups contributed to the significant difference of observed and expected values for the chi square goodness of fit tests, where standardized residuals with an absolute value greater than 2 were considered significant (Agresti, 2007; Sharpe, 2015). We repeated this to investigate if there were any differences between taxon mortality counts (i.e., mammals, birds, reptiles, amphibians).

Buffer scale. We performed an independent sample t-test to assess if direction of road (north-south or east-west) had a significant effect on mortality in the buffers. We ran one-way Analysis of Variance (ANOVA) to test for differences between the mortalities found in buffers among the three park areas (Kitty Todd Nature Preserve, Oak Openings Preserve, Maumee State Park). We conducted Tukey HSD post-hoc analyses to evaluate significant differences between groups. All statistics were run in IBM SPSS Statistics (Version 28.0.1.1).

We investigated the effects of the above variables on the total mortality count recorded in each buffer across all road surveys. We also considered the effects of variables on each taxon by examining each taxon mortality count separately. As an additional outcome variable for evaluation, we created a diversity measurement, which was the number of different taxa (1-4) found in each buffer.

For the outcome variable, mortality count, we combined all mortalities recorded across all years within each buffer. Correlations between bat call counts, micro- and macrohabitat variables and the mortality counts were assessed for the buffers. We removed highly correlated variables, i.e., Spearman $\rho \geq 0.70$, (Dormann et al., 2013) and variables not significantly correlated with the outcome (mortality count) variable. This process was repeated for each buffer outcome variable (mammal mortality count, bird mortality count, reptile mortality count, amphibian mortality count, and diversity measurement).

To accommodate overdispersion or issues with homogeneity of variance, negative binomial distribution generalized linear modeling (GLM), a preferred method for accommodating this type of count data (O'Hara & Kotze, 2010), was utilized to determine which variables explained the mortality counts within the buffers. We further reduced the number of variables in the model by removing those with a standard coefficient $|\beta| < 0.10$ (MacKinnon et al., 2005). We assessed models based on Akaike's Information Criteria adjusted for small sample size (AICc), identifying the best model as the one with the lowest AICc (Burnham & Anderson, 2002; Symonds & Moussalli, 2011). These steps were repeated for reptile mortality count within buffers because, similar to the total vertebrate mortality, reptile mortality count did not meet the assumptions of regression and had over dispersed data.

Mammal mortality counts, amphibian mortality counts, bird mortality counts and taxon diversity within the buffers were investigated using multiple stepwise regression models to determine which predictor variables explained the mortality counts within the buffers, as these outcome variables did not violate the assumptions of regression (Ramette, 2007). We assessed models based on adjusted R-squared values, as these take the number of predictors into account and, therefore, describe the most parsimonious models (Onyutha, 2020). Taxa interaction variables were not considered as predictors of diversity measurement.

Road scale. For the road scale mortality count, we combined all mortalities recorded within all buffers along the same road and divided by the number of buffers on that road, to normalize data as not every road was the same length with equal number of buffers. The road diversity measurement was considered the number of different taxa (1-4) found along each road. We used the normalized count data to investigate the possible relationship with the predictor variables described above. Correlations were conducted for the road count. We removed highly correlated variables (Spearman $\rho \geq 0.70$) (Dormann et al., 2013) and variables not significantly correlated with the outcome (mortality count) variable.

Once the number of predictor variables was reduced, we investigated the relationship with the outcome variable using multiple stepwise regression models to determine which predictor variables explained the mortality counts for the roads, as these outcome variables met the assumptions of regression (Ramette, 2007). We assessed models based on adjusted R^2 values, as these take the number of predictors into account and, therefore, provide the most parsimonious models. We repeated these steps for the remaining outcome variables: road mammal count, road bird count, road reptile count, road amphibian count, and road diversity measurement.

Results

We traveled a total of 2,731 kilometers by bicycle for data collection between 2020-2022, requiring approximately 700 hours of collection. We recorded a total of 654 vertebrate mortalities across the 1,764 km of road surveyed, approximately 0.37 per kilometer. Out of the 654 vertebrate mortalities, 318 were mammals, 124 were birds, 120 were reptiles, and 92 were amphibians. A one-way ANOVA revealed a significant difference between the mortalities found by taxon, F (3,8) = 11.34. $p < 0.05$ (Figure 1.7). Post-hoc analysis revealed that there were significantly more mammal mortalities than any other taxa ($p < 0.05$). Bird, reptile, and amphibian mortalities did not significantly differ from one another.

Mortality Point Scale

At the scale of individual mortality points, we found that there was no significant association between mortality occurrence and protected area χ^2 (2, N = 654) = 4.96, p = 0.08 (Figure 1.8 & Figure 1.9). However, when looking at the specific taxon there was a significant association between amphibian mortality occurrence and protected area status, χ^2 (2, N = 92) = 7.18, $p < 0.05$, where there were fewer amphibian mortalities (N = 53) than expected (N = 64) with unprotected land on both sides of the road (Figure 1.10).

There was a significant association between total mortality occurrence and land use, χ^2 (5, N = 654) = 54.79, $p < 0.001$ (Figure 1.11). There appears to be sufficient evidence to suggest that mortality occurrence differs between land use categories. More mortality occurred than expected with natural habitats on both sides of the road or developed land use on one side and natural land use on the other side of the road (R = 4.67, R = 2.77). Fewer mortalities than expected occurred when there was agricultural land use on both sides or agriculture on one side with natural habitats on the other side of the road (R = -2.10, R = -3.97, respectively). When examining the individual taxon, there was a significant association between mammal mortality occurrence and land use, χ^2 (5, N = 654) = 46.63, $p < 0.001$ (Figure 1.12), and amphibian mortality occurrence and land use, χ^2 (5, N = 654) = 46.63, $p < 0.001$ (Figure 1.13). More mammal mortalities occurred than expected when the road had natural on both sides, developed land use on one side and natural land use on the other side; and fewer mammal mortalities than expected occurred when the road had agricultural land use on both sides (R = 3.18, R = 2.09, R =

-2.69, respectively) Like mammals, amphibian mortalities occurred more than expected when the road had developed land use on one side and natural land use on the other side (R = 2.18) (Figure 1.13).

Buffer Scale

The direction of the road did not have an effect on the buffer mortality count, t (119) = -2.31, p = 0.25. There was a significant effect at this buffer scale of the nearby park on mortality counts, F (2,118) = 5.37. p < 0.05. Significantly more mortalities were found near Kitty Todd Nature Preserve in the north of the study area than near Maumee State Forest in the south of the study area (p < 0.05).

For total mortality count we found annual average daily traffic volume (AADT), road width, percent reptile, percent amphibian, maximum percent canopy cover, minimum percent canopy cover, maximum number of saplings, land use category 1 (natural both sides), land use category 2 (natural/developed), proportion perennial ponds, proportion upland prairie, number of patches, and average water capacity were significantly positively related to the total buffer mortality count, while land use category 5 (developed/agriculture), land use category 6 (agriculture both sides), and proportion cropland, were significantly negatively related to buffer mortality count (p < 0.05) (Table 1.3). We reduced the original list of predictor variables to these 16 for the GLM models.

GLM AICc comparisons indicated the best model for buffer vertebrate mortality included 10 of the originally considered 16 predictor variables: AADT, road width, percent reptile, percent amphibian, maximum percent canopy cover, minimum percent canopy cover, maximum number of saplings, land use category 1 (natural both sides), land use category 2 (natural/agriculture), land use category 5 (agriculture/developed), land use category 6 (agriculture both sides), proportion perennial ponds, proportion upland prairie, proportion cropland, number of patches, and average water capacity (Table 1.4). The likelihood ratio test indicated a significant improvement in model fit compared to the null model (χ^2 (10) = 43.49, $p <$ 0.001). The results revealed that road width was a significant predictor of buffer vertebrate mortality (B = 0.35, SE = 0.08, $Wald$ = 21.46, $p < 0.001$). A unit increase in road width was associated with a 1.4-fold increase in the expected mammal mortality count. According to the model, land use category 5 (agriculture/developed) was the only other significant predictor of buffer mortality (B = -0.86, SE = 0.36, $Wald$ = 5.89, $p < 0.05$). A unit increase in the proportion of land use category 5 (agriculture/developed) is associated with a 57.8 percent decrease in the expected mortality count.

Mammals. Annual average daily traffic volume (AADT), road width, average canopy cover, proportion residential/mixed landcover, and average NDVI score were significantly positively correlated to buffer mammal mortality count, while proportion of land use category 5 (agriculture/developed) and proportion cropland were significantly negatively correlated to buffer mammal mortality count ($p < 0.05$) (Table 1.3). We reduced the original list of predictor variables to these 7 for the subsequent models.

The multiple regression model that had the most explanatory power ($R^2_{adj} = 0.31$) when predicting buffer mammal mortality counts included AADT, average canopy cover, and proportion of residential/mixed landcover ($R^2 = 0.58$, F (3, 112) = 18.02, $p < 0.001$). Average annual daily traffic, average canopy cover, and proportion of residential/mixed landcover significantly predicted buffer mammal mortalities, t (112) = 5.81, $p < 0.001$, t (112) = 2.19, $p <$ 0.05, and t (112) = 1.98, $p < 0.05$, respectively (Table 1.4). Specifically, these variables were positively predictive of buffer mammal mortality counts. For every unit increase in the AADT there was a predicted increase of 0.002 in mammal mortalities ($\beta = 0.48$; $B = 0.002$, $SE = 0.000$). Mammal mortalities were predicted to increase by 0.03 for every percent increase in canopy cover. ($\beta = 0.17$; $B = 0.03$, $SE = 0.015$). Finally, for every unit increase in the proportion of residential/mixed landcover the mammal mortality was predicted to increase by 2.071 ($\beta = 0.16$; $B = 2.07$, $SE = 1.04$).

Reptiles. For reptiles, we found percent amphibian mortality, road width, minimum percent canopy cover, maximum percent canopy cover, maximum number of saplings, minimum number of saplings, proportion of land use category 1 (both natural), proportion of land use category 2 (natural/developed), proportion of protected area 2 (protected/unprotected), proportion perennial ponds, proportion swamp forest, proportion upland prairie, number of patches, and average water capacity were significantly positively correlated to the buffer reptile mortality count ($p < 0.05$); while percent mammal mortality, proportion of land use category 5 (agriculture/developed), proportion of land use category 6 (agriculture both sides), maximum patch area, and proportion cropland were significantly negatively correlated to the buffer reptile mortality count ($p < 0.05$) (Table 1.3). We reduced the original list of predictor variables to these 19 for subsequent modelling.

GLM AICc comparisons indicated the best model for buffer reptile mortality included 5 predictor variables: road width, land use category 5 (agriculture/developed), land use category 6 (agriculture both sides), proportion of upland prairie landcover, and percentage mammal mortality (Tables 1.4 & 1.6). The likelihood ratio test indicated a significant improvement in model fit compared to the null model (χ^2 (5) = 55.78, $p < .001$). Road width and proportion of upland prairie landcover were significant positive predictors of buffer reptile mortality ($B = 0.47$, $SE = 0.15$, $Wald = 9.16$, $p < 0.05$ and $B = 3.00$, $SE = 1.38$, $Wald = 20.10$, $p < .05$, respectively).

Proportion of land use category 5, land use category 6, and percent mammal mortality were significant negative predictors of buffer reptile mortality (*B* = -2.41, *SE* =0.87, *Wald* = 7.61, $p <$ 0.05, *B* = -2.98, *SE* = 0.93, *Wald* = 10.24, $p \leq 0.05$, and *B* = -1.64, *SE* = 0.55, *Wald* = 8.81, $p <$ 0.05, respectively).

Amphibians. For amphibians we found road width, percent reptile mortality, buffer bat calls, average percent canopy cover, average number of saplings, proportion of land use category 1 (natural on both sides), proportion of protected area, proportion wet prairie, proportion wet shrubland, proportion swamp forest, proportion upland prairie, and average water capacity were significantly positively correlated to the amphibian mortality count ($p < 0.05$) while speed limit, percent mammal mortality, proportion of land use category 5 (agriculture/developed), maximum patch area, proportion residential/mixed area, proportion upland deciduous forest, proportion sand barrens, and maximum NDVI score were significantly negatively correlated to the buffer amphibian mortality count ($p < 0.05$) (Table 1.3). We reduced the original list of predictor variables to these 20 for subsequent modelling.

The multiple regression model that had the most explanatory power ($R^2_{adj} = 0.23$) when predicting buffer amphibian mortality counts included road width, proportion of swamp forest, percent mammal mortalities, proportion of land use category 5 (agriculture/developed), and maximum NDVI scores as predictor variables explaining a significant portion of the total variance in the counts ($R^2 = 0.27$, F (5, 120) = 8.35, $p < 0.001$) (Table 1.4). Road width, percent mammal mortalities, and proportion of land use category 5 significantly predicted buffer amphibian mortalities, t (120) = 3.24, $p < 0.05$, t (120) = -3.73, $p < 0.001$, and t (120) = -2.34, $p < 0.05$, respectively. Specifically, road width was positively predictive of buffer amphibian mortalities of buffer amphibian mortalities ($\beta = 0.27$; *B* = 0.28, *SE* = 0.09). However, both percent mammal mortalities (β = -0.31; B = -1.01, SE = 0.27) and proportion of land use category 5 (β = -0.20; B = -0.88, SE = 0.38) were negatively predictive of buffer amphibian mortalities. Maximum NDVI scores were marginally negatively predictive of buffer amphibian mortalities (β = -0.16; B = -3.16, SE = 1.79), while proportion of swamp forest was marginally positively predictive of buffer amphibian mortalities (β = 0.16; B = 1.11, SE = 0.63).

Birds. For birds, we found AADT, road width, and average water capacity were significantly positively related to the buffer bird mortality count ($p < 0.05$); while percent mammal mortalities (cross taxa effect of the percentage of buffer mortalities that were mammals), proportion sand barrens, and depth to water table were significantly negatively correlated to the buffer bird mortality ($p < 0.05$) (Table 1.3). We reduced the original list of predictor variables to these 6 for subsequent modelling.

The multiple regression model that had the most explanatory power ($R^2_{adj} = 0.12$) when predicting buffer bird mortality counts included road width and percent mammal mortalities and explained a significant portion of the total variance in the counts ($R^2 = 0.13$, $F(2, 112) = 8.45$, $p < 0.001$). Road width and percent mammal mortalities significantly predicted buffer bird mortalities, $t(112) = 3.43$, $p < .001$ and $t(112) = -2.25$, $p < 0.05$, respectively (Table 1.4). Specifically, road width was positively predictive (β = 0.30; B = 0.38, SE = 0.11) of the outcome. On the other hand, percent mammal mortalities was negatively predictive of buffer bird mortalities (β = -.200; B = -.696, SE = .309).

Diversity. Taxa diversity for the buffers was similar to the total mortality count in relation to predictor variables. For buffer diversity we found annual AADT, road width, bat count, minimum percent canopy cover, maximum number of saplings, land use category 1 (both natural), land use category 2 (natural/developed), proportion of protected area category 1 (both protected), percent of protected area, number of patches, proportion perennial ponds, proportion swamp forest, proportion upland prairie, and average water capacity were significantly positively related to the buffer taxa diversity, while land use category 5 (agriculture/developed), land use category 6 (agriculture both sides), proportion sand barrens, and proportion cropland, were significantly negatively related to buffer diversity ($p < 0.05$) (Table 1.3). We reduced the original list of predictor variables to these 18 for subsequent modelling.

The multiple regression model that had the most explanatory power ($R^2_{adj} = 0.30$) when predicting buffer diversity had road width, proportion of upland prairie, proportion of land use category 5 (agriculture/developed), and proportion of land use category 6 (agriculture both sides) as predictor variables explaining a significant portion of the total variance in the counts ($R^2 = 0.32$, F (4, 112) = 12.94, $p < 0.001$). Specifically, road width ($\beta = 0.35$; $B = 0.44$, $SE = 0.10$) and proportion of upland prairie landcover ($\beta = 0.18$; $B = 1.89$, $SE = 0.84$) were positively predictive buffer diversity (Table 1.4). Both proportion of land use category 5 ($\beta = -0.31$; $B = -1.45$, $SE = 0.38$) and proportion of land use category 6 ($\beta = -0.28$; $B = -1.34$, $SE = 0.38$) were negatively predictive of buffer diversity (Table 1.4).

Road Scale

At the largest scale for total road mortality count we found AADT, road width, available water capacity, and available water storage were significantly related to the total mortality count ($p < 0.05$) (Table 1.7). The multiple regression model that had the most explanatory power ($R^2_{adj} = 0.49$) when predicting road mortality counts contained two variables, AADT and average water capacity, and explained a significant portion of the total variance in the counts ($R^2 = 0.54$, F (2, 21) = 11.27, $p < 0.001$). Annual average daily traffic volume and average water capacity significantly predicted road mortalities, t (21) = 3.50, $p < 0.05$ and t (21) = 2.75, $p < 0.05$, respectively (Table 1.8). Specifically, both variables positively predicted ($\beta = 0.55$; $B = 0.003$, $SE = 0.001$ and $\beta = 0.43$; $B = 89.74$, $SE = 32.62$, respectively) of the total mortality count at the road scale.

Mammals. Annual average daily traffic volume (AADT), road width (RW), average water capacity (AWC), and available water storage (AWS) were significantly positively correlated with mammal mortality count at the road scale (Table 1.7). The multiple regression model that had the most explanatory power ($R^2_{adj} = 0.76$) when predicting road mortality counts only contained AADT and explained a significant portion of the total variance in the mammal mortality counts ($R^2 = 0.78$, F (1, 21) = 68.71, $p < 0.001$) (Table 1.8). Annual average daily traffic volume significantly predicted road scale mortalities for mammals, t (21) = 8.29, $p < 0.001$, $\beta = 0.88$; $B = 0.003$, $SE = .000$.

Reptiles. For road scale reptile mortality count we found that road width (RW), proportion of perennial ponds, proportion of upland prairie, and average water capacity were significantly positively correlated to the mortality count ($p < 0.05$) (Table 1.7), while percent bird mortalities was significantly negatively correlated to road reptile mortalities.

The multiple regression model that had the most explanatory power ($R^2_{adj} = 0.39$) when predicting road scale reptile mortality counts contained two variables, UP and AWC, and explained a significant portion of the total variance in the road reptile counts ($R^2 = 0.67$, F (2, 22) = 8.03, $p < 0.05$). Annual average daily traffic volume and average water capacity significantly predicted road mortalities, t (22) = 2.93, $p < 0.05$ and t (22) = 1.91, $p < 0.05$, respectively (Table 1.8). Specifically, proportion upland prairie and average water capacity positively predicted ($\beta = 0.50$; $B = 5.52$, $SE = 1.88$ and $\beta = 0.33$; $B = 17.16$, $SE = 8.96$, respectively) the reptile mortality count.

Amphibians. For road scale amphibian mortality count we found percent reptile mortality, proportion of protected category 2 (protected/unprotected), proportion swamp forest, proportion upland prairie, and average water capacity, were significantly positively correlated to the amphibian mortality count ($p < 0.05$), while percent mammal mortality, patch area minimum, patch area maximum, and average NDVI score were significantly negatively correlated to the road amphibian mortality count ($p < 0.05$) (Table 1.7). We reduced the original list of predictor variables to these 9 for subsequent modelling.

The multiple regression model that had the most explanatory power ($R^2_{adj} = 0.66$) when predicting road scale amphibian mortality counts had percent mammal mortalities, proportion of protected area 2 (protected/unprotected), proportion of upland prairie, and average water capacity as predictor variables, explaining a significant portion of the total variance in the counts ($R^2 = 0.85$, F (4, 22) = 11.75, $p < 0.001$). Proportion of protected area 2 ($\beta = 0.39$; $B = 1.12$, $SE = 0.36$), proportion of upland prairie ($\beta = 0.34$; $B = 3.14$, $SE = 1.27$), and average water capacity ($\beta = 0.29$; $B = 12.46$, $SE = 5.76$) significantly positively predicted road amphibian mortalities, t (22) = 3.12, $p < 0.05$, t (22) = -2.48, $p < 0.05$, and t (22) = 2.16, $p < 0.05$, respectively (Table 1.8). Conversely, percent mammal mortalities ($\beta = -0.39$; $B = -1793.23$, $SE = 652.31$) was negatively predictive of the road scale amphibian mortality outcome t (22) = -2.75, $p < 0.05$ (Table 1.8).

Birds. Average water capacity (AWC) was significantly positively correlated to the road scale bird mortality count ($p < 0.05$) (Tables 1.7 & 1.8). The regression revealed AWC explained a significant portion of the total variance in the road bird counts ($R^2 = 0.28$, F (1, 22) = 8.19, $p < 0.05$).

Diversity. For taxa diversity along the road, we found average percent canopy cover, minimum vegetation, number of different landcover class present, and number of patches, were significantly negatively related to buffer diversity ($p < 0.05$) (Table 1.7). We reduced the original list of predictor variables to these 18 to begin modelling.

The multiple regression model that had the most explanatory power ($R^2_{adj} = 0.51$) when predicting road scale taxa diversity only had one variable, number of landcover classes, as a predictor variable explaining a significant portion of the total variance in the counts ($R^2 = 0.73$, F (1, 22) = 23.94, $p < 0.001$). Specifically, number of landcover classes ($\beta = 0.73$; $B = 0.001$, $SE = 0.000$) was positively predictive buffer diversity (Table 1.8).

Discussion

The number of mortalities per kilometer we found in our study (0.37) was similar to other studies that found approximately 0.4 vertebrate mortalities per kilometer (Glista & DeVault, 2008; Garriga et al., 2012). However, our study differed from previous studies where amphibians and reptiles dominated the mortalities (Glista et al., 2008; Garriga et al., 2012), as the majority of mortalities were mammals in our study.

We found that an increase in road width and AADT was associated with higher mortalities; this is consistent with previous studies that indicate the road becomes more dangerous when more cars are present and there is a greater journey to exit that danger zone (Skórka et al., 2013; Canal et al., 2019). We also found that natural areas (areas classified as natural on one side of the road and either natural or developed on the other) were associated with increased vertebrate mortalities. Even if developed areas are acting as habitat, those areas are dominated by mowed yards with limited resources, making it less likely that vertebrates were concentrated within this habitat long term without the need to disperse for some additional resources (Belaire et al., 2016). In other words, animals in developed areas would be expected to have to move around more to find suitable resources and thus be more likely to suffer the consequences of limited permeability. The positive relationship identified between vertebrate mortality and the natural areas suggests that either there are more vertebrates present in these areas or vertebrates are moving toward and using these natural areas for resources. Given the highly fragmented nature of the landscape, the roads could be bisecting areas necessary for resources, meaning there is more movement because the habitat patches are too small and disconnected for vertebrates to persist within just one (Janin et al.,2012). Our findings are consistent with other studies that found increased road mortalities near protected natural areas (Garriga et al., 2012).

Upland prairie tends to occur in small, isolated patches within the OOR (Martin & Root, 2020). This provides an explanation for why upland prairies are associated with increased vertebrate mortalities, as again, animals must move among the small, isolated patches in order to obtain the necessary resources (Janin et al.,2012). This result could also be an illustration of the habitat amount hypothesis (Gardiner et al. (2018), where the amount of habitat within a given area is directing the occupancy and dispersal of these vertebrates, regardless of whether roads are fragmenting it. We also found the proportion of perennial pond areas to be positively associated with vertebrate mortality. This is logical since ponds provide fundamental resources to wildlife and may attract movement towards them (Hassall, 2014; Ancillotto et al., 2019; Hill et al. 2021).

The negative relationship we found that agricultural land use (areas classified as agricultural on one side of the road and either agricultural or developed on the other) had with mortality count suggests that vertebrates are not using agricultural (cropland) for habitat or could be avoiding them. An alternative explanation is that vertebrates that utilize these may have sufficient resources and do not need to disperse, which is unlikely given past studies have illustrated that cropland provided diminished habitat qualities (Egli et al., 2018; Stanton et al., 2018; Şekercioğlu et al., 2019).

We found that the taxa diversity for all vertebrates at the buffer scale was consistent with the total mortality count at the buffer scale, in that it increased with increasing road width and proportion of upland prairie and decreased with increasing proportions of land use category 5 (agriculture/developed) and land use category 6.

Mammal mortalities at the buffer scale were influenced by increases in traffic (AADT), average canopy cover, and developed land cover. These findings are consistent with other studies on mammal roadkill which found canopy cover can act as a visual barrier, reducing visibility for both wildlife and drivers (Gleason & Jenks, 1993). Over 50% of the mammals we found were nocturnal mesopredators (opossum, skunk, and raccoon), which exacerbates the impact of lowered visibility. In addition, mesopredators have exhibited adaptability to residential areas (Červinka et al., 2014; Rodriguez et al., 2021). Road width positively predicted the mortalities of the remaining taxa. While the road width varied from 4-9 m, 68% of the roads were between 6-7 meters wide. It is possible these small variations do not have much of an effect on larger organisms, such as the mammals within our study area.

The variables affecting reptile mortality at the buffer scale were consistent with those affecting total vertebrate mortality and directly reflected those that affected overall diversity of mortality. We found the increase in road width and proportion of upland prairie within the buffer and the decrease in the proportion of agricultural land use predicted more reptile mortalities. Agricultural land and upland prairie may represent important thermal habitats for these ectotherms. Previous studies have found reptiles have difficulty crossing wider roads (Meek, 2009). The variables that predicted reptile mortality at the road scale differed from those at the buffer scale. Soil average water capacity and proportion of upland prairie predicted reptile mortalities at the road scale.

Amphibian mortalities were consistent with other taxa models that predicted more mortality when there was less agricultural land. We also found that an increase in the proportion of swamp forest land class and decreased maximum NDVI scores predicted an increase in amphibian mortalities. Swamp forest is likely acting as habitat for amphibians, having the necessary elements for at least some of their life stages (Baldwin et al., 2006). Although, because of their varied life stage requirements, dispersal to and from the swamp forest landcover may be required. The negative relationship with productivity index (NDVI) could suggest that their resource requirements are being met in areas with higher productivity, negating the necessity to disperse to new areas or be related to thermal requirements for ectotherms. Slightly different variables predicted amphibian mortality at the road scale, including soil average water capacity and proportion of upland prairie, coincided with those predicting reptile road mortalities. In addition, the proportion of area that had protected area on one side of the road and unprotected area on the other predicted amphibian mortalities at the road level. Previous research has linked the number of amphibians killed on roads with protected areas (Garriga et al., 2012).

Road width was the only spatial factor that contributed to the predictive model for bird mortality at the buffer scale, which is consistent with previous research (Forman & Alexander, 1998), but this variable explained less than 10% of the variance in bird mortality count. This suggests that bird mortalities are more complicated than the variables measured in this study could explain. This could be attributable to how different bird movements are compared to the other taxa and the frequent use of roadside areas by birds (Forman & Alexander, 1998; Dean & Milton, 2003; Morelli et al., 2014). The only variable contributing to predicting bird mortality at the road scale was average water capacity, explaining 28% of the variance in the bird mortality counts. This suggests that the larger scale variables are more appropriate predictors of bird mortality counts, but still suggest there are components to bird mortality that were not accounted for in this study.

Our results illustrate the importance of scale to the assessment of mortalities. As expected, much of the detail gets lost on the larger scale. Mortality counts at the road scale were associated with variables that were obtained at a larger scale, traffic and soil average water capacity. The measurements of annual average daily traffic were commonly spaced at least 1-1.5 km between points (ODOT). The soil surveys, which provided the average water capacity information, were mapped at 1:15,800 scale (NRCS, 2007). There are far fewer variables that contribute to explaining the variance in total vertebrate mortality at the road scale (i.e., 2) compared to the number of variables that contribute to explaining the variance in total vertebrate mortality at the buffer scale (i.e., 10).

Our study does not address how decreased permeability affects populations or communities, but we can reason that if areas with insufficient resources require the need for organisms to move around and this movement is killing them (i.e., road mortalities) then that impacts the fitness of individuals, which could affect the fitness of the population overall. This study also does not attempt to answer how or why vertebrates are moving across the landscape. We would argue that the areas in which the potential movement is hindered (i.e., low permeability) are more important to direct management. However, our results can be coupled with knowledge about dispersal, foraging tendencies, and habitat selection to make logical assumptions about the answer to these two questions. We assumed that our vertebrate mortality counts were representative and not biased, or at least equally biased across the landscape, by low carcass detectability or high removal rates because of the extent, frequency, and type of sampling (Santos et al., 2011; Ratton et al., 2014; Santos et al., 2016). Our study attempted to control for detectability differences by surveying by bicycle to reduce the influence of small carcass size on detection (Garrah et al., 2015). The effectiveness of this was demonstrated by the fact we recorded the mortalities of reptiles and amphibians that were only the size of a quarter.

As we expected, road characteristics, such as AADT and road width, were associated with mortality. While this information may contribute to planning future roads, as in reducing road width and occurrence of roads, particularly around protected areas, alterations to factors such as road width and traffic density are not necessarily practical. Managing human behavior is much more difficult and has not demonstrated to be an effective method of reducing roadkill. Research has found that driving speed on roads is dictated more by the speed the driver feels is safe and socially acceptable and less by the posted speed limit. (Elliott et al., 2005; Mannering, 2009), indicating a reduction in speed limit is likely to suffer from a lack of compliance, and therefore, is not an actual reduction in drive speed. This was the case in a previous study that investigated reducing speed limits to mitigate roadkill and found that there was only a 3-5 mph speed reduction as opposed to the 15-mph posted reduction, which resulted in no change to the risk of vehicle collisions for the wildlife (Treves et al., 2006).

We determined several spatial factors that impact the number of vertebrate mortalities along a road and that the impact of these factors differ based on taxon and scale. As we expected, landscape composition and structure influenced mortality. Fragmentation will continue to occur if steps are not taken to prevent it, which translates to increases in smaller habitat patches requiring increased dispersal by organisms to find resources and increases the likelihood of encountering roads. This is particularly important in and around protected areas that are acting as source areas, but where habitat may occur in smaller patches, as land managers seek to maximize diversity, that require dispersal between them and outside of the park boundaries. However, managers can alter the landcover and habitat patch size in areas of concern to mitigate these effects. Optimally, we want roadless natural protected areas, barring that we need to at least consider the structural and vegetation characteristics that border those roads. Therefore, altering structural and vegetation characteristics is proposed as a more effective strategy than attempting to manage human behavior with a particular focus on configuration as well as composition.

Our research demonstrates the validity of employing road surveys as a reliable method for gaining insights into the locations of vertebrate mortalities on roadways and the spatial factors influencing the corresponding lack of permeability across the landscape. Roads and their corresponding mortalities should be considered when determining how effective protective areas are at accomplishing protection. Our findings also reveal that vertebrate mortalities are not uniformly distributed throughout the region and vary across taxonomic groups. We were focused on general patterns and due to the issues with identifying vertebrates to species, caution should be taken when trying to apply these results specifically to rare or vulnerable species, considering the vertebrates we were able to identify to species were more common generalist species (e.g., Virginia opossum, *Didelphis virginiana*, and raccoon, *Procyon lotor*). There may also be spatial factors that were not investigated in this study that contribute to vertebrate mortality counts. There was also a large amount of variation within the buffers, so further analysis at smaller scales may be necessary for investigating the complexity in specific areas. Additionally, we demonstrate the results vary depending on the scale of analysis. These outcomes imply that for a more comprehensive understanding of vertebrate mortality and landscape permeability, adopting a multi-taxa and multi-scale approach is important.

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CHAPTER II. THE EFFECTS OF TEMPORAL VARIABILITY ON PERMEABILITY PATTERNS OF VERTEBRATES WITHIN THE OAK OPENINGS REGION

Introduction

In complex environments, the temporal variation in biotic and abiotic factors can shape the distribution of species on the landscape. These effects can differ depending on the temporal scale considered, especially across taxa that vary in their feeding, breeding, and shelter needs. Movement patterns are represented by temporal changes, in addition to the spatial changes, associated with resource availability, life history stages, and habitat use (Grilo et al., 2009; Philcox et al.,1999). The resources organisms need, and therefore their movements to meet those needs, vary across time. For example, movements and distributions associated with the shifting from summer foraging to winter hibernacula represent seasonal changes (Larsen, 1987; Web & Shine, 1997). These seasonal changes in how organisms are distributed across the landscape have been well documented (da Rosa & Bager, 2012; Sassi et al., 2011; Van Gelder, 1973). Vertebrates have varying life histories; however, many of them are longer lived species, which provides a way to look at ecological effects across a variety of temporal scales. Multi-year studies can capture variability in distribution and relative abundance (Brockie et al., 2009) as well as variability in ecosystem dynamics (Sinclair et al., 2007; Jones & Driscoll, 2022). These long-term studies allow for more informed comparisons of effects and ecological responses (Turner et al., 2003; Orwig et al., 2008).

There are year-to-year differences in environmental variables and resource availability. Interannual variation in aboveground net primary productivity has been recorded, although the degree of variation depends on the type of habitat being investigated, with the greatest variability found in grasslands and the least amount of variability found in forest habitats (Knapp & Smith, 2001). Annual fluctuations in environmental variables, such as water availability, have been associated with variation in functional trait patterns in plants, such as seed mass (Carmona et al., 2015). Interannual variation in abundance has been associated with maximum snow depth, summer temperature and precipitation, and winter temperatures (Bowden et al., 2018). The reproduction success of several amphibian species depends on the combination of the timing, frequency, and duration of precipitation (Greenberg et al., 2017). Without the required hydrological regime, amphibian populations could go several years without breeding (Hansen, 1958). The annual temporal stability of raccoon populations was found to be associated with habitat patches with streams (stable water source) and greater plant diversity (stable food availability) (Beasley et al., 2011).

Climate change has already had substantial documented effects on the structure of ecosystems and communities (Newbold, 2018). Land use changes cause local climatic changes. For example, altered land, such as forested areas converted to agricultural areas, frequently experience more temperature extremes and are drier as compared with the original natural habitats, partly due to the removal of climate-buffering canopy cover (Frishkoff et al., 2016). Land use changes can cause habitat degradation or loss as well as fragmentation, which alter the landscape and redistribute or even remove resources. Therefore, land use change can have a significant effect, positive or negative, on the diversity, abundance, and distribution of vertebrates over time (Coetzee & Chown, 2016; Ficetola et al., 2010; Gross et al., 2009). Environmental factors that vary temporally, such as mean day and night time temperatures, humidity, and precipitation have also been demonstrated to contribute to the presence of roadkill (Zhang et al., 2018; Carvalho et al., 2017; Costa et al., 2020). Roadkill, or vertebrate mortality, denotes a lack of permeability, or decreased ability to move across the landscape, as roads pose a serious threat to movement between patches (van der Ree et al., 2015; Ceia-Hasse et al., 2017). Decision makers most often use the identification of clusters of road mortalities to implement mitigation on roads (Santos et el., 2015).

Our current research is focused on the larger temporal scale (i.e., annual, or more long term as compared with seasonal) and the changes underlying patterns of resources. Many of the vertebrates in the current study have longer life histories, so the focus of the project is on the longer-term changes and potential cross generational impacts. In the context of this study permeability is the ease at which vertebrates can move from patch to patch through the mixed disturbance landscape; since the study area is so fragmented by roads, vertebrates will need to cross the roads to accomplish this movement between patches. Therefore, we are considering vertebrate mortality, specifically from roads, as a tool for assessing permeability. Vertebrate mortality will illuminate how animals are moving through the area in relation to each other and their environment, as it is important to understand the real tradeoffs that animals confront as they move through altered ecosystems. Our approach allows us to evaluate how different types of factors that vary temporally influence vertebrate permeability.

Research Questions

1. How does vertebrate mortality differ across time?
2. How do spatial, structural, and environmental features change over time?
3. What temporal changes can predict the differences in vertebrate mortality?

Methods

Road Surveys

We surveyed a total of 23 roads, covering 49.0 kilometers (km). The roads were situated near one of three natural areas, Kitty Todd Nature Preserve, Maumee State Forest, or Oak

Openings Preserve. We surveyed a total of 8 roads (15 transects), covering 19.57 km, near Kitty Todd Nature Preserve; a total of 7 roads (9 transects), covering 13.71 km near Maumee State Forest; and a total of 9 roads (10 transects), covering 15.77 km, near Oak Openings Preserve. The roads were divided into transects at designated intersections; therefore, the resulting 34 road transect lengths ranged from 0.8 km – 2.0 km. All roads were two lane, paved roads, with speed limits that vary between 56.3 kph (35 mph) and 88.5 kph (55 mph).

Roads within the study area were surveyed for all dead terrestrial vertebrates, e.g., amphibians, birds, mammals, and reptiles. We surveyed all transects every other week from May to October in 2020, 2021, and 2022, totaling 36 surveys (12 per year). We conducted the surveys by bicycle, riding 16.1 - 24.9 kph (10.0 - 15.5 mph), similar to Garrah et al., (2015). As walking the transects was not feasible, bicycling was used as car surveys tend to underestimate roadkill, especially reptiles and amphibians (Langen et al., 2007). However, car surveys were conducted when weather was unsafe to complete the survey by bicycle (approximately 10% of the total number of surveys). When car surveys were employed, we evaluated all transects by car to keep the data consistent across transects for that survey.

For each vertebrate mortality found, we recorded the location with a Garmin Etrex GPS, identified the organism to taxonomic class (and species, if possible), took photographs of the organism and surrounding areas, noted where and in what condition the organism was, as well as recorded a variety of environmental and spatial variables (Table 2.1 & 2.2). Due to the quality of the carcass, most identifications were left to class. Animal carcasses were not removed, duplicate entries of persistent carcasses were avoided by referring to prior data before recording new entries.

Spatial, Structural, and Productivity Features of Road Areas

Along each transect sampling points were placed approximately every 400 meters to evaluate the heterogeneity along the road segment. We created 200-meter buffers (radius from fixed sampling point) around each sampling point, resulting in a 160,000 square meter area to provide an evaluation of landscape context. Spatial and structural features were measured for all roads at these fixed points and at all mortality points (Figure 1.1). The features we measured included microhabitat data, such as canopy cover, adjacent vegetation height, understory presence, and water presence. These features were measured at each mortality point and during point surveys in June 2020, May-October 2021, and June and October 2022 (Table 2.1). For all continuous variables, the minimum, maximum, and average values were investigated.

The height of vegetation directly adjacent to the road was measured on both sides in centimeters at each sampling point. The presence of an understory within 10 m of the road was recorded for each side of the road, and the sampling point was classified as no presence, present on one side, or present on both sides.

Canopy cover data was collected at taken on each side of the road and was estimated by taking photographs and analyzing them in Canopeo, which measures canopy cover by calculating fractional green canopy cover, turning images black and white and measuring the shade values as the amount of white pixels vs. black pixels, where the white pixels are areas covered by canopy (Patrignani & Ochsner, 2015). The minimum, maximum, and average percent canopy cover values from each side were recorded. The change in vegetation height and canopy cover from the beginning of sampling (June) period to the end of the sampling period (October) were calculated for each 2021 and 2022.

We also collected macrohabitat data, such as normalized difference vegetation index (NDVI), moisture stress index (MSI), normalized difference water index (NDWI), enhanced vegetation index (EVI_2) (Table 2.2).

NDVI, EVI_2, MSI, and NDWI indices were calculated from Landsat-8 images for our study area (Path 20, Row 31) acquired from the U.S. Geological Survey from early spring, summer, and fall and then combined across all three years. The indices were then calculated from the aggregate image in ESRI ArcGIS version 10.8.1. NDVI and EVI_2 use the satellite data to evaluate biomass patterns, while MSI and NDWI use the satellite data to evaluate water content (Valerio et al., 2022). In addition to the minimum, maximum, and average values per year that were considered, the minimum, maximum, and average for the summer (i.e., July) was evaluated for each indices, as peak values are expected during the summer (Chun & Guldmann, 2018; Naifet al., 2020).

Road features we measured included traffic volume (AADT). The annual average daily traffic volume (AADT) was acquired from the Ohio Department of Transportation Data Management System (https://odot.public.ms2soft.com/tcds/tsearch.asp?loc=Odot&mod=) once a year, following the year of sampling. Road speed limits, in mi/h, were obtained for each road during the study from either Fulton County Auditor’s Office, Henry County Sheriff’s Office, or Lucas County Engineer's Office. The roads were then converted to kilometers per hour (kph).

Environmental Features

Environmental features were recorded for the study area. This data included: temperature (C°), precipitation (cm), wind speed (km/h), humidity (%), dew point (C°), and barometric pressure (mmHg). Daily data for environmental features was obtained from two weeks before the first sampling event to the last date of sampling for each year. Daily precipitation, temperature, and wind speed were obtained from the NOAA weather data (https://ncdc.noaa.gov/). Humidity (%), dew point (C°), and barometric pressure (mmHg) were obtained from Weather Underground (https://www.wunderground.com/). The Toledo Express Airport weather station was used for both NOAA and Weather Underground data.

Analysis

We evaluated correlations between spatial, structural, and environmental variables for the buffers to reduce the number of variables. We removed highly correlated variables i.e., Spearman $\rho \geq 0.70$ (Dormann et al., 2013) from further investigations.

We ran one-way Analysis of Variance (ANOVA) to test the effect of time on vertebrate mortality. We conducted post-hoc Tukey's test to evaluate significant differences between groups. We also ran ANOVAs to test differences between our spatial, structural, and environmental variables over time. When necessary, Welch's F was used to accommodate violations to homogeneity of variance (Field, 2013). We conducted post-hoc Tukey's test to evaluate significant differences between groups. Games-Howell post-hoc test was employed instead of Tukey's when homogeneity of variance was not met (Field, 2013).

We used Spearman's correlation analysis to investigate the trends and significance between spatial, structural, environmental features and mortality counts within the buffered areas. Additionally, we tested for interaction effects between mortality count and year for total mortality counts as well as individual taxon mortality counts. We further investigated the relationship of any significant correlations with mortality using negative binomial distribution generalized linear modeling (GLM). This type of GLM is a preferred method for accommodating overdispersion and issues with homogeneity of variance when using count data (O'Hara &

Kotze, 2010). All statistical tests were conducted using SPSS version 28.0 (IBM SPSS Statistics).

Results

We found the largest total of vertebrate mortalities in 2021, with 246 mortalities (Table 2.3). Mammal mortalities dominated the mortalities found in 2020 and 2022, while the number of mortalities in 2021 were more evenly spread across the taxa (Table 2.3; Figure 2.1 & 2.2). There were no significant differences found between the years for total mortality or for any single taxon mortality.

We detected differences in structural, productivity, and environmental variables over time. We found a significant difference between the maximum number of saplings recorded between the years, Welch's F (2, 228.10) = 3.73, $p < 0.05$. Our post-hoc analysis revealed that the maximum number of saplings in 2020 was significantly greater than in 2022 ($p < 0.05$). There was a significantly greater change in average vegetation height over the sampling period in 2021 compared to 2022 (Welch's F (1, 215.61) = 34.64, $p < 0.001$). There were significant differences found between years for both the summer maximum NDVI, F (2, 362) = 13.330, $p < 0.001$, and average NDVI, F (2, 362) = 4.47, $p < 0.05$ (see Figures 2.3 and 2.4, respectively). Post-hoc analyses revealed that the values for both the summer maximum and summer average NDVI were significantly higher in 2020 and 2022 as compared to 2021 ($p < 0.05$), although values for 2020 and 2022 did not significantly differ. We found a significant difference between the maximum humidity between the years, Welch's F (2, 724.08) = 4.31, $p < 0.05$. Post hoc tests indicated that maximum humidity in 2022 was significantly lower in either 2020 or 2021 ($p < 0.05$). Although not significantly related to the mortality counts, there were peak times for humidity that coincided with or directly preceded spikes in monthly vertebrate mortality for certain taxon (Figure 2.5). There was a significant difference found between 2021 and 2022 for the change in average vegetation height (change in average vegetation height for the two sides of the road over the sampling season), Welch's F (1, 213.82) = 31.20, $p < 0.001$. The change in average vegetation height was greater in 2021 than in 2022.

While there were no significant differences found between years for either average temperature or precipitation, it should be noted that the contribution of each taxon to the total mortality was more evenly distributed in 2021, compared to 2020 and 2021 where mammal mortality was much greater than the other groups (Figure 2.1). The highest average temperature and total annual precipitation were recorded in 2021 (Figure 2.6 & 2.7, respectively).

Of the structural and productivity variables that had significant changes over time, we only found two to be significantly related to vertebrate mortality counts. The maximum number of saplings was positively related to vertebrate mortality counts ($r_s(363) = 0.21$, $p < 0.001$). The summer average NDVI was positively related to vertebrate mortality counts ($r_s(363) = 0.16$, $p < 0.01$). These variables were also significantly positively correlated to mammal ($r_s(363) = 0.16$, $p < 0.01$ and $r_s(363) = 0.17$, $p < 0.01$, respectively) and reptile mortality counts ($r_s(363) = 0.20$, $p < 0.001$ and $r_s(363) = 0.13$, $p < 0.05$, respectively), while only the maximum number of saplings was related to amphibian mortalities ($r_s(363) = 0.12$, $p < 0.05$). None of the variables that were significantly different between years were correlated with bird mortalities.

GLM AICc comparisons indicated the best model for vertebrate mortality (i.e., lowest AICc) included both maximum number of saplings and summer average NDVI (Table 2.4). The likelihood ratio test indicated a significant improvement in model fit compared to the null model (χ^2 (2) = 15.20, $p < 0.001$). Summer average NDVI was the only significant predictor of vertebrate mortality, as an increase in summer average NDVI was associated with an increase in the expected vertebrate mortality count ($B = 4.09$, $SE = 1.65$, $Wald = 6.17$, $p < 0.05$). The GLM model only including the summer average NDVI was also substantially supported as the change in AICc < 2 units compared to the best model (Table 2.4).

GLM AICc comparisons indicated the best model for mammal mortality (i.e., lowest AICc) only included summer average NDVI (Table 2.5). The likelihood ratio test indicated a significant improvement in model fit compared to the null model (χ^2 (1) = 13.65, $p < 0.001$). Summer average NDVI was a significant predictor of mammal mortality, as an increase in summer average NDVI is associated with an increase in the expected mammal mortality count ($B = 6.858$, $SE = 1.90$, $Wald = 12.99$, $p < 0.001$). The GLM model including both the summer average NDVI and the maximum number of saplings is also substantially supported as the change in AICc < 2 (Table 2.5).

GLM AICc comparisons indicated the best model for reptile mortality (i.e., lowest AICc) included both maximum number of saplings and summer average NDVI (Table 2.6). The likelihood ratio test indicated a significant improvement in model fit compared to the null model (χ^2 (2) = 15.77, $p < 0.001$). Maximum number of saplings was the only significant predictor of reptile mortality, as an increase in maximum number of saplings was associated with an increase in the expected reptile mortality count ($B = 0.08$, $SE = 0.03$, $Wald = 8.20$, $p < 0.05$). The GLM model only including the maximum number of saplings was also substantially supported as the change in AICc < 2 units compared to the best model (Table 2.6).

GLM AICc comparisons indicated the best model for amphibian mortality (i.e., lowest AICc) only included maximum number of saplings (Table 2.7). The likelihood ratio test indicated a significant improvement in model fit compared to the null model (χ^2 (1) = 9.458, $p < 0.05$). Maximum number of saplings was a significant predictor of amphibian mortality, as an increase in maximum number of saplings was associated with an increase in the expected amphibian mortality count ($B = 0.076$, $SE = 0.02$, $Wald = 9.66$, $p < 0.005$). The GLM model including both the maximum number of saplings and summer average NDVI was also substantially supported as the change in AICc < 2 units compared to the best model (Table 2.7).

Discussion

Our results demonstrated how temporally variable factors influence vertebrate mortality, corresponding to decreased permeability of those areas of landscape. We focused on annual, or longer term as compared with seasonal, differences and the changes underlying patterns of resources.

We identified temporal variations in structural, productivity, and environmental variables. The differences found across the years in the maximum number of saplings, change in average vegetation height (change in average vegetation height for the two sides of the road over the sampling season), the summer maximum NDVI, and annual average NDVI all suggest there are differences in resource availability across the years. This is consistent with previous research that has found differences between years in primary productivity (Knapp & Smith, 2001; Oesterheld et al., 2001) and other measures of ecosystem functionality, such as soil respiration (Ruehr et al., 2010). Stability in the availability of resources is important for vertebrate population persistence (Beasley et al., 2011). However, these differences did not translate into a significant difference between total annual vertebrate mortalities.

We found a significant difference between the maximum humidity between the years, but it was not significantly related to the mortality counts. While there were no significant differences found between years for either average temperature or precipitation, we did find that the highest average temperature and total annual precipitation were recorded when mammal mortality was much greater than the other taxa. Previous studies have found that temperature and precipitation are strong predictors of road mortality (Zhang et al., 2018; Carvalho et al., 2017; Costa et al., 2020). These interannual differences in temperature and precipitation have manifested into variations in organism abundances across years as well (Carmona et al., 2015; Bowden et al., 2018).

Although we found some interesting patterns in annual variation in temperature and precipitation that may be affecting mortality, it should be noted that the majority of the studies finding these differences occur in the tropics, which have distinct wet and dry season (Bombardi et al., 2020). Studies have demonstrated that there are increasing changes in precipitation patterns most likely resulting from climate change; not only is the intensity changing, but there has been a shift in the start and duration of 'wet' seasons (Feng et al., 2013). Similar shifts have been documented in the United States as well, as there is an increase in intense and frequent rainfall (Motha & Baier, 2005), but these changes are more impactful at the short time scale and the variability decreases when examining longer, interannual time scales (Zhang et al., 2021). More variability within a year than between years suggests that small time scales are less important to longer term ecological processes or to the larger landscape. It is also possible our scale was not sensitive enough to detect a difference, if a significant difference was in fact present. It is also possible that there are lag effects at greater temporal scales, but exploration of these would require a much longer study and multiple weather stations.

Of the structural and productivity variables that were significantly different between years, only the maximum number of saplings and summer average NDVI were positively related to vertebrate mortality counts. Summer average NDVI was a significant predictor of total vertebrate and mammal mortality, as an increase in summer average NDVI was associated with an increase in the expected vertebrate mortality counts. Maximum number of saplings was a significant predictor of reptile and amphibian mortality, as an increase in maximum number of saplings is associated with an increase in the expected mortality counts. The changes in localized structural components are influencing mortalities of the ectotherms that have difficulty moving through areas of thicker understories (Brisson et al., 2003; Homan et al., 2010), which could be exemplified by the number of saplings. Neither of these temporally varying structural factors were correlated with birds. Perhaps our measurements were not sensitive enough to capture the differences with this taxon at this time scale.

Previous research has emphasized that the success of strategies to mitigate vertebrate road mortalities are contingent upon understanding not only the spatial components affecting mortality, but the temporal ones as well (Clevenger et al., 2003; Santos et al, 2015). Although there were few significant differences found across the years, there were intra-annual fluctuations within the years which may be of importance if managers are interested in employing temporary mitigations measures, such as closing certain roads during a particular season (Hobday & Minstrell, 2008; Garriga et al., 2012). In addition, there could be lag effects in mortality that are not accounted for in this study, although for longer-lived organisms it may be that the time scale of the study was not long enough to detect temporal influences.

While the resources organisms need, and therefore their movements, vary across time, our study suggests that these variations are seen more within years than between. Research has found movement patterns to reflect temporal changes (Grilo et al., 2009). Conducting multi-year studies allows for an examination of variability in the distribution and relative abundance of organisms and ecosystem dynamics (Sinclair et al., 2007; Brockie et al., 2009; Jones & Driscoll, 2022). Extended investigations provide a robust foundation, enabling more informed comparisons of effects and ecological responses (Turner et al., 2003; Orwig et al., 2008). However, our consistent findings across years suggests that the spatial components were influencing mortality differences more than temporal differences and the intra-year differences do not impact total mortality in a way that would steer long term mitigation. Our methodology could be useful for management without the concern that the data would only be relevant in the short term before any application of mitigation measures could be put into effect.

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CHAPTER III. MORTALITY HOTSPOT ANALYSIS: WHERE THE DEAD POOL

Introduction

Protecting species, their natural habitats, and ensuring their connection across the landscape are conservation strategies that are threatened by roads cutting through that landscape, fragmenting habitat. Understanding and predicting the impact of roads on wildlife population connectivity is a key goal of road ecology (Kroll, 2015). The substantial amount of wildlife road mortality that occurs has created a need to reduce the interactions that wildlife has with roads in order to minimize the number of collisions (Gomes et al., 2009).

Animal mortality studies are used to determine mitigation measures that might alleviate some of the negative ecological effects of roads (Coffin et al., 2021). The impact of roads could be managed if we were able to locate particular problem areas. The connection of wildlife to specific habitats and surrounding land use types implies that landscape spatial patterns are likely to influence the rates and locations of mortality incidents (Forman & Alexander, 1998).

Any management has associated costs, and it is not feasible to mitigate the entirety of the road. Therefore, it is important to determine cost effective ways to mitigate the road impacts by determining areas where the most lives can be saved for the least cost. The most common practice to achieve this is to determine locations of mortality 'hotspots' (Langen et al., 2012; Santos et al., 2017; Spanowicz et al., 2020). Hotspot analysis identifies clustering of spatial data, is useful for identifying local spatial autocorrelation, and measures the extent to which points close to a given point share similar values (Getis & Ord, 1992).

In this study we identify where vertebrate road mortality is concentrated across the study area. We also determine whether patterns of mortality in these areas are consistent over time. In the context of this study permeability is the ease at which vertebrates can move from patch to patch through the mixed disturbance landscape; since the study area is so fragmented by roads, vertebrates will need to cross the roads to accomplish this movement between patches. Therefore, we are considering vertebrate mortality, specifically from roads, as a tool for evaluating permeability. A map of vertebrate mortality will highlight where animals are having difficulty moving through the area (i.e., lower permeability). Our approach allows us to evaluate how these different types of factors that are varying spatially and/or temporally influence vertebrate permeability. Our objective is to assess how spatial and temporal heterogeneity influences mortality patterns of vertebrates.

Research Questions

1. How do these mortality concentrations change in space and time?
2. How do these mortality concentrations differ among taxa?

Methods

Road Surveys

We surveyed a total of 23 roads, covering 49.0 kilometers (km). The roads were situated near one of three natural areas, Kitty Todd Nature Preserve, Maumee State Forest, or Oak Openings Preserve. We surveyed a total of 8 roads (15 transects), covering 19.57 km, near Kitty Todd Nature Preserve; a total of 7 roads (9 transects), covering 13.71 km near Maumee State Forest; and a total of 9 roads (10 transects), covering 15.77 km, near Oak Openings Preserve. The roads were divided into transects at designated intersections; therefore, the resulting 34 road transect lengths ranged from 0.8 km – 2.0 km. All roads were two lane, paved roads, with speed limits that vary between 56.3 kph (35 mph) and 88.5 kph (55 mph).

Roads within the study area were surveyed for all dead terrestrial vertebrates, e.g., amphibians, birds, mammals, and reptiles. We surveyed all transects every other week from May to October in 2020, 2021, and 2022, totaling 36 surveys (12 per year). We conducted the surveys by bicycle, riding 16.1 - 24.9 kph (10.0 - 15.5 mph), similar to Garrah et al., (2015). Walking the transects was not feasible, so bicycling was used as car surveys tend to underestimate roadkill, especially reptiles and amphibians (Langen et al., 2007). However, car surveys were conducted when weather was unsafe to complete the survey by bicycle (approximately 10% of the total number of surveys). When car surveys were employed, we evaluated all transects by car to keep the data consistent across transects for that survey.

For each vertebrate mortality found, we recorded the location with a Garmin Etrex GPS, identified the organism to taxonomic class (and species, if possible), took photographs of the organism and surrounding areas, noted where and in what condition the organism was, as well as recorded a variety of environmental and spatial variables (Table 1.1). Due to the quality of the carcass, most identifications were left to class. Animal carcasses were not removed, duplicate entries of persistent carcasses were avoided by referring to prior data before recording new entries.

Acoustic Surveys

We used the same 34 road transects for acoustic surveys of bat activity. The bat call surveys were conducted during the peak activity timeframe, which starts approximately 30 minutes after sunset and ends approximately 3 hours after sunset (Hayes, 1997; Sewald, 2012). We completed the surveys by extending an Anabat SD2 acoustic monitor with an attached GPS unit out the car window while driving along the transect at approximately 32 km/hr (Fisher-Phelps et al., 2017). The calls were continuously recorded while their geolocations were marked. All transects were surveyed within the same day once a month in 2020 and 2021 either on or shortly after the new moon, which is the time in the lunar cycle when bats are most active (Lang, 2006). Acoustic surveys were conducted twice a month in 2022, once on or shortly after the new moon and either two weeks before or two weeks after the new moon, depending on the occurrence of the new moon within the month. Acoustic surveys were only conducted when conditions were appropriate, which includes when wind speeds were less than 15 mph and chance of rain was less than 50% during the survey time.

Analysis

Along each transect sampling points were placed approximately every 400 meters to evaluate the heterogeneity along the road segment. We created 200-meter buffers (radius from fixed sampling point) around each sampling point, resulting in a 160,000 square meter area to provide an evaluation of landscape context. Total vertebrate mortality per 160,000 square meter buffer area (Figure 1.1) was used to conduct hotspot analysis and check for associations with collected variables within the buffer areas (Figures 3.1, 3.2, & 3.3). Total bat activity, derived from the acoustic monitoring, was also calculated per buffer area and was compared with the vertebrate mortality data. We performed hotspot analysis using Getis-Ord G*i** in ArcGIS to examine spatial clustering patterns using the buffers (vers.10.8.1; Earth Systems Research Institute, 2020). Getis-Ord G*i** identifies statistically significant clusters of high values (hot spots) and low values (cold spots) which works by looking at each feature within the context of its neighboring features (Mitchell, 2009). To be statistically significant ($p < 0.1$), a feature had to be surrounded by other features with those values as well. Hotspots were considered positive clustering at the $p < 0.1$ to $p < 0.01$ (based on positive z-scores), whereas coldspots were considered negative (low) clustering based on negative z-scores at $p < 0.1$ to $p < 0.01$ (Mitchell, 2009). Hotspots were areas that had a clustering of high mortalities, where coldspots were areas that were less likely than average to have mortality clusters.

Results

For total mortality for all years, we identified a total of 10 hotspots and 4 coldspots (Table 3.1, Figure 3.4). All coldspots were located within the study area surrounding the Maumee State Forest, whereas 4 hotspots were identified surrounding Kitty Todd Preserve and 6 hotspots were found surrounding Oak Openings Preserve.

The lowest number of hotspots for total mortality was found in 2020 (Table 3.2; Figure 3.5); the number of hotspots for total mortality found in 2021 and 2022 was similar, with 12 and 13, respectively (Tables 3.3 & 3.4; Figure 3.5). No coldspots were identified within the individual years. The majority of the hotspots found in 2020 and 2022 were located near the Oak Openings Preserve area, whereas the hotspots were evenly distributed between the Oak Openings Preserve and the Kitty Todd Nature Preserve areas in 2021. There were no hotspots found in the Maumee State Park area in 2021, but a similar number of hotspots was found in 2020 and 2022, with 2 and 3, respectively.

We found some common hotspot areas across the years and some areas that were in adjacent locations along the same transect or road among years. There was one hotspot, KT8A located in the Kitty Todd Nature Preserve area, that was consistently identified across all years (Figure 3.5). Three hotspots from 2020 were consistent in either 2021 or 2022 (Figure 3.5). Five of the hotspots identified in 2021 that were not found at or around the same locations in the other two years: KT8D, KT9C, KT9D, OO10B, and OO10C. There were 4 hotspots in 2022 that did not occur within similar areas in 2020 or 2021: OO5A, OO5B, OO7C, MP1B.

Mammal mortality hotspots were identified around all three OOR natural areas, but more were concentrated within the study area around Oak Openings Preserve: 2 near Kitty Todd Nature Preserve, 3 near Maumee State Forest, 7 near Oak Openings Preserve (Table 3.6, Figure

3.6). Only mammal mortalities were found in the Maumee State Forest study area. No mammal morality hotspots overlapped with any other taxa hotspots; however, the two mammal hotspots within the Kitty Todd Nature Preserve area (KT2B and KT2C) alternated with bird hotspots (KT2A and KT2C); of note, the east bird hotspot was also a reptile hotspot (Figure 3.6).

We identified 6 reptile mortality hotspots and all but one, OO9A, is in the Kitty Todd Nature Preserve area (Table 3.7, Figure 3.6). The one reptile mortality spot that was located within the Oak Opening Preserve, OO9A, overlapped with a bird mortality hotspot. One of the reptile mortality hotspots, KT8A, overlapped with an amphibian mortality hotspot. We identified 12 amphibian mortality hotspots (Table 3.9, Figure 3.6). One hotspot for amphibians was adjacent to the shared reptile and bird hotspot, OO9A. The remaining 11 amphibian mortality hotspots were located near Kitty Todd Preserve. We identified 9 bird mortality hotspots, evenly distributed between Kitty Todd Nature Preserve and Oak Opening Preserve areas. (Table 3.9, Figure 3.6).

There were 9 hotspots and 7 coldspots identified based on the acoustic bat monitoring data (Table 3.12, Figure 3.7). There were 2 hotspots of bat activity that were adjacent to total vertebrate mortality hotspots, KT8B and OO9B. Two of the bat activity hotspots overlapped with reptile mortality hotspots. There was one bat activity hotspot that overlapped with a total vertebrate mortality coldspot, MF6B. Three of the bat activity coldspots have been identified as bird mortality hotspots, KT1C, KT1D, and OO9C. Another bat activity coldspot was adjacent to an amphibian mortality hotspot.

Discussion

Based on the results from the previous chapters, we expected to find mortality hotspots in areas with wider roads, high productivity (NDVI), and near greater amounts of upland prairie.

Coldspots were expected in areas with narrower roads, near agricultural land use, and lower productivity (NDVI).

We did find hotspots along the areas with the widest roads, 9 m wide (transects KT1 and KT2). This is consistent with previous studies that have found increases in road width associated with higher mortalities (Skórka et al., 2013). However, the coldspots found were not along the narrowest road, 4 m wide (transects MP8 and MP9), which may be more related to the low traffic densities found on these roads. Coldspots were found on roads that were 5 m wide, which was below the mean road width of 6.4 m. The coldspots also were found in areas that had a large amount of cropland in the surrounding area, while the hotspots were not found near these areas of cropland/agriculture, suggesting avoidance of cropland areas that lack adequate resources or limited movement in and around these land use types (Lark et al., 2020).

Significant hotspots of vertebrate mortality were found, however, but varied year-to-year, as well as taxon-to-taxon, in their occurrence and locations. Taxon do not respond equivalently to the landscape; therefore, examining these taxa separately is informative because they do not necessarily exhibit synchronous responses. The hotspots do demonstrate nonrandom patterns in vertebrate mortality across pace and time. In turn, these nonrandom patterns can be used by managers to alter the landscape to reduce the effects of decreased permeability. It is essential to incorporate both spatial and temporal patterns, as in this type of analysis, to address permeability issues. These areas could be important for both common and threatened species given the demonstrated decreased permeability across the landscape in these areas. Goals for managing the landscape may be different, depending on a specific taxon. Our results also indicate that the detrimental impacts on permeability may be greater around protected areas, which is particularly concerning for rare and threatened species.

It is assumed that our vertebrate mortality counts are representative and not biased by low carcass detectability or high removal rates (Santos et al., 2011; Ratton, et al, 2014; Santos et al., 2016). While we attempted to control for the influence of small carcass size on detectability differences by surveying by bicycle (Garrah et al., 2015), we were unable to control for high removal rates. Scavengers, weather and traffic volume are the main factors that influence carcass permanency on the road, which affect removal differently depending on when the mortality event occurs (Ratton et al., 2014). In our study, the majority of reptiles and amphibians were removed by the next survey; however, birds and mammals tended to persist at least until the next survey. Therefore, there may be higher amounts of amphibian and reptile mortalities that are not accounted for in our data. However, it is unclear whether this strengthens our understanding of these taxa by making our results even more robust, or whether the unaccounted-for mortalities would have been found in different areas altogether.

We expected an overlap between bat activity hotspots and mortality hotspots based on productivity: areas with greater productivity would have greater activity, and in turn higher mortality associated with it. Given how infrequently these two types of hotspots overlapped, there is more influencing these patterns than some measure of productivity. Previous results (e.g., Chapters 1 and 2) illustrated the relationship between productivity (NDVI) and mortality, and while important, NDVI was not the main contributor. This strengthens our argument that landscape context and structural variables are also important components to consider.

Previous research has found that bat activity hotspots are associated with ponds, upland prairie, and overall forest cover, and had negative associations with cropland (Russo-Petrick & Root, 2023). The hotspot areas found in the current study did not align with those factors. A number of our hotspots occurred in areas with large amounts of cropland, but that association might be explained by the proximity of streams or ponds to the road in those areas. It is interesting to note that 43% of the coldspots for bat activity corresponded to bird mortality hotspots, possibly indicating an inverse relationship between how these organisms use or navigate the landscape, such niche partitioning. Previous studies have found increased bat activity to occur within and above the forest canopy (Kalcounis et al.,1999), while bird abundance was greatest at the middle canopy layer (Dinanti et al., 2018).

There is limited stability over time and space even for the extreme ends of the clustering spectrum. This indicates a dynamic system that requires adaptive management and will not be mitigated by static solutions. Other studies that have examined road mortality hotspots have also found hotspots to vary between taxonomic groups (Barthelmess, 2014) and locations from year to year (Lutterschmidt et al., 2019). While the specific locations of these hotspots change or shift over time, our results illustrate that the effects of these areas are robust enough across time and space to indicate general areas where an effect is regular enough that it keeps occurring. This suggests a good place to start a more comprehensive assessment of the main components associated with these areas. In addition, taxon specific hot spots can be used to identify where areas of concern are for each taxon and could be used to parse out if there are hot spots for species of concern. Our study differs from other hotspot studies that direct recommendations for culverts, land bridges, fencing, or other methods of diverting road crossings (Dodd et al., 2004; Ng et al., 2004; Patrick et al., 2010; Smith et al., 2015; Colley et al., 2017), or temporary measures such as closing roads or reducing speed limits for certain time periods (Shepard et al., 2008; Crawford et al., 2014). In fact, a number of our previous results (e.g., Chapters 1 and 2) suggest that there may be ways to manage the landscape without using anthropogenic structures to mitigate mortality, such as managing the vegetation clutter along park boundaries.

Other hotspot studies have found speed and traffic intensity to be the main influencers of these clustered areas (Clevenger et al., 2003; Eberhardt et al., 2013; Grilo et al., 2015; Canal et al., 2019; Ferreguetti et al., 2020; Rendall et al., 2021). However, our system has demonstrated to be more dynamic than these two factors suggest.

A major advantage of hotspot analysis is how easy it is to visualize where these clustered areas are, as opposed to trying to determine which clump of points are important. It also places these mortalities in the broader landscape, allowing associations to be made with the surrounding landscape. Hotspot analysis, like the one used in this study, not only takes density into account, but spatial clustering of that density. It gives a more thorough understanding of the broader area of concern. This can also help direct managers to areas where there is concern for disruptions in connectivity, rather than just mitigating isolated conflict points on the road. In this study, we originally had some transects with buffers that hung off the edge of the road being investigated, meaning half of the buffer covered an area that was not sampled. One way to address the issue of scale-dependent results would be to conduct hotspot analysis across a range of scales.

A drawback, however, of the hotspot method is this clustering does not directly relate to the environment. Understanding the dynamics of how vertebrates interact with the environment is a crucial aspect of conservation ecology (Maskey Jr & Sweitzer, 2020; Howze & Smith 2015; Roe & Georges, 2008). Therefore, without additional information, these hotspots offer little guidance as to what critical components influence these effects or what the most appropriate approach to mitigation in those areas should be. In addition, because of the nature of the Getis Gi* analysis, areas near the edge of the study area would have fewer neighbors, which may skew results further down the road (Mitchell, 2009). In other words, because of the artificial truncation, the analysis is not as accurate at the edges.

These hotspot areas explain the extreme end of the mortality spectrum, but the areas of little or no spatial clustering are messy, and much more complicated. However, this does give managers a good place to start, since the extreme mortality areas are the ones that require the most improvement to permeability. We recommend that hotspot analyses be paired with additional investigations to link these areas to the specific ecological components that are influencing these patterns.

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CHAPTER IV. MODELING CURRENT AND FUTURE LANDSCAPE PERMEABILITY FOR VERTEBRATES

Introduction

Species distribution modeling (SDM) is a method of estimating the relationship between species records and the environmental and/or spatial characteristics of those sites (Franklin, 2009). Critical areas of habitat can be identified through these models as well as predicting past, present and future species distributions (Bellamy et al., 2013; Scharf & Fernandez, 2018). Larger spatial and temporal patterns and the factors that determine them can be investigated through modeling. SDM can be generated from small data sets, is easy to interpret and can include various interactions between organisms and their environment (Bellamy et al., 2013). The maximum entropy method (Maxent) is one method of modeling species geographic distributions using presence-only data (Elith et al., 2011).

In order to build predictive models and to propose mitigation measures, especially in heterogeneous landscapes, it is important to identify relationships between the landscape and activity patterns. De Freitas et al. (2015) found that the strength of the associations between landscape features and roadkill occurrence for each of three medium-sized mammals not only varied by scale but by species. Habitat selection behavior is also scale dependent. At larger spatial scales, more complex models may be required to predict habitat selection patterns than the simple habitat-selection models that do reasonably well at predicting individual behavior and spatial distributions at smaller spatial scales (McGarigal et al., 2016).

Studies with modeling have typically focused on one or more species within a single taxon (Hansen et al., 1993; Murray et al., 2014) or only a few taxa at a time (Anunciação, et al., 2021; Rich et al., 2019). More recently, studies have included all terrestrial vertebrate taxa in the modeling to predict the effects of habitat and climate changes on distribution (Kuipers et el., 2021; Powers & Jetz, 2019; Warren et al., 2014). The inclusion of all four terrestrial vertebrate taxa, as in this study, allows for the interaction between taxa to be considered as a factor in the changing environment. For example, predation and competition have been found to have effects on distribution models (Tompkins & Veltman, 2006; Trainor et al., 2014). Also, it is worth considering that predicting land use change impacts is much more urgent than predicting those of climate change (Dale, 1997). Land use change is more immediate and does not allow as much time for adaptation and acclimation as for climate changes, especially for longer lived vertebrates.

To evaluate these types of effects, Maxent models use occurrence data and their associated attributes to extrapolate the probability of occurrence and have higher predictive power using presence-only data than other modeling approaches (Elith et al., 2010; Warren & Seifert, 2011). Previous research has demonstrated how these Maxent models can be used to establish where wildlife populations are at increased risk of vehicle encounters, as well as modelling current and future distributions of wildlife across landscapes (Garrote et al., 2018; Ha & Shilling, 2018; Newbold, 2018). For example, Ha and Shilling (2018) used Maxent modeling to determine that the total forest area and road density within a 500-meter buffer were the primary factors that explained the road mortality locations they observed.

The current study incorporates the variables from Chapter 1 and 2 into a modeling framework that features the critical structural, environmental, and landscape factors that are associated with vertebrate mortality. This is different from the visualization of hotspots that were explored in Chapter 3 as these models extrapolate across the landscape; in other words, we are predicting (spatially) out across the entire landscape based on the current study sites. These models show the current distribution patterns of these vertebrate taxa mortalities and how they may change due to the predicted changes in the variables used to build the model. The current habitat changes occurring throughout the Oak Openings Region (OOR) highlight the importance of creating these distribution models. The goal is to build models predicting areas with high probability of mortality (i.e., lower permeability), which increase our ecological understanding of these vertebrates and their distribution patterns across the landscape. In the context of this study permeability is the ease at which vertebrates can move from patch to patch through the mixed disturbance landscape; since the study area is so fragmented by roads, vertebrates will need to cross the roads to accomplish this movement between patches. Therefore, we are considering vertebrate mortality, specifically from roads, as a tool for determining permeability. Vertebrate mortality will help build a picture of where animals are having difficulty moving through the area (i.e., lower permeability).

Research Questions

1. What are the critical variables that influence the current distribution of vertebrate mortalities that can be extrapolated across the landscape?
2. How do predicted landscape and environmental changes impact vertebrate distribution across the OOR?

Methods

Model Development

We assessed continuous variables such as landcover class percentage (PLAND), available water capacity (AWC), available water storage (AWS), water table depth (WTD), normalized difference vegetation index (NDVI), moisture stress index (MSI), normalized difference water index (NDWI), and enhanced vegetation index (EVI_2) (Table 4.1).

We used the full supervised classification landcover for the entire Landsat frame and clipped to an area that extended 300 m from the OOR boundary to encompass the entirety of our sampling sites, since our sites extended slightly beyond the original Brewer and Vankat (2004) area. Landcover types were identified using the Oak Openings Region land cover map (Martin & Root, 2020). There were 15 landcover classes: upland prairie, upland savanna, sand barrens, upland deciduous forest, upland coniferous forest, wet prairie, swamp forest, floodplain forest, wet shrubland, perennial pond, Eurasian meadow, cropland, and turf/pasture. We converted the categorical map to continuous data to estimate area of landcover classes in FRAGSTATS ver. 4.2.598 (McGarigal & Marks, 1995). Proportions of area of each landcover class were calculated using a moving window analysis with a 120-m buffer, four times the 30-m raster cell size, applying an 8-cell patch neighbor rule (Schetter et al., 2013). We chose the 120-m extent to avoid overlap among 400-m x 400-m study area buffers.

Available water capacity, available water storage and water table depth information was acquired from the Web Soil Survey database from United States Department of Agriculture Natural Resources Conservation Service (USDA NRCS, 2019). Available water capacity is measured in centimeters of water per centimeter of soil and refers to the quantity of water that the soil is capable of storing for use. Available water storage is the water capacity times the thickness of the soil. Water table depth is the distance between the soil surface and the saturated zone, known as the water table.

NDVI , EVI_2, MSI, and NDWI indices were calculated from Landsat-8 images for our study area (Path 20, Row 31) acquired from the U.S. Geological Survey from early spring (March or April), summer (June), and fall (September or October) and then combined across all three study years (2020-2022). The indices were then calculated from the aggregate image in

ESRI ArcGIS version 10.8.1. NDVI and EVI_2 use the satellite data to evaluate biomass patterns, while MSI and NDWI use the satellite data to evaluate water content (Valerio et al., 2022).

The data that we collected 2020-2022 were used to extrapolate across the entire study area in the OOR, relying on continuous biotic and abiotic variables that are associated with the mortality of vertebrates to provide models of current high death probability distribution areas using Maximum Entropy Species Distribution Modeling (Maxent version 3.4.4).

Default settings in Maxent were used initially to run models with 10 replicates (Phillips, 2017). However, the default setting of removing duplicate presence records was unchecked to ensure the weight of multiple taxa being found in a single spot over years was applied. Models were developed for total mortality (all taxa combined). The model predictions were tested with additional independent sampling (described below).

We developed models for individual taxon, as well as for individual years. For individual years, the composite NDVI for that year was used as opposed to the composite NDVI for all three years used in the total vertebrate mortality model.

Model Evaluation

The Maxent model for total mortality was used to select 36 400-m transects to evaluate the model, 12 of each high, middle, and low mortality probabilities. In order to make Maxent results comparable to previous sampling efforts and hotspots from Chapter 3, we converted the 30x30 pixels to 400x400 pixels using the raster resample tool, with bilinear interpolation, which is suitable for continuous data. The raster was converted to a polygon to produce 400x400 m grids that could be chosen to use as potential sampling sites (transects) based on their Maxent output values. We randomly chose 6 grids that contained the hotspots delineated in Chapter 3, corresponding to 4 high, 2 middle Maxent values, and 6 of the non-hotspot transect areas identified in Chapter 3, corresponding to1 high, 2 middle, and 3 low Maxent values. For the remaining 24 transects, we used the continuous values obtained from the Maxent model to obtain an equal number of high probability (top 10% of values), middle probability (middle 80%), and low probability (bottom 10%) grids. We randomly selected 12 values from each of the top, middle, and bottom groups. Grids were thrown out if a complete road was not found in the grid. We verified on the map if random selections were on surveyable roads, and continued selection until we obtained a list of 36 road transects (12 high, 12 middle, 12 low). The 36 road transects were then investigated according to the protocol outlined in Chapter 1, except only six surveys on each transect were conducted from May to August in 2023.

Analysis

Model development. Multicollinearity was tested in ArcMap and only variables below the set 0.7 threshold for correlations were included in the models (see Table 4.2 for models considered). We evaluated the models using the area under the receiver operator curve (AUC) (Phillips et al., 2006). AUC values greater than 0.9 indicate an excellent model, with 1.0 being perfect, values 0.8–0.89 indicate a good test, and values lower than 0.8 indicate, at best a fair test, and at worst a test of no value (AUC = 0.5) (Hanley & McNeil, 1982; Carter et al., 2016). The model with the highest AUC was used for field testing, referred from here on as the best model. We also assessed the measure of variable importance to models by examining permutation importance, as opposed to percent contribution. While percent contribution assessments evaluate how much the variable adds to the model based on the path of Maxent calculations, i.e., the order the variables are entered, permutation importance evaluates the importance based on the final model regardless of the path (Phillips, 2017). The effect graphs produced by Maxent provided an additional tool of variable evaluation, while illustrating the relationship the variable had with the mortality probability.

We analyzed the best model for robustness across taxa, parsing out each taxon, as well as across individual years. For individual years, the composite NDVI for that year was used as opposed to the composite for all three years.

Model evaluation. We investigated the predictability of the best model using the independent set of mortalities recorded in 2023. We utilized a chi square goodness of fit test of the expected mortalities based on if there were no differences between the areas in each category to the mortalities that were observed. We employed a one-way ANOVA to test the effects that the classification of high, middle, and low transect groups had on mortalities.

Results

Model Development

The best model of the likelihood of mortality (AUC = 0.883 +/-0.022; Table 4.2) included: 1) percentage of each landcover class from the full supervised landcover map (minus turf) derived from FRAGSTATS; 2) soil available water storage; 3) soil water table depth; and 4) NDVI scores (Table 4.3; Figure 4.1). The importance of the permutation of the predictor variables to the Maxent model is shown in Table 4.3 and Figure 4.2. The main contributing variables for the best model (i.e., highest AUC) were residential/mixed area (35.5% contribution to model), dense/urban area (10.5 % contribution), soil available water storage (9.3 % contribution) (Table 4.3). Each of the remaining variables contributed less than 7% to the model. The probability of mortality occurrence was predicted to generally increase with the increase in residential/mixed area (Figure 4.3). The probability of mortality occurrence was predicted to generally decrease with the increase in dense/urban area (Figure 4.4). The probability of mortality occurrence was predicted to fluctuate as the soil available water storage increases, beginning with a sharp increase before peaking and then drastically decreasing (Figure 4.5). The probability of mortality occurrence increased slightly before dramatically decreasing again as the proportion of cropland increases (Figure 4.6).

To compare the influence of individual taxon, we examined models for each of the four taxa separately. When the parameters of the best model were used for the individual taxon, the individual models for mammal mortalities alone (AUC = 0.895 +/-0.021) and reptile mortalities alone (AUC = 0.915 +/- 0.035) were better models compared to the model for total taxa mortalities, while the model for amphibian mortalities alone (AUC = 0.864 +/-0.085) and bird mortalities alone (AUC = 0.854 +/-0.047) were not quite as strong as the total taxa mortality model (Table 4.4, Figure 4.7).

Residential/mixed landcover area was one of the top two influential variables in all of the individual taxon mortality models (Figure 4.8). We found that all species showed an increasing probability of mortality as the percentage of residential/mixed landcover area increased. There was variability in the rest of the main contributing variables for each taxon. We found as the area of dense/urban landcover class increased the predicted mammal mortalities decreased (8.4% contribution to model), all other variables contributed less than 8% to the model (Table 4.5). The additional top contributing variables for the best model for reptile mortalities were upland prairie area (13.8% contribution to model) and swamp forest area (10.8%), while all other variables contributed less than 10% to the model (Table 4.6). As the area of upland prairie area increased the probability of reptile mortality increased until peaking then the likelihood of reptile mortality would decrease, whereas the probability of reptile mortality sharply increased with the increase in the area of swamp forest area until there was a peak and then the reptile mortality probability started to decrease (Figure 4.8). We found that for the best model of amphibian mortalities, the additional top contributing variables were cropland area (20.6 % contribution) and swamp forest area (10.5% contribution) (Table 4.7). As the area of cropland area increased the probability of amphibian mortality slightly increased until peaking then the probability of amphibian mortality would decrease; the probability of amphibian mortality responded similarly with the increase in the area of swamp forest area (Figure 4.8). The probability of bird mortalities increased not only with the increase in residential/mixed area, but with the increase in the other top contributing variables as well: cropland area (14.5% contribution to model) and upland prairie area (10.3%), before beginning to decrease (Table 4.8, Figure 4.8).

The best model was an excellent predictor for 2020 mortalities (AUC = 0.918 +/-0.020), 2021 mortalities (AUC = 0.917 +/-0.016), and 2022 mortalities (AUC = 0.902 +/-0.028) (Figure 4.10; Table 4.9). Residential/mixed landcover class area was the top contributing variable in all the individual year models. The composite NDVI for the individual year was the next most influential variable for each of the individual year models. However, there was variability in the rest of the main contributing variables for each year. We found the additional top contributing variables for the best model using 2020 mortalities to be cropland area (7.5% contribution) and swamp forest area (7.4% contribution) (Table 4.10). For 2021 mortalities, the additional top contributing variables were upland prairie area (8% contribution) and dense/urban area (6% contribution (Table 4.11). The same additional top contributing variables were found using 2022 mortalities as using 2020 mortalities: swamp forest area (7.7% contribution) and cropland area (4.9% contribution) (Table 4.12).

Model Evaluation

In our independent data set to evaluate the model, we recorded a total of 23 vertebrate mortalities across the six road surveys, requiring 270 miles of cycling. Out of the 23 vertebrate mortalities, 19 were mammals, 4 were birds, and no reptiles or amphibians were found. We found 12 mortalities in areas classified as high probability, 8 mortalities in areas classified as middle probability, and 3 mortalities in areas classified as low probability. There were 5 mortalities found in previously sampled areas. Two of the mortalities occurred in areas established as a total vertebrate hotspot in Chapter 3. The remaining 3 mortalities occurred in one area, which was considered a non-hotspot for total vertebrate mortality, but was a hotspot in 2021 and for amphibians.

We found that the observed mortalities were trending towards significance ($p = 0.07$) based on the mortality probability categorization. There was not a significant difference in vertebrate mortalities between areas classified as high, middle, or low probability (F (2, 33) = 1.14, $p = 0.33$).

Discussion

Mortality was well predicted by the best model and these results were robust across taxa and years. Although not significant, the results of the model testing were trending in the expected direction, additional sampling would clarify the accuracy of the model in regards to a specific taxon. In addition, these data could be used to further refine the model as desired. The best model fit well for each taxon. However, we did not find any reptiles or amphibians when field testing the model. Therefore, additional field testing would help to explore these relationships further. Residential/mixed landcover area was the main contributing variable for all taxa and this was robust across years. The same key contributing variables appeared across years, meaning that even if responses varied across taxa or location in a given year, the contributing variables were relatively stable. The model provides a broadly predictable set of factors that can be used to make predictions into the future. This modeling framework is also very flexible and can readily accommodate exploration and expansion with additional data.

Land use/land cover (LULC) change is consider one of the greatest threats to the environment and is one of the leading causes of biodiversity loss (Foley et al., 2005; Davison et al., 2021). Changes in natural landcover often lead to a reduced availability of habitat for wildlife, and the persistence of species largely relies on the connectivity of these natural habitats (Cuarón, 2000). These LULC changes can force organisms to disperse to new areas to find resources. LULC change is more immediate and does not allow as much time for adaptation and acclimation as does climate changes; therefore, predicting LULC change impacts is much more urgent than predicting those of climate change (Dale, 1997), especially for longer lived vertebrates. Our model provides a way to see how these LULC changes affect the probability of vertebrate mortality, as in the following example.

If the Oak Openings Region continues to experience landcover changes as those found between Schetter and Root (2011) and Martin and Root (2020) analyses (map years 2006-2016), our model predicts a substantial increase in expected road mortalities. Residential/mixed landcover class areas increased approximately 5% over the 10 years between studies (Martin & Root, 2020) and this landcover class was the strongest predictor of road mortality probabilities. The expected increase in mortality probability with the increased residential/mixed landcover class is expected across all taxa as well.

Many studies that attempt to predict wildlife mortality focus on local factors such as road width and type (Chyn et al., 2021), local vegetation plots (Blais et al., 2023), and prey mortality presence (Barrientos & Bolonio, 2009) and have tried to make regional predictions based on those local variables. Our study provides evidence that local predictions can be made using larger scale, landscape variables, as in our top-down approach versus the more common bottom-up approaches. This can have a tremendous impact on the way data is gathered in future conservation planning. A significant advantage of a reliance on larger-scale data for developing these types of models is that many of these measurements can be extracted from readily available satellite images that are available across large spatial scales and occur at frequent intervals.

As in any modeling framework, there are assumptions made when using Maxent modeling; however, we avoid one of the main assumptions through our particular application. Unlike most studies that utilize Maxent models, we are not trying to predict suitable habitat; therefore, we are not making an assumption that the use of habitat (presences) implies preferable or high-quality habitat. In our study, the occurrence data indicated mortality or where the organism died. However, presence-only modeling, such as those developed using Maxent, are susceptible to sample selection bias; the area that was chosen for sampling is not evenly or randomly distributed, which can lead to environmental bias when the model chooses background points for the modeling (Phillips et al., 2009). There are ways to evaluate the impact on the results of these assumptions, though, to further strengthen the value of the output. For example, if we are concerned that all of the occurrence sampling was on roads, we can directly evaluate the model with and without roads to estimate the contribution to the outcome. Similarly, you can subsample the occurrence points to evaluate the contribution of particular locations or sets of data to the model output.

The model indicates that if these vertebrates move, this predicted mortality is a possible consequence. To understand the impacts of this mortality, you would need to investigate the consequences at a population level, which is outside the scope of this study. However, the methodology outlined in this study is highly adaptable to context and desired application. It could be altered to investigate a wide variety of taxa, but could also be used to predict future impacts related to landcover or climate change. This methodology is a useful tool in monitoring species, choosing locations for or designing restoration or management projects across a variety of temporal and spatial scales.

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CHAPTER V. CONCLUSIONS AND IMPLICATIONS

This study explores how vertebrate mortality could be used as a lens to understand the factors influencing the distribution of vertebrates and the critical factors impacting the consequences of permeability (i.e., movement from patch to patch through the landscape) in a mixed disturbance landscape. Vertebrate mortality is a possible consequence of low permeability. The greater the mortality, the less successful the movement between patches of habitat has been.

Chapter 1 examines the spatial factors, such as the structural components of roads themselves, land use classification, landscape matrix, and proximity to protected areas, associated with vertebrate patterns of mortality. We found several spatial factors that impact the number of vertebrate mortalities along a road and that the impact of these factors differs based on taxa and scale. As we expected, landscape composition and structure were associated with mortality. Our research demonstrates the validity of employing road surveys as a reliable method for gaining insights into the locations of vertebrate mortalities on roadways and the spatial factors influencing the corresponding lack of permeability across the landscape.

Chapter 2 focuses on the temporal differences, e.g., annual, such as precipitation, temperature, and green vegetation availability, in vertebrate mortality patterns across the study area. Our results showed temporal variations in structural, productivity, and environmental variables. However, our consistent findings across years suggests that the spatial components were influencing mortality differences more than temporal differences and the intra-year differences do not impact total mortality in a way that would steer long term mitigation of permeability issues.

In Chapter 3 we map vertebrate mortality hot spot locations across the study area. Using this analysis, we found significant hotspots of vertebrate mortality that varied year-to-year in their occurrence and locations. However, there was one common hotspot across all three years, which was influenced by the reptile and amphibian mortalities that shared that area as a hotspot. There were other hotspot areas that were shared between birds, reptiles, or amphibians; however, mammals, who constituted almost 50% of our mortalities, did not share any hotspots with the other taxa. We demonstrated that it is essential to incorporate both spatial and temporal patterns, as in this type of analysis, to address permeability issues.

The purpose of Chapter 4 is to develop spatially explicit models for predicting current vertebrate mortality probabilities across the entire Oak Openings Region. We found the main determinant of mortality occurrence probability was the proportion of residential/mixed landcover area. We found mortality was well predicted and the results of the same key contributors were robust across taxa and years. The models developed can serve as an assessment tool for evaluating conservation and management to improve landscape permeability.

We attempted to use camera trap surveys as an additional independent line of sampling. After the first sampling season data was analyzed, we applied hotspot analysis (Getis Ord Gi* in ArcMap 10.8) to choose sites in pairs based on spatial characteristics (totaling 20 sites). Hotspots were paired with non-hotspots based on spatial factors including land use category, vegetation type, and understory presence. Once locations were chosen, we contacted landowners for permission to place Moultrie A-900I camera traps on the property. Once permission was granted (permission was only granted for 6 of the sites), we placed cameras on a tree near the roadside 1 meter from ground level to maximize detection of the largest range of animal sizes without having to alter the surrounding vegetation and faced the camera diagonally across the road to capture a wider area of potential crossings. We collected data from June 1 to October 13, 2021, and checked cameras every week. The data captured could not be analyzed due to incredibly low numbers of images with actual vertebrate activity. Cameras are particularly challenging when working with spatial scales that vary. These challenges are exacerbated when working with private landowners. To accommodate the desired spatial scale, it would have required a larger number of cameras in addition to willing participation by private land owners in order to obtain useable data within our study area.

Overall, our study contributes to the understanding, and potentially the predictability, of spatial and temporal dynamics in ecology. This project also contributes to the growing field of road ecology, as we explored additional ways to assimilate road related effects. This research identifies the factors that affect where vertebrates are concentrated and how that can lead to road mortality, which is applicable to other regions where natural areas are heavily affected by human activity and fragmentation. These types of studies are particularly important to understand the variety of effects that habitat loss, isolation, degradation and fragmentation can have on wildlife as they seek out necessary resources. The group of vertebrates that contributed to mortalities may not be representative of the full suite of organisms that are present within the area; therefore, strong conclusions cannot be made about individual species. Additionally, we were focused on general patterns and many of the mortalities that were found in this study were of more common species, so caution should be taken when trying to apply these results specifically to rare or vulnerable species.

In addition, understanding the factors associated with these distribution patterns can help predict how that pattern will change as those associated variables change. This provides a sense of what the critical areas/components are and where conservation efforts should be focused. An understanding and anticipation of the distribution of these resources and organisms' movement needs could help managers better protect crucial corridors or particular source areas for resources. The results of this research provide a tool that land managers could use to increase permeability and connectivity across the landscape and prioritize areas needed between protected areas to close gaps in the currently protected network or, if necessary, to facilitate direct mitigation of road mortality. For example, managing roadside vegetation structure and composition (e.g., number of shrubs or saplings) can influence the permeability of the landscape and mitigate the consequences of road mortality. The methodology and models we have developed could potentially aid management and conservation activities to minimize the negative impacts on native vertebrates. Roads are a cost-effective way to track wildlife. The information can be used to gain insight into where and when these organisms are moving. This provides information on connectivity, permeability, and other aspects of fragmented landscapes.

The results can be used as a static map but are intended to be used as a fluid tool that allows not only for the exploration of ecological connectivity between phenomena, but for conservation planning to be adapted for targeted systems, areas, or species. Now that areas of diminished permeability have been identified; efforts can be made to improve these areas. Effort can also be made to target areas that have high permeability and productivity but are not currently part of the protected network. Specified targets can be made and progress can be evaluated through refinement of the model with updated data. This tool also allows you to vary degrees of conservatism and sensitivity based on the quality of the data you have and what is intended for the output.

Our methodology is not limited to the Oak Openings Region; it can be applied anywhere with sufficient mortality and environmental data to address the ecological question of interest. It has the added benefit of being scalable, adaptable, and easily updated, making it an accessible approach to address a wide variety of conservation challenges.

APPENDIX A. TABLES

Table 1.1. Microhabitat variables measured in this study with name and units, description of the variable, and the frequency of measurement.

Variable Name (unit)	Description	Measurement frequency
AADT	Annual average daily traffic volume obtained through the Ohio Department of Transportation	Once a year
SL (kph)	Road speed limit	Once
RW (m)	Road width, measured from road edge to edge	Once
topography	Topography, classified as raised, buried, level, buried-raise, part buried, part raised	Once & noted with roadkill
ditch	classified as present or not	With site survey & roadkill
markings	Markings present on the roadway or not	Once & with roadkill
passing	If road marking present, was there passing	Once & with roadkill
edge lines	If road marking present, were there edge lines	Once & with roadkill
shoulder	Classified as present or not	Once & with roadkill
shoulder material	what material shoulder was, if present	Once & with roadkill
water	Classified as present or not along either side of road	With roadkill
canopy cover (%)	Measured from each side of road	With site survey & with roadkill
understory presence	Layer of plant growth below the forest canopy classified as not present, on one side, or on both	With site survey & with roadkill
VCH (cm)	Vegetation cover height of verge vegetation at each sampling point on both sides of road	With site survey & with roadkill
#_SAP	Average of count of saplings on both sides of road	With site survey & with roadkill
TL	Telephone lines classified as not present, on one side, or on both	Once & with roadkill
RQ	Road quality, based on the rideability of the road, classified as poor, fair, or good	With site survey & with roadkill

Table 1.2. Macrohabitat variables measured in this study with name, a description of the variable and the frequency of measurement.

Variable Name (units)	Description	Measurement frequency
LU	Land use type where roadside habitat is delineated as natural, developed, agricultural, natural/developed, natural/agricultural, or developed/agricultural	Once a year
LC	Landcover percent of 15 classifications determined by Martin & Root (2020) map	Once
AWC (cm)	Available water capacity obtained from USDA NRCS, quantity of water that the soil is capable of storing for use by plants	Once
AWS (cm)	Available water storage obtained from USDA NRCS, computed as AWC times the thickness of the soil	Once
WTD (cm)	Water table depth obtained from USDA NRCS, saturated zone in the soil	Once
NDVI	Normalized difference vegetation index derived from Lidar data, ratio of Near-Infrared and Red of satellite data that measures vegetation biomass	Three times per year: early spring, summer, fall
MSI	Moisture stress index derived from Lidar data, ratio of Near-Infrared and Red of satellite data that measures landscape water content	Three times per year: early spring, summer, fall
NDWI	Normalized difference water index derived from Lidar data, ratio of Near-Infrared and Red of satellite data that measures vegetation water content	Three times per year: early spring, summer, fall
EVI_2	enhanced vegetation index derived from Lidar data, ratio of Near-Infrared and Red of satellite data that measures vegetation biomass	Three times per year: early spring, summer, fall

Table 1.3. The buffer variables investigated in this study and the relationship to the buffer count outcome variables of total mortality, mammal mortality, reptile mortality, amphibian mortality, and bird mortality according to Spearman correlation analysis ($N = 121$).

Variable	Total Mortality	Mammal Mortality	Reptile Mortality	Amphibian Mortality	Bird Mortality	Diversity
AADT	+**	+**	+	-	+**	+**
Road width	+**	+**	+**	+**	+*	+**
Percent mammal	+		-**	-**	-*	
Percent bird	+	+**	-	-		
Percent reptile	+**	-		+**	+	
Percent amphibian	+**	+	+**		+	
Bat call count	+	-	+	+**	+	+*
Average canopy cover	+**	+	+**	+*	+	+**
Maximum canopy cover	+**	+*	+**	+	+	+**
Minimum canopy cover	+**	+**	+*	+	+	+**
Average number saplings	+**	+*	+**	+**	+	+**
Maximum number saplings	+**	+	+**	+**	+	+**
Minimum number saplings	+	+	+*	+	-	+
Average vegetation height	+	+	+	+	-	+
Maximum vegetation height	+	+	+	+	-	-
Minimum vegetation height	-	-	+	-	-	+
Natural/natural land use	+*	+	+**	+**	-	+**
Natural/developed land use	+**	+	+**	+	+	+**
Natural/agricultural land use	-	+	-	-	-	-
Developed/developed land use	+	+*	-	-	+	+
Developed/agricultural land use	-**	+*	-**	-**	-	-**
Agricultural/agricultural land use	-*	-	-**	+	-	-*
Proportion of protected area both sides	+	+	+	+*	-	+*
Proportion of protected area one side only	+	+	+	+	+	+
Proportion of unprotected area both sides	-	-	-**	-*	+	-
Proportion of protected area	+	+	+**	+**	+	+**
Number of landcover classes	-	-	+	-	-	-

Variable	Total Mortality	Mammal Mortality	Reptile Mortality	Amphibian Mortality	Bird Mortality	Diversity
Number of patches	+*	+	+**	+	+	+**
Average patch area	+*	-	-**	-	+	-**
Maximum patch area	-	+	-**	-**	+	-
Minimum patch area	-	-	+	-	-	-
Proportion of wet prairie	+	-	+	+*	+	+
Proportion of residential/mixed	+	+**	-	-**	+	+
Proportion of perennial ponds	+*	+	+*	+	+	+*
Proportion of upland savannah	-	-	+	+	-	-
Proportion of wet shrubland	+	-	+	+*	-	+
Proportion of swamp forest	+	+	+**	+*	-	+*
Proportion of upland conifer forest	+	-	+	-	+	+
Proportion of upland deciduous forest	-	+	-	-*	-	-
Proportion of floodplain forest	+	+	+	+	+	+
Proportion of sand barrens	-	+	-	-**	-*	-*
Proportion of Eurasian meadow	+	+	-	-	+	+
Proportion of upland prairie	+*	-	+**	+**	+	+**
Proportion of dense urban	-	-	-	-	-	-
Proportion of cropland	-**	-*	-**	-	-	-**
AWC	+**	+	+*	+**	+*	+**
AWS	+*	+	+	+**	+	+*
Depth to water table	-*	-	-	-**	-*	-**
Average NDVI	+	+*	+	-**	+	-
Maximum NDVI	-	+	-	-**	+	-
Minimum NDVI	-	+	+	-	-	-

* Significant correlations at $p < 0.05$
**Significant correlations at $p < 0.01$
+ Positive correlation
- Negative correlation
AADT = annual average daily traffic
AWC = available water capacity
AWS = available water storage
NDVI = normalized difference vegetation index

Table 1.4. Relationship between final set of predictor buffer variables and the buffer count outcome variables of total mortality, mammal mortality, reptile mortality, amphibian mortality count, and bird mortality count.

Variable	Total Mortality	Mammal Mortality	Reptile Mortality	Amphibian Mortality	Bird Mortality	Diversity
AADT		+**				
Road width	+**		+*	+*	+*	+**
Percent mammal			-*	-*	-*	
Percent reptile	+					
Percent amphibian	+					
Average canopy cover		+*				
Natural/natural land use	+					
Natural/developed land use	+					
Developed/agricultural land use	-*		-*	-*		-**
Agricultural/agricultural land use	-		-*			-*
Proportion of residential/mixed		+*				
Proportion of perennial ponds	+					
Proportion of swamp forest				+*		
Proportion of sand barrens					-*	
Proportion of upland prairie	+		+*	+		+**
Proportion of cropland	-	-				
Average NDVI		+				

* Significant correlations at $p < 0.05$
**Significant correlations at $p < 0.01$
\+ Positive correlation
\- Negative correlation
AADT = annual average daily traffic
NDVI = normalized difference vegetation index

Table 1.5. General linear models for buffer vertebrate mortality counts with Akaike Information Criterion adjusted for small sample size (AICc) and the difference in AICc scores between the best model (i.e., model with the lowest value) and the model being compared (Δ AICc).

Model	AICc	Δ AICc
RW + %_rep + %_amph + LU1 + LU2 + LU5 + LU6 + PP + UP + C	602.602	0
AADT + RW + %_rep + %_amph + %_CC_Max + %_CC_Min + #_SAP_Max + LU1 + LU2 + LU5 + LU6 + PP + UP + C + #_P + AWC	610.080	7.478
RW + LU5	630.606	28.004
Intercept	658.729	56.127

RW = road width
%_rep = percent reptile
%_amph = percent amphibian
LU1 = natural/natural roadside land use
LU2 = natural/developed roadside land use
LU5 = developed/agricultural roadside land use
LU6 = agricultural/agricultural roadside land use
PP = proportion perennial ponds
UP = proportion upland prairie
C = proportion cropland
%_CC_Max = maximum percent canopy cover
%_CC_Min = minimum percent canopy cover
#_P = number of patches
#_Sap_Max = maximum count of saplings
AWC = available water capacity

Table 1.6. General linear models for buffer reptile mortality counts with Akaike Information Criterion adjusted for small sample size (AICc) and the difference in AICc scores between the best model (i.e., the model with the lowest value) and the model being compared (Δ AICc).

Model	AICc	Δ AICc
RW + %_mamm + LU5 + LU6 + UP	291.047	0
RW + %_mamm + %_amph + LU5 + LU6 + UP	291.848	0.801
RW + %_mamm + %_amph + LU5 + LU6 + PA_2 + PP + UP + C + AWC	299.895	8.848
RW + %_mamm + %_amph + LU5 + LU6 + UP	302.374	11.327
RW + %_mamm + LU5	305.703	11.327
RW + %_mamm + %_amph + LU2 + LU5 + LU6 + PA_2 + PP + SF + UP + C + + AWC	304.869	13.822
%_mamm + LU5	307.880	16.833
RW + %_mamm + %_amph + %_CC_Max + %_CC_Min #_SAP_Max + #_SAP_Min + LU1 + LU2 + LU5 + LU6 + PA_2 + PP + SF + UP + C + #_Patch + maximum patch area + AWC	316.614	25.567
Intercept	336.126	45.079

RW = road width
%_rep = percent reptile
%_amph = percent amphibian
LU1 = natural/natural roadside land use
LU2 = natural/developed roadside land use
LU5 = developed/agricultural roadside land use
LU6 = agricultural/agricultural roadside land use
PP = proportion perennial ponds
UP = proportion upland prairie
C = proportion cropland
%_CC_Max = maximum percent canopy cover
%_CC_Min = minimum percent canopy cover
#_P = number of patches
#_Sap_Max = maximum count of saplings
AWC = available water capacity

Table 1.7. The road variables investigated in this study and the relationship to the road count outcome variables of total mortality, mammal mortality, reptile mortality, amphibian mortality, and bird mortality according to Spearman correlation analysis (N = 121).

Variable	Total Mortality	Mammal Mortality	Reptile Mortality	Amphibian Mortality	Bird Mortality	Diversity
AADT	+**	+**	+	-	+	+
Road width	+**	+**	+**	+	+	-
Percent mammal	+		-	-**	-	
Percent bird	-	-	-*	-		
Percent reptile	+	-		+*	-	
Percent amphibian	+	-	+		+	
Bat call count	-	-	+	+	+	-
Average canopy cover	+	+	+	-	+	-*
Maximum canopy cover	+	+	+	+	+	-
Average number saplings	+	+	+	+	+	-
Maximum number saplings	+	+	+	+	+	-
Average vegetation height	-	-	+	+	+	-
Maximum vegetation height	-	+	-	-	+	-
Minimum vegetation height	-	-	-	+	-	+*
Natural/natural land use	-	-	+	+	+	+
Natural/developed land use	+	-	+	+	+	-
Natural/agricultural land use	+	-	+	-	+	-
Developed/developed land use	+	+*	-	-	+	-
Developed/agricultural land use	-	-	-	-	-	-
Agricultural/agricultural land use	-	-	-	+	+	-
Proportion of protected area both sides	+	+	+	+	-	-
Proportion of protected area one side only	+	+	+	+*	+	+
Proportion of unprotected area both sides	-	-	-	-*	-	-
Proportion of protected area	+	-	+	+	+	+
Number of landcover classes	+	+	-	-	-	+**
Number of patches	+	+	-	-	+	+*
Average patch area	-	-	-	-	+	+

Variable	Total Mortality	Mammal Mortality	Reptile Mortality	Amphibian Mortality	Bird Mortality	Diversity
Maximum patch area	+	+*	-	-**	-	-
Minimum patch area	-	-	-	-*	-	-
Proportion of wet prairie	-	-	+	+	+	-
Proportion of residential/mixed	+	+*	-	-	+	+
Proportion of perennial ponds	+	+	+**	+	+	+
Proportion of upland savannah	-	-	-	+	-	-
Proportion of wet shrubland	-	-	+	+	-	-
Proportion of swamp forest	+	-	+	+**	+	-
Proportion of upland conifer forest	-	-	-	-	-	+
Proportion of upland deciduous forest	-	+	-	-	+	-
Proportion of floodplain forest	+	+	+	+	+	-
Proportion of sand barrens	-	+	-	-	-	-
Proportion of Eurasian meadow	+	+	+	+	+	+
Proportion of upland prairie	+	-	+**	+*	+	+
Proportion of dense urban	-	-	-	-	-	-
Proportion of cropland	-	-	-	+	-	-
AWC	+*	+	+**	+*	+*	-
AWS	+*	+	+	+	+	-
Depth to water table	-	-	-	-*	-	+
Average NDVI	+	-	-	-	+	+
Maximum NDVI	+	+	-	-	+	-
Minimum NDVI	-	+	-	-*	-	+

* Significant correlations at $p < 0.05$
**Significant correlations at $p < 0.01$
+ Positive correlation
- Negative correlation
AADT = annual average daily traffic
AWC = available water capacity
AWS = available water storage
NDVI = normalized difference vegetation index

Table 1.8. Relationship between final set of predictor road variables the road count outcome variables of total mortality, mammal mortality, reptile mortality, amphibian mortality count, and bird mortality count.

Variable	Total Mortality	Mammal Mortality	Reptile Mortality	Amphibian Mortality	Bird Mortality	Diversity
AADT	+*	+**				
Percent mammal				-*		
Proportion of protected area one side only				+*		
Number of landcover classes						+**
Proportion of upland prairie			+*	+*		
AWC	+*		+*	+*	+*	

* Significant correlations at $p < 0.05$
**Significant correlations at $p < 0.01$
\+ Positive correlation
\- Negative correlation
AADT = annual average daily traffic
AWC = available water capacity

Table 2.1. The spatial, structural, and productivity variables measured in this study with a description of the variable and the frequency of measurement.

Variable Name (unit)	Description	Measurement frequency
AADT	Annual average daily traffic volume obtained through the Ohio Department of Transportation	Once a year
canopy cover (%)	Percentage of canopy cover measured from each side of road	With site survey & with roadkill
VCH (cm)	Vegetation cover height of verge vegetation at each sampling point on both sides of road	With site survey & with roadkill
change VCH (cm)	change in average vegetation height for the two sides of the road over sampling period	Once in 2021 and 2022
#_SAP	Average of count of saplings on both sides of road	With site survey & with roadkill
NDVI	Normalized difference vegetation index derived from Lidar data, ratio of Near-Infrared and Red of satellite data that measures vegetation biomass	Three times per year: early spring, summer, fall
MSI	Moisture stress index derived from Lidar data, ratio of Near-Infrared and Red of satellite data that measures landscape water content	Three times per year: early spring, summer, fall
NDWI	Normalized difference water index derived from Lidar data, ratio of Near-Infrared and Red of satellite data that measures vegetation water content	Three times per year: early spring, summer, fall
EVI_2	enhanced vegetation index derived from Lidar data, ratio of Near-Infrared and Red of satellite data that measures vegetation biomass	Three times per year: early spring, summer, fall

Table 2.2. The environmental variables measured in this study with a description of the variable and the frequency of measurement.

Variable Name (unit)	Description	Measurement frequency
temperature (C°)	temperature obtained from the NOAA weather data	Daily
humidity (%)	humidity obtained from the NOAA weather data	Daily
precipitation (cm)	total precipitation during the sampling months (May-October)	Daily
wind (m/s)	average daily wind speed obtained from the NOAA weather data	Daily
dew point (C°)	Dew point obtained from Weatherbug	Daily
barometric pressure (mmHg)	Barometric pressure obtained from Weatherbug	Daily

Table 2.3. The total vertebrate mortality count and mortality count for each taxon for each year of the study.

Year	Mammal Mortality	Reptile Mortality	Amphibian Mortality	Bird Mortality	Total Mortality
2020	122	29	31	41	223
2021	84	64	51	47	246
2022	112	27	10	36	185

Table 2.4. Best general linear models for buffer vertebrate mortality counts and intercept only model with Akaike Information Criterion adjusted for small sample size (AICc) and the difference in AICc scores between the best model and the model being compared (Δ AICc).

Model	AICc	Δ AICc
maximum # saplings + summer average NDVI	1316.29	0
summer average NDVI	1317.95	1.66
Intercept	1327.43	11.13

NDVI = normalized difference vegetation index

Table 2.5. Best general linear models for buffer mammal mortality counts and intercept only model with Akaike Information Criterion adjusted for small sample size (AICc) and the difference in AICc scores between the best model and the model being compared (Δ AICc).

Model	AICc	Δ AICc
summer average NDVI	901.91	0
maximum # saplings + summer average NDVI	903.65	1.74
Intercept	913.54	11.63

NDVI = normalized difference vegetation index

Table 2.6. Best general linear models for buffer reptile mortality counts and intercept only model with Akaike Information Criterion adjusted for small sample size (AICc) and the difference in AICc scores between the best model and the model being compared (Δ AICc).

Model	AICc	Δ AICc
maximum # saplings + summer average NDVI	488.60	0
maximum # saplings	489.24	0.64
Intercept	500.31	11.71

NDVI = normalized difference vegetation index

Table 2.7. Best general linear models for buffer amphibian mortality counts and intercept only model with Akaike Information Criterion adjusted for small sample size (AICc) and the difference in AICc scores between the best model and the model being compared (Δ AICc).

Model	AICc	Δ AICc
maximum # saplings	552.63	0
maximum # saplings + summer average NDVI	554.57	1.94
Intercept	560.06	7.43

NDVI = normalized difference vegetation index

Table 3.1. Locations of hotspots and coldspots with associated *p*-value significance levels for total vertebrate mortality combined over 2020, 2021, and 2022. Buffer name are sample sites coded where the first two letters of buffer name indicate the natural area associated with the sampling, the number indicates the transect, and the letter is the buffer location along the transect.

Buffer Name	Hotspot or Coldspot	*p*-value
KT1D	hotspot	0.1
KT2B	hotspot	0.1
KT2C	hotspot	0.1
KT8A	hotspot	0.01
OO4C	hotspot	0.05
OO4D	hotspot	0.01
OO5A	hotspot	0.1
OO9A	hotspot	0.01
OO10B	hotspot	0.05
OO10C	hotspot	0.05
MF5C	coldspot	0.1
MF6A	coldspot	0.1
MF6B	coldspot	0.1
MF6C	coldspot	0.1

Table 3.2. Locations of hotspots with associated *p*-value significance levels for total vertebrate mortality for 2020. Buffer name are sample sites coded where the first two letters of buffer name indicate the natural area associated with the sampling, the number indicates the transect, and the letter is the buffer location along the transect.

Buffer Name	*p*-value
KT8A	0.01
OO4A	0.01
OO4B	0.01
OO8A	0.05
OO9A	0.01
MP1C	0.05
MP1D	0.05

Table 3.3. Locations of hotspots with associated *p*-value significance levels for total vertebrate mortality for 2021. Buffer name are sample sites coded where the first two letters of buffer name indicate the natural area associated with the sampling, the number indicates the transect, and the letter is the buffer location along the transect.

Buffer Name	*p*-value
KT1D	0.1
KT2D	0.1
KT8A	0.05
KT8D	0.05
KT9C	0.05
KT9D	0.01
OO4C	0.05
OO4D	0.01
OO9A	0.01
OO9B	0.01
OO10B	0.05
OO10C	0.05

Table 3.4. Locations of hotspots with associated *p*-value significance levels for total vertebrate mortality for 2022. Buffer name are sample sites coded where the first two letters of buffer name indicate the natural area associated with the sampling, the number indicates the transect, and the letter is the buffer location along the transect.

Buffer Name	*p*-value
KT2B	0.01
KT2C	0.01
KT8A	0.05
OO4C	0.05
OO4D	0.01
OO5A	0.05
OO5B	0.1
OO7C	0.1
OO8B	0.1
OO9D	0.1
MF1B	0.01
MF1C	0.05
MF1D	0.05

Table 3.5. Locations of hotspots with associated *p*-value significance levels for mammal mortality combined over 2020, 2021, and 2022. Buffer name are sample sites coded where the first two letters of buffer name indicate the natural area associated with the sampling, the number indicates the transect, and the letter is the buffer location along the transect.

Buffer Name	*p*-value
KT2B	0.01
KT2C	0.01
KT8A	0.05
OO4C	0.05
OO4D	0.01
OO5A	0.05
OO5B	0.1
OO7C	0.1
OO8B	0.1
OO9D	0.1
MF1B	0.01
MF1C	0.05
MF1D	0.05

Table 3.6. Locations of hotspots with associated *p*-value significance levels for reptile mortality combined over 2020, 2021, and 2022. Buffer name are sample sites coded where the first two letters of buffer name indicate the natural area associated with the sampling, the number indicates the transect, and the letter is the buffer location along the transect.

Buffer Name	*p*-value
KT3A	0.01
KT3B	0.01
KT3C	0.05
KT8A	0.05
KT8D	0.01
OO9A	0.1

Table 3.7. Locations of hotspots with associated *p*-value significance levels for amphibian mortality combined over 2020, 2021, and 2022. Buffer name are sample sites coded where the first two letters of buffer name indicate the natural area associated with the sampling, the number indicates the transect, and the letter is the buffer location along the transect.

Buffer Name	*p*-value
KT1C	0.05
KT1D	0.01
KT2D	0.1
KT7C	0.1
KT8A	0.1
KT9B	0.05
KT9C	0.05
KT9D	0.05
KT12B	0.01
KT12C	0.05
KT12D	0.05
OO9B	0.05

Table 3.8. Locations of hotspots with associated *p*-value significance levels for bird mortality combined over 2020, 2021, and 2022. Buffer name are sample sites coded where the first two letters of buffer name indicate the natural area associated with the sampling, the number indicates the transect, and the letter is the buffer location along the transect.

Buffer Name	*p*-value
KT1C	95
KT1D	95
KT2A	95
KT2D	95
OO6C	0.1
OO6D	0.1
OO8A	99
OO9B	99
OO9C	95

Table 3.9. Locations of hotspots and coldspots with associated *p*-value significance levels for bat acoustic monitoring calls combined over 2020, 2021, and 2022. Buffer name are sample sites coded where the first two letters of buffer name indicate the natural area associated with the sampling, the number indicates the transect, and the letter is the buffer location along the transect.

Buffer Name	Hotspot or Coldspot	*p*-value
KT1B	coldspot	99
KT1C	coldspot	95
KT1D	coldspot	90
KT3A	hotspot	99
KT3B	hotspot	99
KT6D	hotspot	99
KT8B	hotspot	90
KT9A	coldspot	95
OO3A	coldspot	90
OO9B	hotspot	90
OO9C	hotspot	90
MF5C	hotspot	90
MF6B	hotspot	90
MF7C	hotspot	90
MF7D	hotspot	90
MF8C	hotspot	90

Table 4.1. Continuous variables measured in this study with name and units, description of the variable, and the frequency of measurement.

Variable Name (units)	Description	Measurement frequency
Landcover	Percentage of each landcover class per 120 m buffer. Categorical landcover of 15 class created by Root & Martin (2020) map converted to continuous data through FRAGSTATS	Once
AWC (cm)	Available water capacity obtained from USDA NRCS, quantity of water that the soil is capable of storing for use by plants	Once
AWS (cm)	Available water storage obtained from USDA NRCS, computed as AWC times the thickness of the soil	Once
WTD (cm)	Water table depth obtained from USDA NRCS, saturated zone in the soil	Once
NDVI	Normalized difference vegetation index derived from Lidar data, ratio of Near-Infrared and Red of satellite data that measures vegetation biomass	Three times per year: early spring, summer, fall
MSI	Moisture stress index derived from Lidar data, ratio of Near-Infrared and Red of satellite data that measures landscape water content	Three times per year: early spring, summer, fall
NDWI	Normalized difference water index derived from Lidar data, ratio of Near-Infrared and Red of satellite data that measures vegetation water content	Three times per year: early spring, summer, fall
EVI_2	enhanced vegetation index derived from Lidar data, ratio of Near-Infrared and Red of satellite data that measures vegetation biomass	Three times per year: early spring, summer, fall

Table 4.2. Maxent models based on contributing continuous variables for total mortality count with corresponding area under the curve (AUC) score and standard deviation (SD). Models are arranged based on the order of investigation.

Model	AUC	SD
Landcover	0.862	0.019
Landcover (minus wet shrubland)	0.859	0.019
Landcover (minus turf)	0.862	0.019
Moisture stress index (MSI)	0.651	0.011
Normalized difference water index (NDWI)	0.635	0.025
Enhanced vegetation index (EVI_2)	0.627	0.022
Normalized difference vegetation index (NDVI)	0.624	0.018
Water table depth (WTD)	0.638	0.037
Available water capacity (AWC)	0.685	0.021
Available water storage (AWS)	0.676	0.017
MSI + Landcover	0.865	0.017
EVI_2 + Landcover	0.863	0.012
NDWI + Landcover	0.865	0.015
NDVI + Landcover	0.868	0.018
WTD + Landcover	0.864	0.018
AWS + WTD + NDVI + Landcover	0.883	0.022
AWC + WTD + NDVI + Landcover	0.879	0.020
AWS + WTD + NDVI + Landcover (minus Turf)	0.883*	0.022
AWS + WTD + NDVI + Landcover (minus Turf & Wet Shrubland)	0.882	0.022

* Indicates best model

Table 4.3. The continuous environmental variables contributing to the final Maxent best model (AWS + WTD + NDVI + Landcover (minus Turf)) with the percent contribution evaluating how much the variable adds to the Maxent model based on the order the variables are entered and permutation importance that evaluates the importance of the variable based on the final model regardless of the path.

Variable	Percent contribution	Permutation importance
Residential mixed area	37.6	35.5
Dense urban area	13.9	10.5
Water table depth	7.6	4.9
Cropland area	6.8	6.3
Available water storage	5.9	9.3
Swamp forest area	5.8	5.3
Upland prairie area	5.8	6.1
Eurasian meadow area	5.7	4.6
NDVI	3.4	4.5
Floodplain forest area	2.8	4.6
Wet prairie area	1.6	2.5
Upland conifer forest area	1.1	1.9
Perennial ponds area	0.8	0.4
Upland deciduous forest area	0.6	1.9
Sand barren area	0.3	0.9
Upland savannah area	0.3	0.7
Wet shrubland area	0.2	0.1

Table 4.4. Final Maxent best model (AWS + WTD + NDVI + Landcover (minus Turf)) for each taxon mortality counts with corresponding area under the curve (AUC) score and standard deviation (SD).

Taxon Model	AUC	SD
Mammal	0.895	0.021
Reptile	0.915	0.035
Amphibian	0.864	0.085
Bird	0.854	0.047

Table 4.5. The continuous environmental variables contributing to the final Maxent best model (AWS + WTD + NDVI + Landcover (minus Turf)) for mammal mortality with the percent contribution evaluating how much the variable adds to the Maxent model based on the order the variables are entered and permutation importance that evaluates the importance of the variable based on the final model regardless of the path.

Variable	Percent contribution	Permutation importance
Residential mixed area	42.9	42.5
Dense urban area	15.9	8.4
Eurasian meadow area	6.6	5.5
Available water storage	6.4	4.6
Cropland area	6.2	5
Water table depth	4.8	4.3
NDVI	4.7	4.5
Swamp forest area	3.1	4.7
Upland conifer forest area	2.3	2.8
Upland prairie area	1.5	3.7
Floodplain forest area	1.4	3.9
Wet prairie area	1.2	2.6
Upland deciduous forest area	1.2	3.9
Sand barren area	0.8	2
Upland savannah area	0.7	1.6
Perennial ponds area	0.5	0
Wet shrubland area	0	0

Table 4.6. The continuous environmental variables contributing to the final Maxent best model (AWS + WTD + NDVI + Landcover (minus Turf)) for reptile mortality with the percent contribution evaluating how much the variable adds to the Maxent model based on the order the variables are entered and permutation importance that evaluates the importance of the variable based on the final model regardless of the path.

Variable	Percent contribution	Permutation importance
Residential mixed area	22.6	33.4
Upland prairie area	18.6	13.8
Swamp forest area	11.9	10.8
Floodplain forest area	9	7.3
Water table depth	6.9	6.6
Cropland area	6.4	4.1
Dense urban area	5.8	4.5
Upland deciduous forest area	5.1	2.3
Available water storage	5.1	4.4
Eurasian meadow area	2.6	5.5
Wet prairie area	2.2	3.1
Sand barren area	1	0.9
Upland conifer forest area	1	1.1
NDVI	0.9	1.1
Perennial ponds area	0.5	0.3
Upland savannah area	0.4	0.9
Wet shrubland area	0	0

Table 4.7. The continuous environmental variables contributing to the final Maxent best model (AWS + WTD + NDVI + Landcover (minus Turf)) for amphibian mortality with the percent contribution evaluating how much the variable adds to the Maxent model based on the order the variables are entered and permutation importance that evaluates the importance of the variable based on the final model regardless of the path.

Variable	Percent contribution	Permutation importance
Residential mixed area	19.6	19.4
Upland prairie area	18.4	5.6
Water table depth	8.5	8.7
Dense urban area	8.4	7.2
Eurasian meadow area	7.9	7.4
Swamp forest area	7.6	10.5
Floodplain forest area	5.2	4.7
Cropland area	4.6	20.6
Wet prairie area	4.3	2.3
NDVI	3.6	5.2
Available water storage	3.4	2.8
Wet shrubland area	2.6	0.3
Upland conifer forest area	1.9	1.1
Upland deciduous forest area	1.7	0.9
Sand barren area	1.4	0.8
Upland savannah area	0.7	2.1
Perennial ponds area	0.3	0.2

Table 4.8. The continuous environmental variables contributing to the final Maxent best model (AWS + WTD + NDVI + Landcover (minus Turf)) for bird mortality with the percent contribution evaluating how much the variable adds to the Maxent model based on the order the variables are entered and permutation importance that evaluates the importance of the variable based on the final model regardless of the path.

Variable	Percent contribution	Permutation importance
Residential mixed area	40.3	37.6
Upland prairie area	9.2	10.3
Dense urban area	9.1	3.4
Water table depth	7.8	2.9
Available water storage	6.5	5.2
Cropland area	6.3	14.5
Wet prairie area	5.7	5
Eurasian meadow area	3.7	3.6
Swamp forest area	2.6	5.1
Floodplain forest area	2.2	4.7
NDVI	1.4	2.4
Perennial ponds area	1.3	0.4
Upland deciduous forest area	1.2	0.9
Wet shrubland area	1.1	0.3
Upland conifer forest area	0.8	0.9
Sand barren area	0.7	2.4
Upland savannah area	0.3	0.4

Table 4.9. Final Maxent best model (AWS + WTD + NDVI + Landcover (minus Turf)) for each year mortality counts with corresponding area under the curve (AUC) score and standard deviation (SD).

Model Year	AUC	SD
2020	0.918	0.020
2021	0.917	0.016
2022	0.902	0.028

Table 4.10. The continuous environmental variables contributing to the final Maxent best model (AWS + WTD + NDVI + Landcover (minus Turf)) for 2020 mortality with the percent contribution evaluating how much the variable adds to the Maxent model based on the order the variables are entered and permutation importance that evaluates the importance of the variable based on the final model regardless of the path.

Variable	Percent contribution	Permutation importance
Residential mixed area	28.8	34.3
NDVI composite for 2020	25	19.9
Swamp forest area	9	7.4
Dense urban area	8.6	3.3
Cropland area	8	7.5
Eurasian meadow area	4.4	3.2
Available water storage	3.7	2.6
Water table depth	2.8	2.1
Upland conifer forest area	1.8	2.2
Wet prairie area	1.8	2.5
Upland prairie area	1.5	4.4
Floodplain forest area	1.3	4.4
Sand barren area	1.2	2.7
Upland savannah area	0.9	0.1
Upland deciduous forest area	0.7	3.4
Wet shrubland area	0.4	0.1
Perennial ponds area	0	0

Table 4.11. The continuous environmental variables contributing to the final Maxent best model (AWS + WTD + NDVI + Landcover (minus Turf)) for 2021 mortality with the percent contribution evaluating how much the variable adds to the Maxent model based on the order the variables are entered and permutation importance that evaluates the importance of the variable based on the final model regardless of the path.

Variable	Percent contribution	Permutation importance
Residential mixed area	21.1	35.8
NDVI composite for 2021	20.2	16.4
Dense urban area	10.2	6
Upland prairie area	9.2	8
Eurasian meadow area	9.1	4.2
Water table depth	6.5	5.4
Cropland area	6.4	4.7
Available water storage	5.4	4.5
Floodplain forest area	3.4	4.1
Sand barren area	2.4	0.5
Swamp forest area	2	3.8
Wet prairie area	1.2	2.1
Perennial ponds area	1	0.4
Upland savannah area	0.6	1.9
Upland deciduous forest area	0.6	2.2
Upland conifer forest area	0.4	0
Wet shrubland area	0.2	0.1

Table 4.12. The continuous environmental variables contributing to the final Maxent best model (AWS + WTD + NDVI + Landcover (minus Turf)) for 2022 mortality with the percent contribution evaluating how much the variable adds to the Maxent model based on the order the variables are entered and permutation importance that evaluates the importance of the variable based on the final model regardless of the path.

Variable	Percent contribution	Permutation importance
Residential mixed area	37.5	40.4
NDVI composite for 2022	17.4	10.6
Dense urban area	12.2	4.5
Swamp forest area	8.4	7.7
Water table depth	4.8	4.4
Available water storage	4.4	4.1
Upland prairie area	3.3	4.4
Eurasian meadow area	2.7	4.5
Floodplain forest area	2.6	4.5
Upland conifer forest area	2.1	2.9
Upland deciduous forest area	1.1	3.3
Cropland area	1.1	4.9
Wet prairie area	0.9	1.7
Perennial ponds area	0.7	0.1
Upland savannah area	0.5	1.1
Sand barren area	0.3	1.1
Wet shrubland area	0	0

APPENDIX B. FIGURES

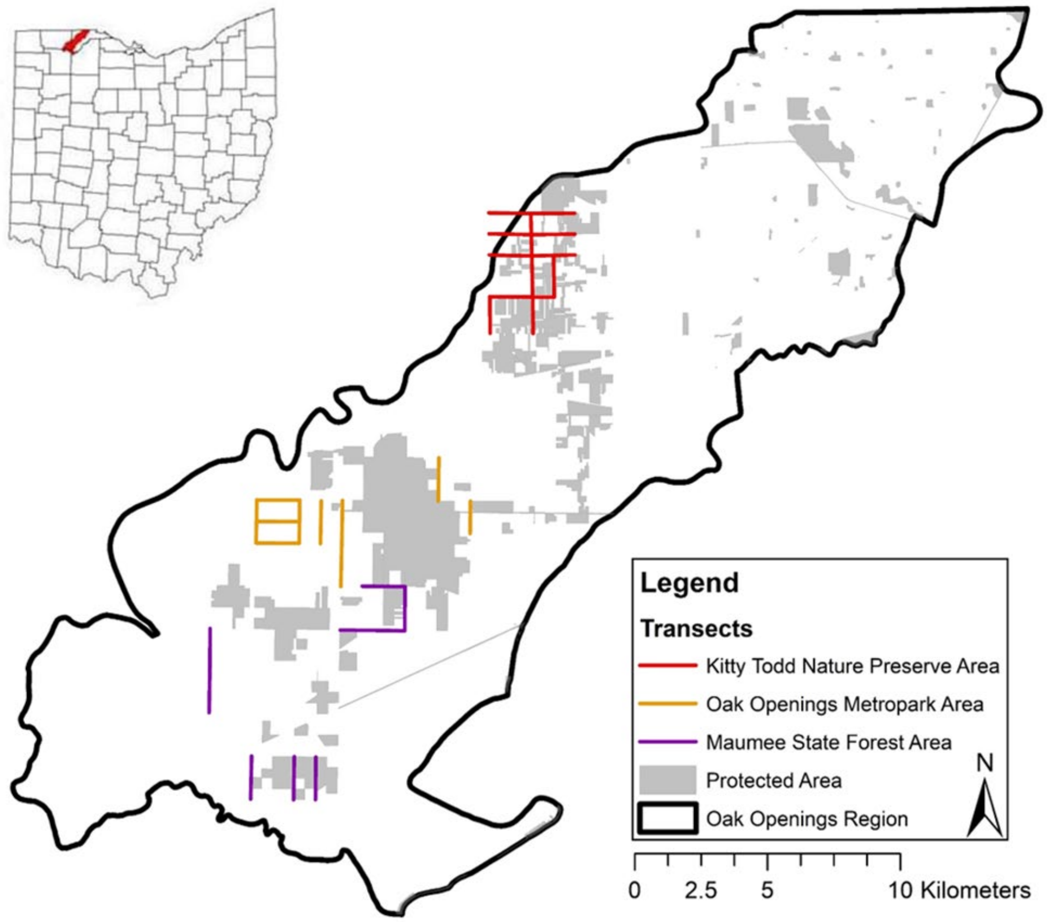


Figure 0.1. Map of the Oak Openings Region Area outlined in black (red in Ohio inset map) located in Northwest Ohio. The study transects are grouped by natural area: Kitty Todd Nature Preserve area (red), Oak Openings Preserve (orange), and Maumee State Forest (purple). Protected areas within the region are highlighted in gray.

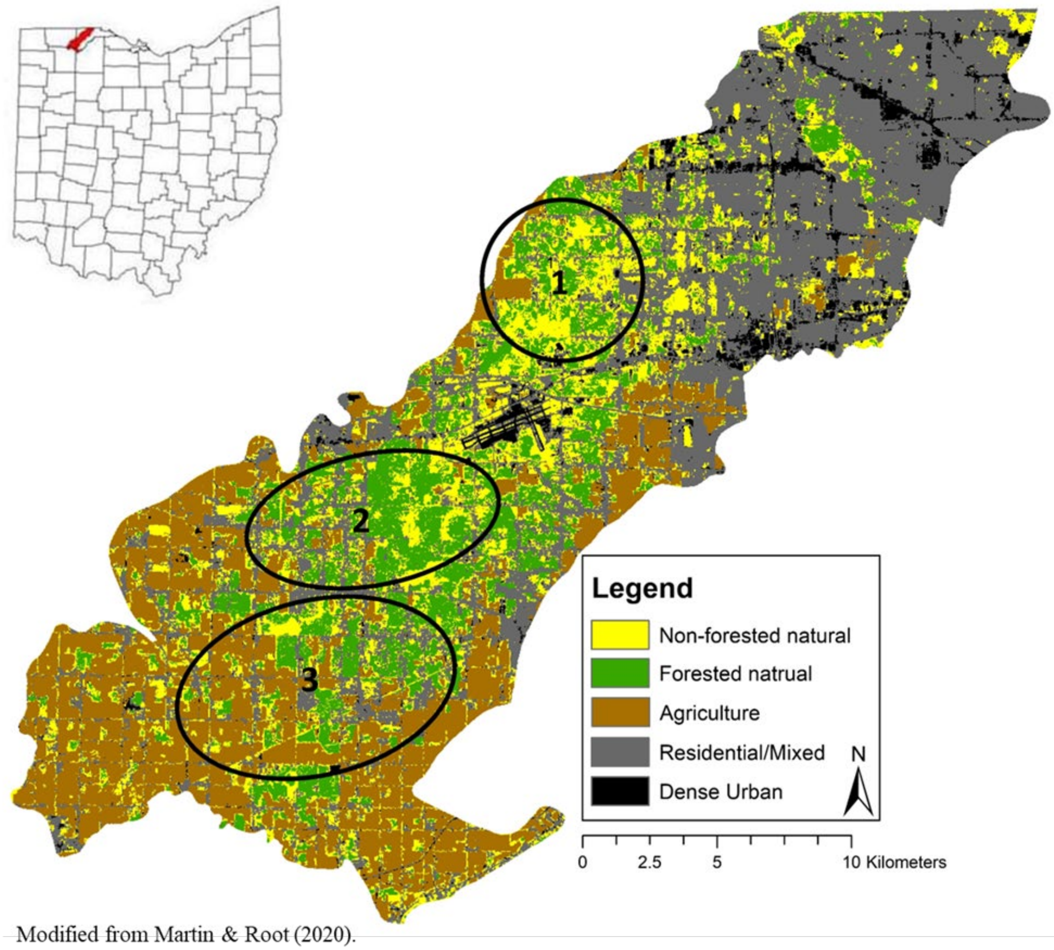


Figure 0.2. Map of the Oak Openings Region in Northwest Ohio (red in Ohio inset map) with the natural areas associated with the study transects circled: Kitty Todd Nature Preserve area (1), Oak Openings Preserve (2), and Maumee State Forest (3). Landcover is classified as non-forested natural (yellow), forested natural (green), agricultural (brown), residential/mixed (gray), or dense urban (black).

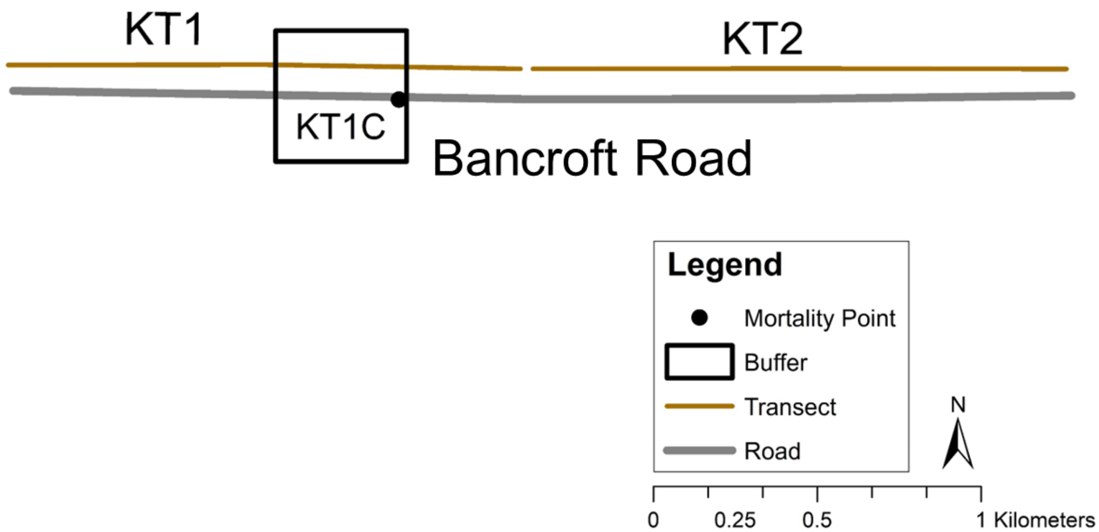


Figure 1.1. Example of the different measurement scales considered in this study: vertebrate mortality point (dot), buffer 200 meters in radius, KT1C (square), transects, KT1 and KT2 (brown lines), and road, Bancroft Road (gray line).

Identifiable to species | Identifiable to class

Figure 1.2. Example photographs of vertebrate mortality found during road surveys. Photos in the left column represent organisms that were able to be identified to species; those in the right column represent organisms that were identified only to class. Taken by the author (S. Rair).

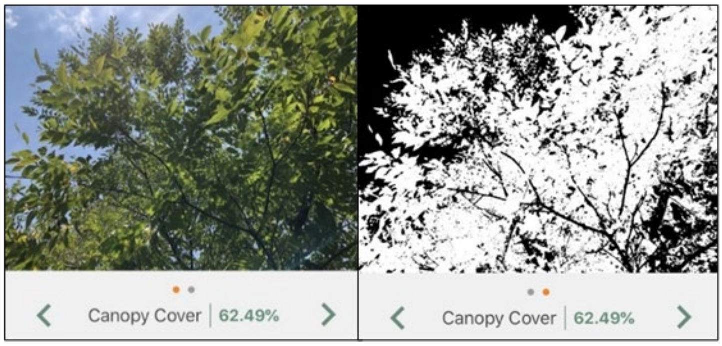


Figure 1.3. Sample of canopy cover measurement taken through Canopeo. The left photo is the color version, and the right is the black and white version with the corresponding amount of canopy cover as a percentage.

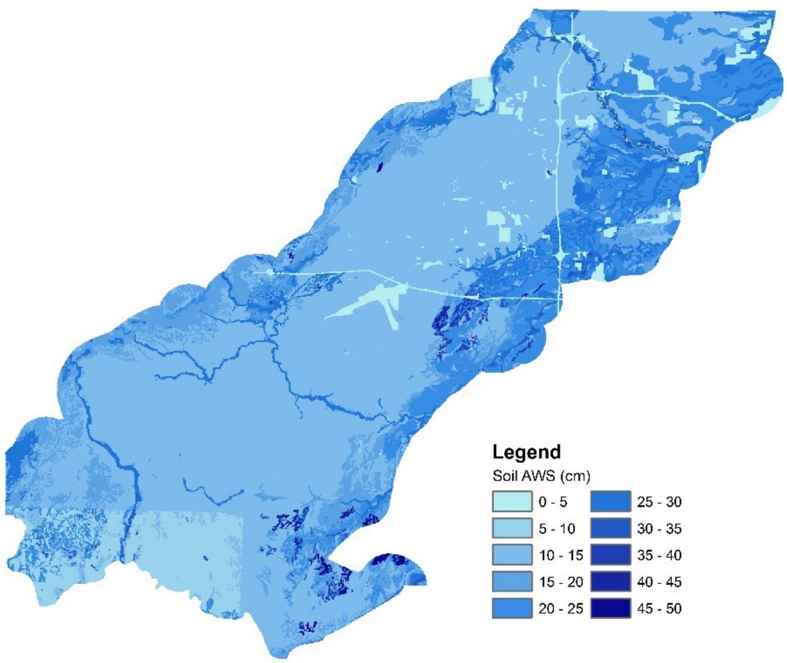


Figure 1.4. Soil available water storage values obtained from USDA NRCS for the Oak Openings Region. The darker the blue the greater the available water storage is for the soil ranging 0-5 centimeters.

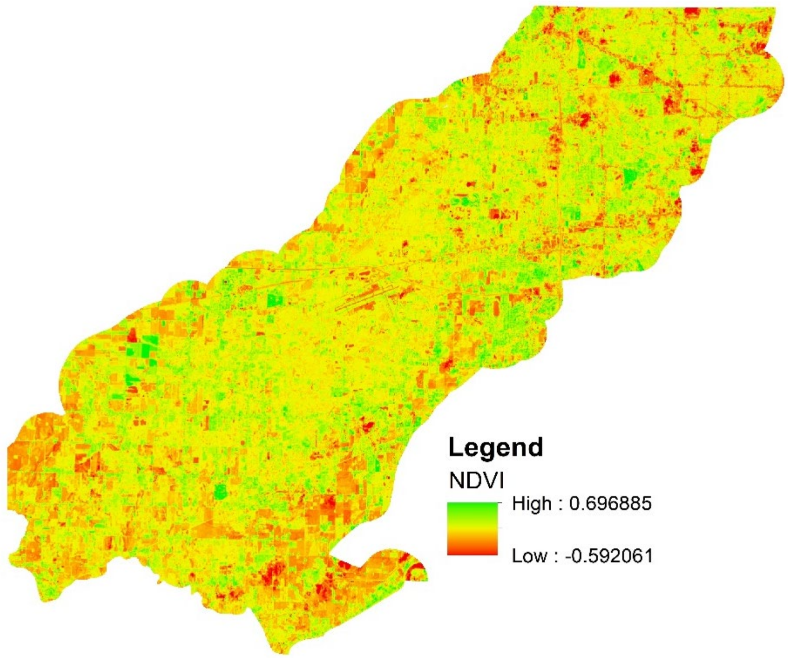


Figure 1.5. Normalized difference vegetation index (NDVI) values for the Oak Openings Region averaged over 2020, 2021, and 2022. Lower values of productive shown in red to orange and greater values shown in yellow to green.

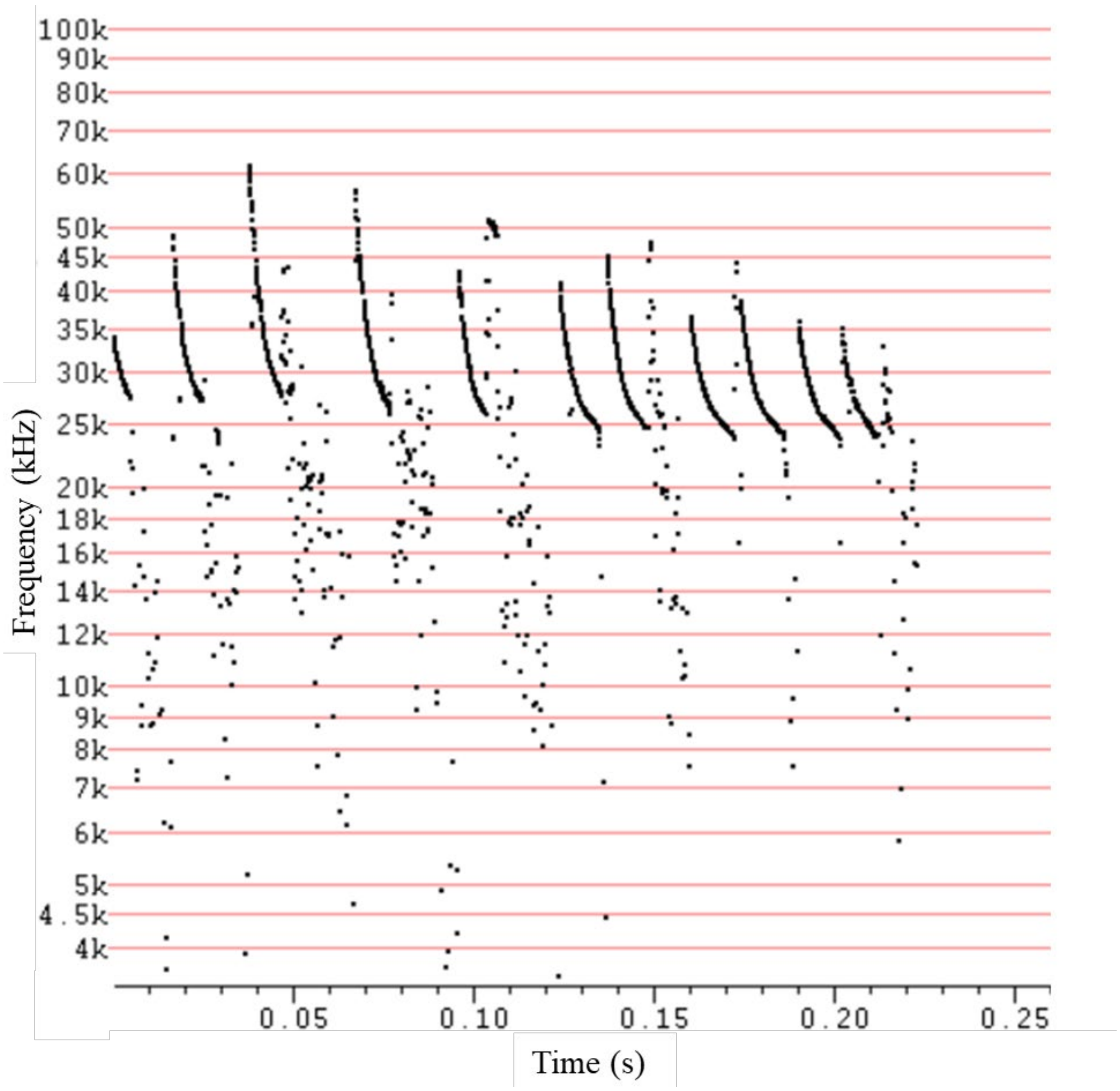


Figure 1.6. Sample of sonogram output from Anabat SD2 in AnalookW with pulses (lines of connected dots) recorded by time in seconds and frequency in kilohertz.

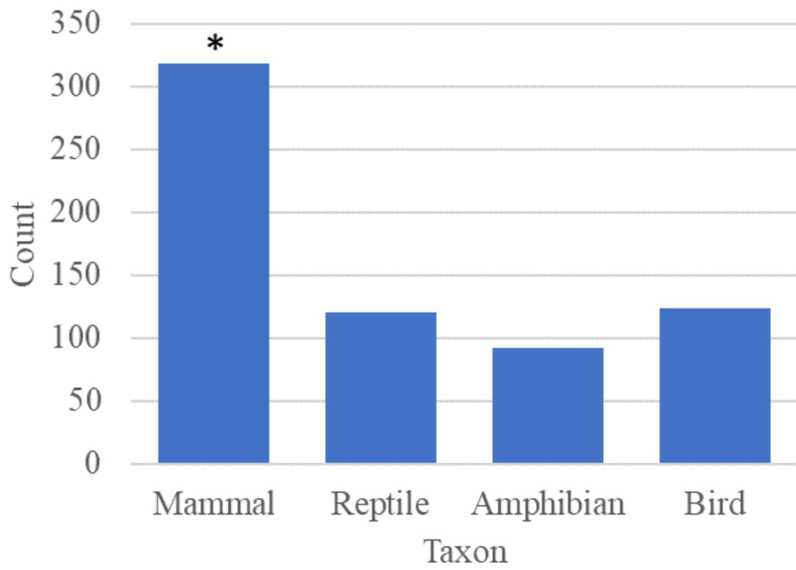


Figure 1.7. Total vertebrate mortality counts for each taxon for entire sampling period. Significant difference at $p < 0.05$ denoted by *.

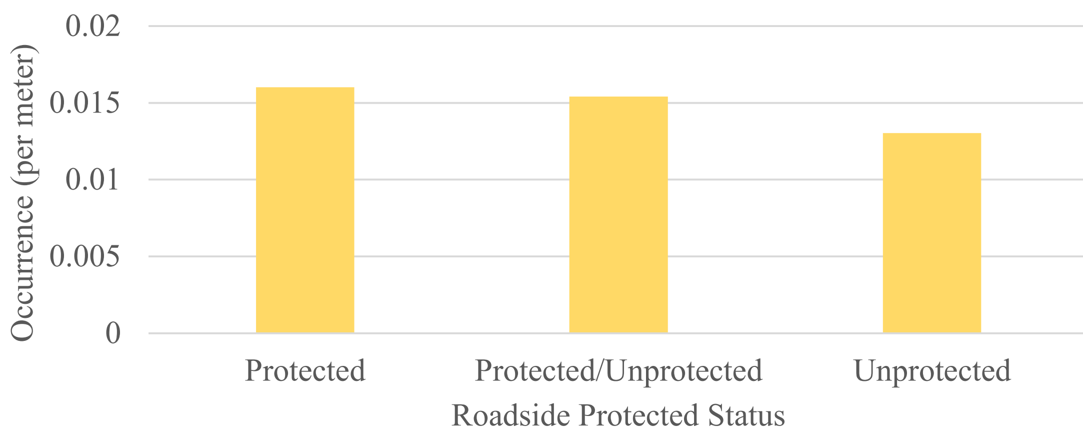


Figure 1.8. The total vertebrate mortality detected for the entire sampling period categorized by the protected status of each side of the road, normalized to occurrence per meter to control for differences in the lengths of roadside protected statuses within the study area.

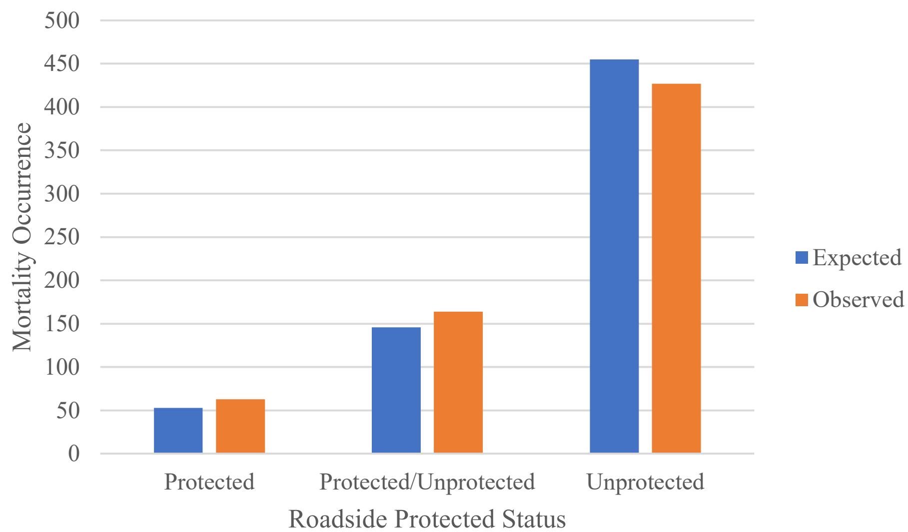


Figure 1.9. Total vertebrate mortality for the entire sampling period is categorized by the protected status of each side of the road. Observed occurrences (orange) are compared to expected occurrences (blue).

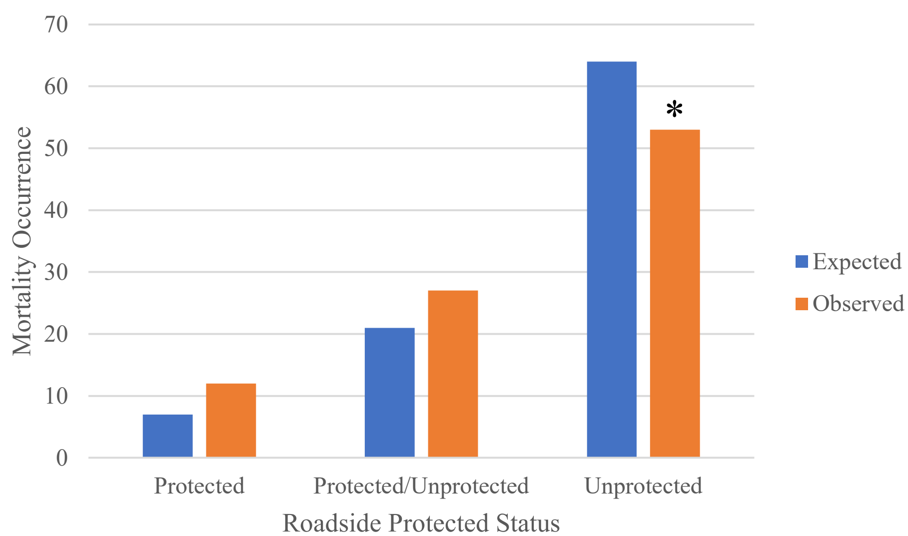


Figure 1.10. Total amphibian mortality for the entire sampling period is categorized by the protected status of each side of the road. Observed occurrences (orange) are compared to expected occurrences (blue).

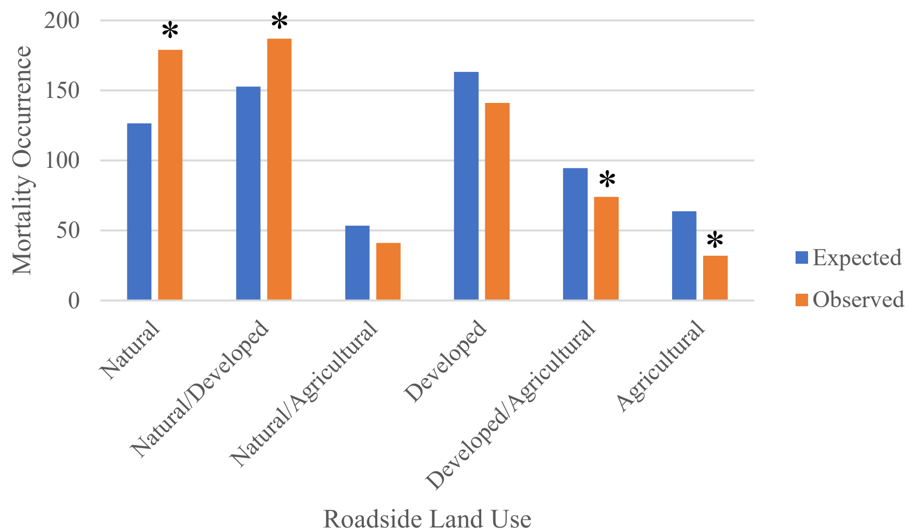


Figure 1.11. Total vertebrate mortality for the entire sampling period is categorized by the land use of each side of the road. Observed occurrences (orange) are compared to expected occurrences (blue). Significant difference at $p < 0.001$ denoted by *.

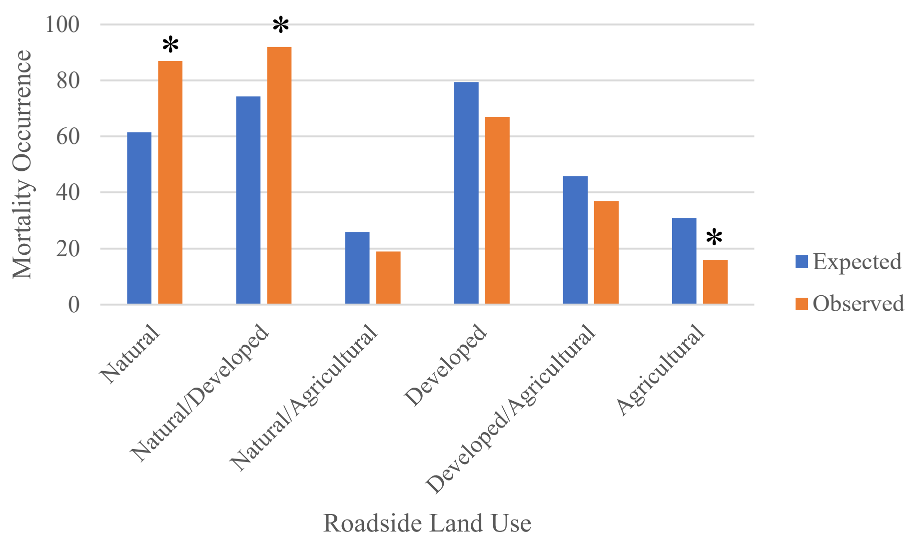


Figure 1.12. Total mammal mortality for the entire sampling period is categorized by the land use of each side of the road. Observed occurrences (orange) are compared to expected occurrences (blue). Significant difference at $p < 0.001$ denoted by *.

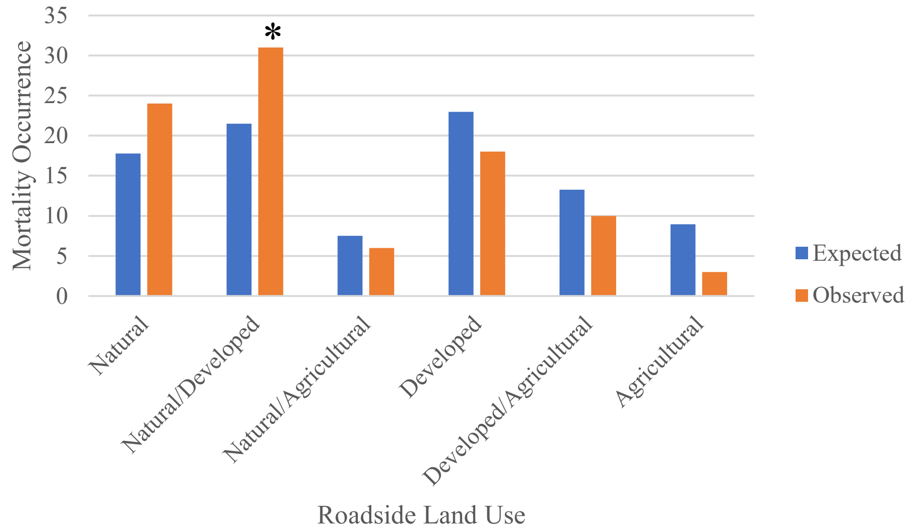


Figure 1.13. Total amphibian mortality for the entire sampling period categorized by the land use of each side of the road. Observed occurrences (orange) are compared to expected occurrences (blue). Significant difference at $p < 0.001$ denoted by *.

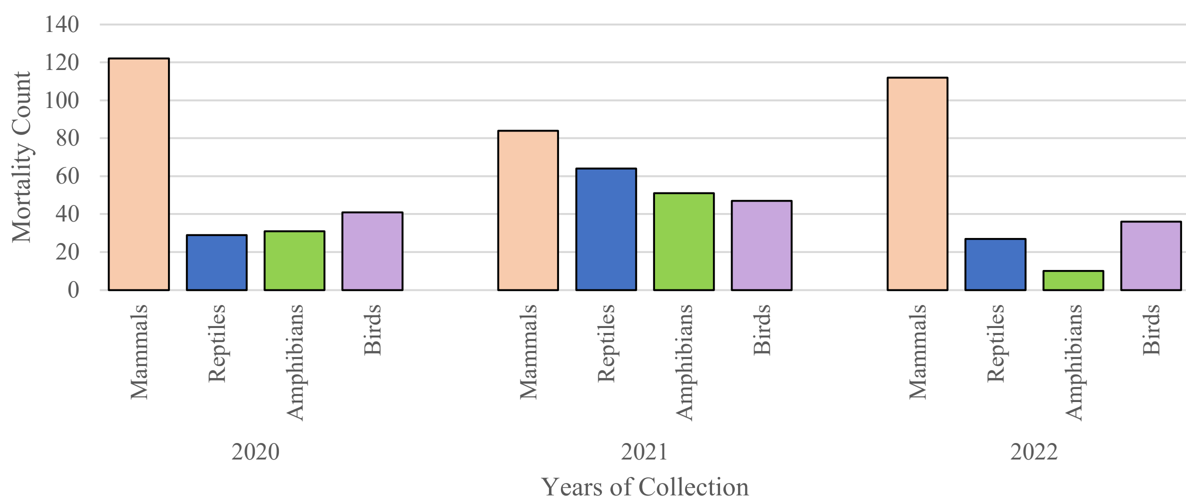


Figure 2.1. Vertebrate mortality detected throughout the 2020, 2021, and 2022 field seasons categorized by totals for each taxon per year. Mammals are represented by orange, reptiles by blue, amphibians by green, and birds by purple.

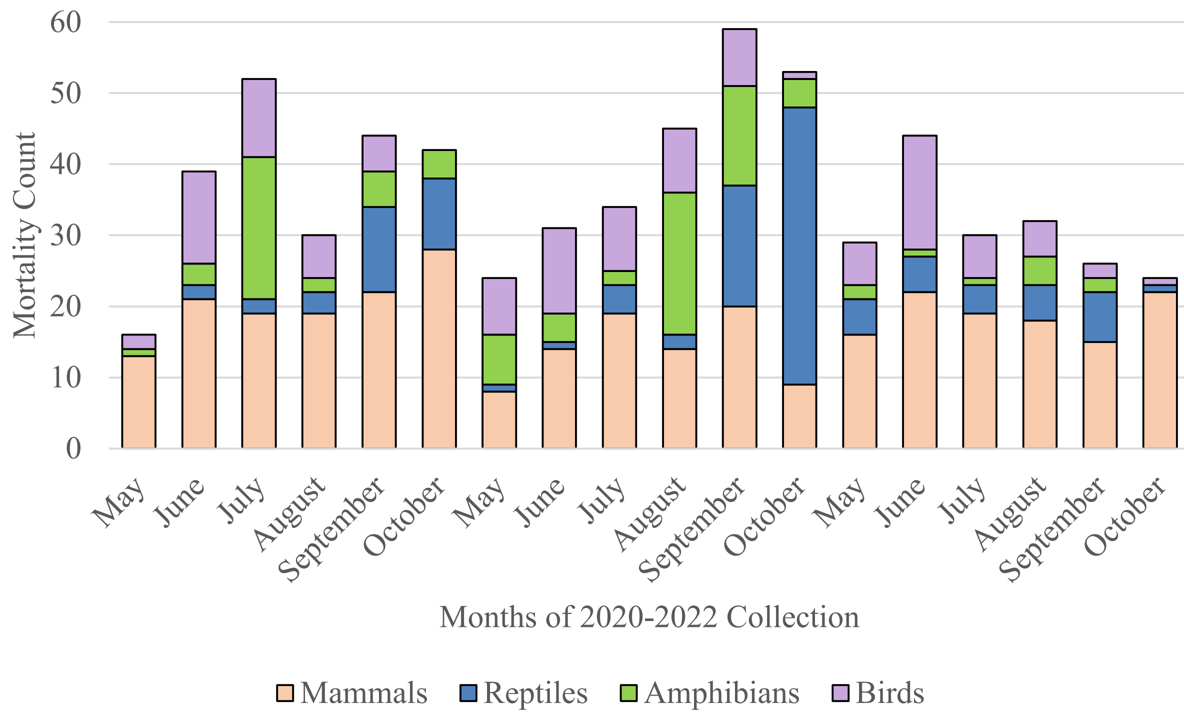


Figure 2.2. Vertebrate mortality detected throughout the 2020, 2021, and 2022 field seasons categorized by stacked totals for each taxon per month. Mammals are represented by orange, reptiles by blue, amphibians by green, and birds by purple.

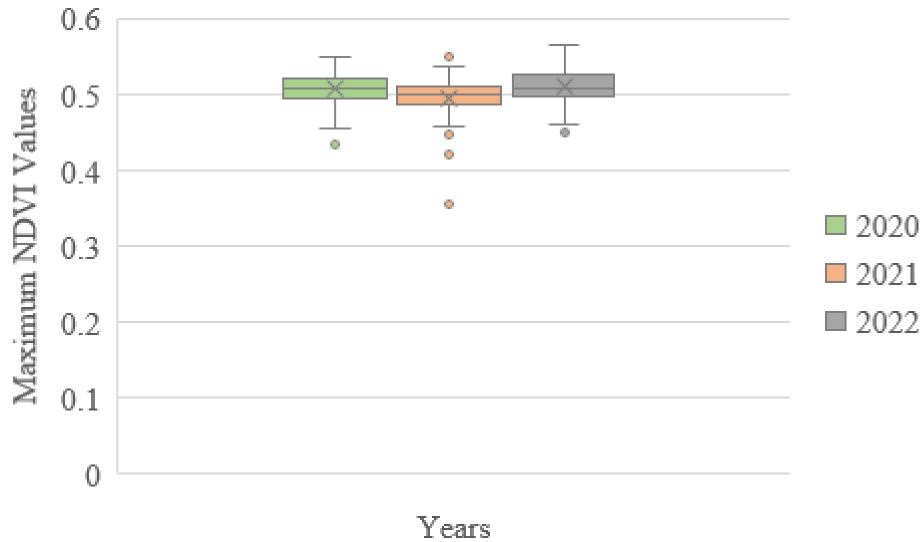


Figure 2.3. Spread of maximum NDVI values across locations recorded across 2020, 2021, and 2022. Values for 2020 are in green, 2021 are in orange, and 2022 are in gray. Average across all sites is shown with an X , the shaded box shows the range of values across sites and the bars represent the standard error in the data in each year.

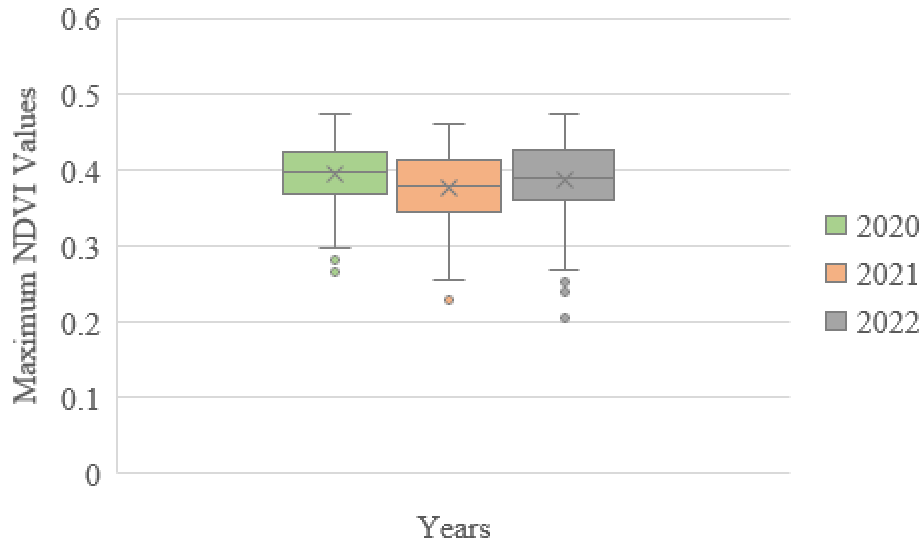


Figure 2.4. Spread of average NDVI values recorded across 2020, 2021, and 2022. Values for 2020 are in green, 2021 are in orange, and 2022 are in gray. Average across all sites is shown with an X , the shaded box shows the range of values across sites and the bars represent the standard error in the data in each year.

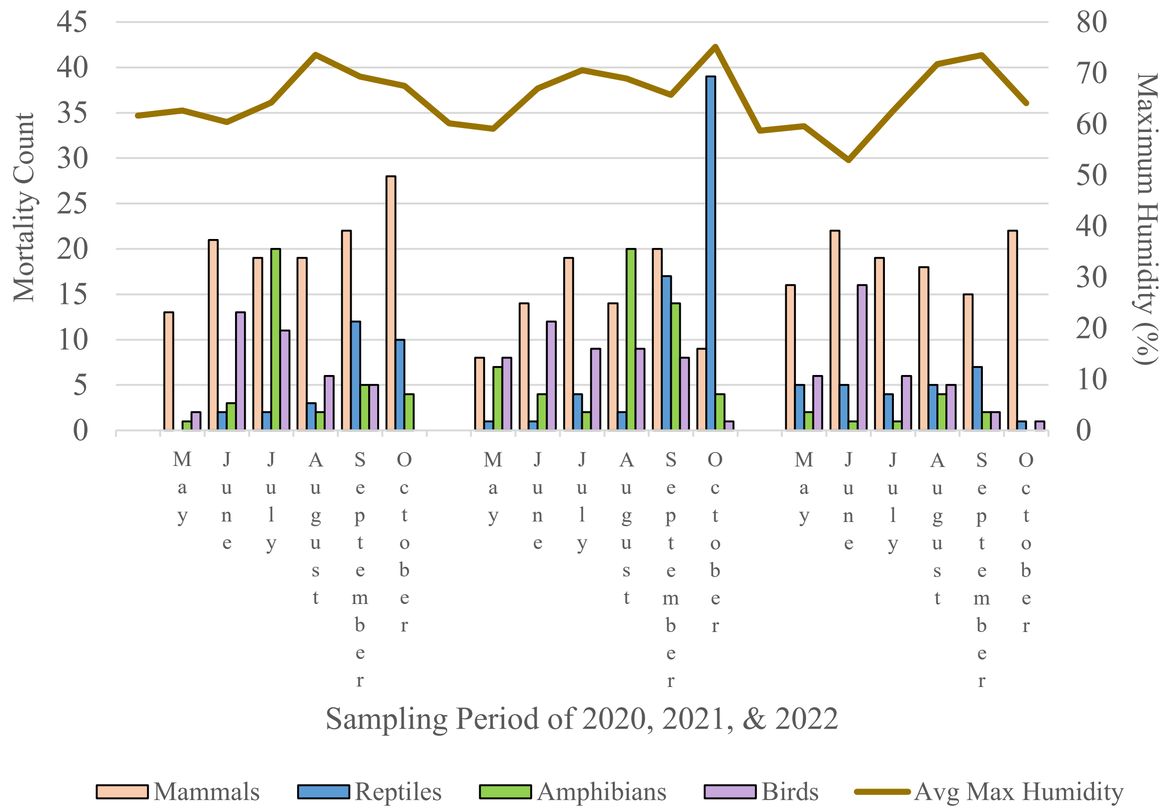


Figure 2.5. Total vertebrate mortality detected categorized by taxon and maximum humidity recorded throughout the 2020, 2021, and 2022 field seasons by month. Mammals are represented by orange bars, reptiles by blue, amphibians by green, and birds by purple. Percent maximum humidity is represented by brown line.

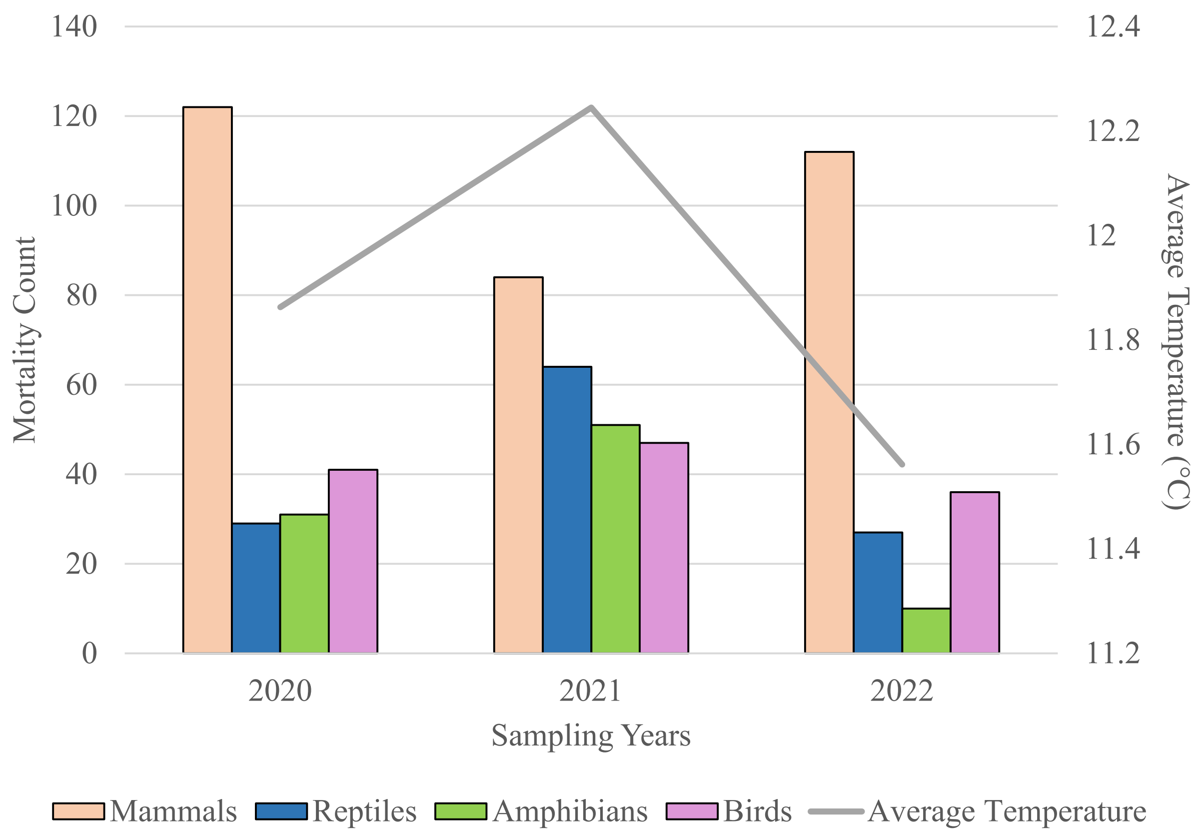


Figure 2.6. Total vertebrate mortality detected categorized by taxon and average temperature (°C) recorded in 2020, 2021, and 2022. Mammals are represented by orange bars, reptiles by blue, amphibians by green, and birds by purple. The average temperature in degrees Celsius is represented by light gray line.

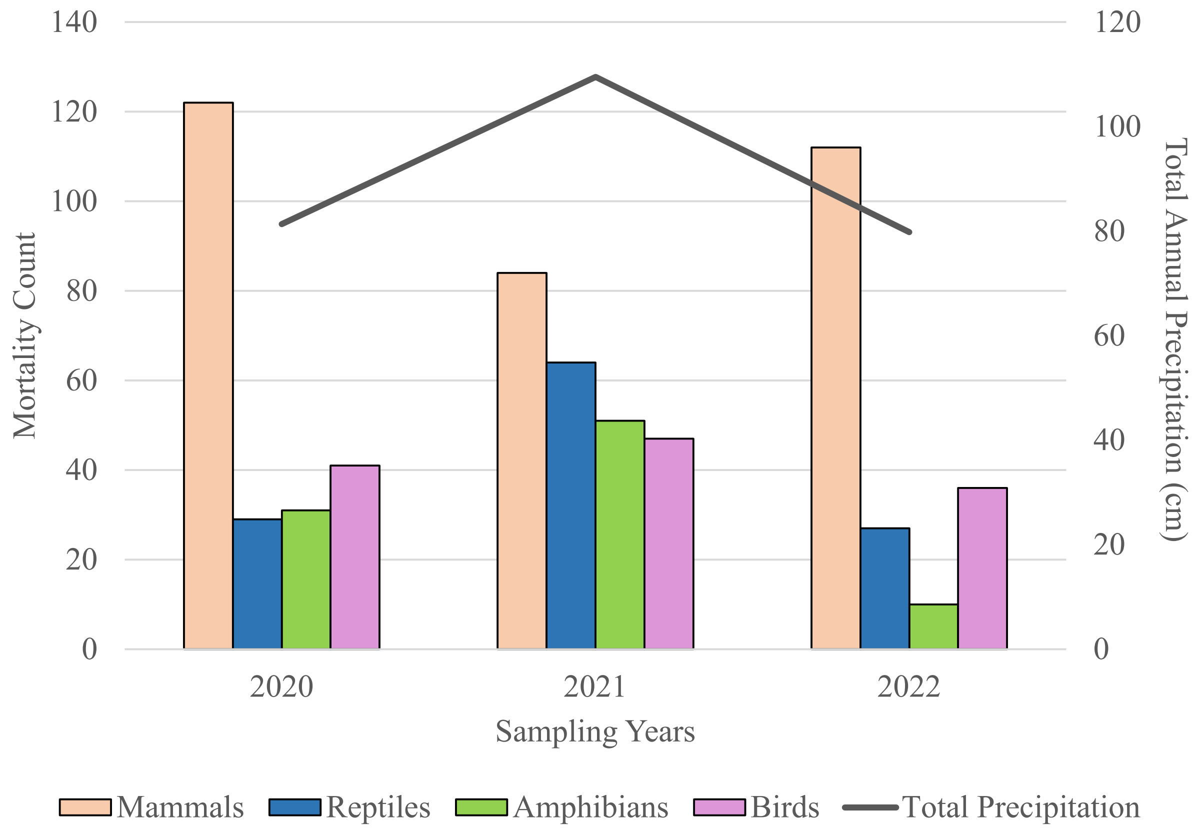


Figure 2.7. Total vertebrate mortality detected categorized by taxon and average temperature (°C) recorded in 2020, 2021, and 2022. Mammals are represented by orange bars, reptiles by blue, amphibians by green, and birds by purple. The total annual precipitation in centimeters is represented by light gray line.

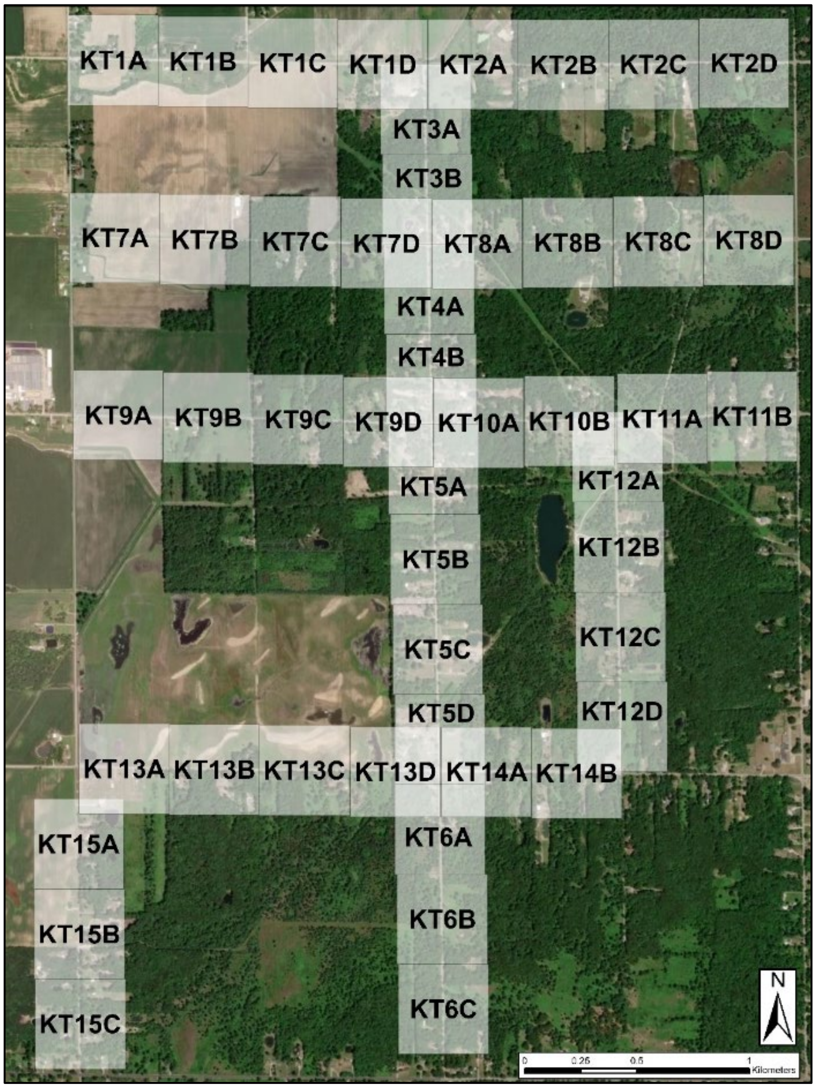


Figure 3.1. Sample areas that were located around Kitty Todd Nature Preserve (KT). Sample sites are coded where the first two letters of buffer name indicate the natural area associated with the sampling, the number indicates the transect, and the letter is the buffer location along the transect, which is coded east to west or north to south.

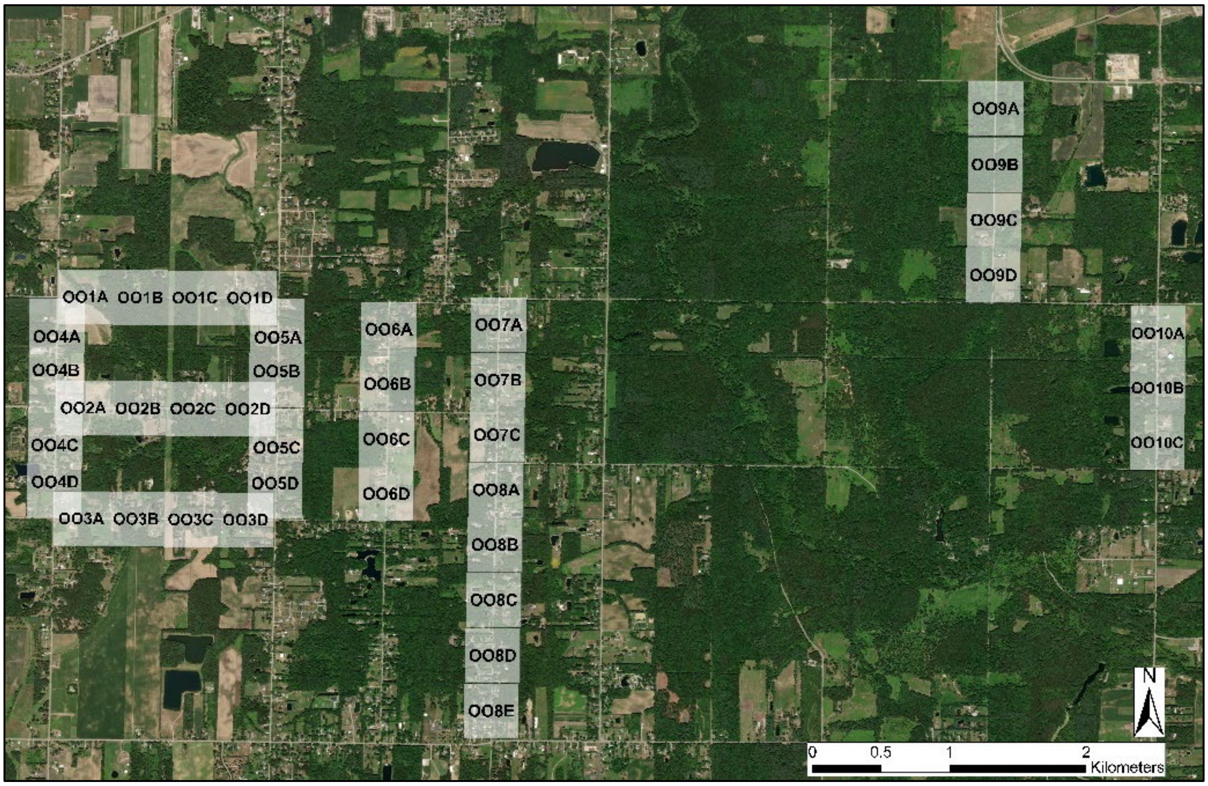


Figure 3.2. Sample areas that were located around Oak Openings Preserve (OO). Sample sites are coded where the first two letters of buffer name indicate the natural area associated with the sampling, the number indicates the transect, and the letter is the buffer location along the transect, which is coded east to west or north to south.

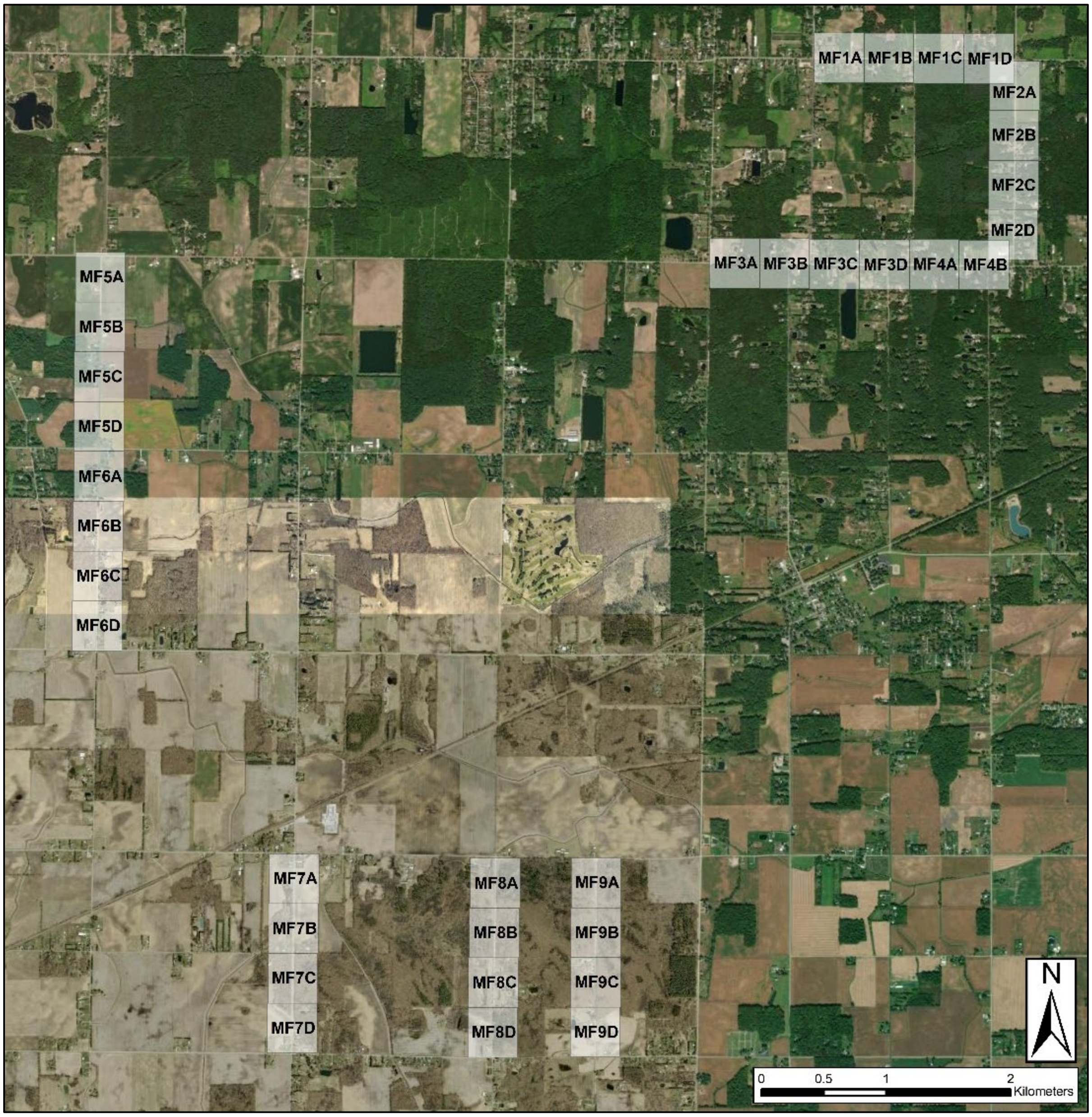
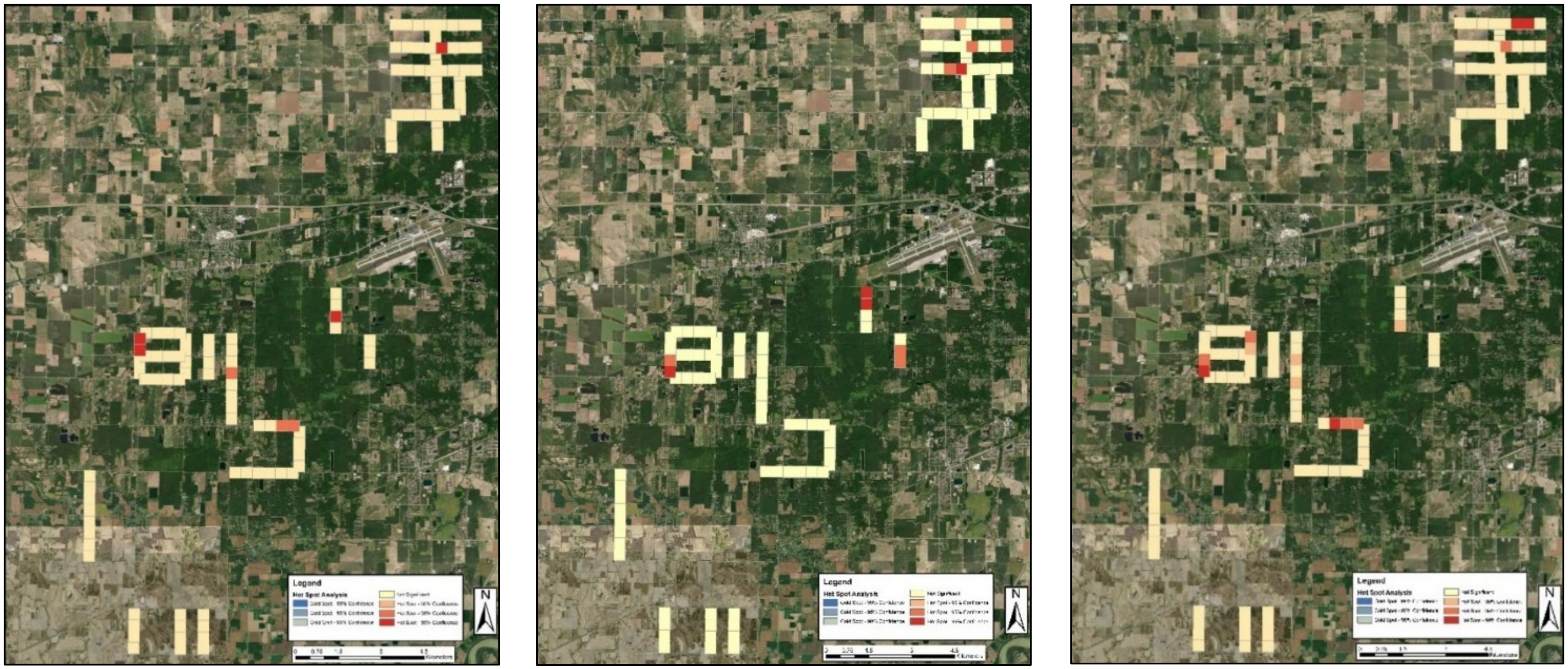


Figure 3.3. Sample areas that were located around Maumee State Forest (MF). Sample sites are coded where the first two letters of buffer name indicate the natural area associated with the sampling, the number indicates the transect, and the letter is the buffer location along the transect, which is coded east to west or north to south.

Figure 3.4. Getis-Ord Gi* hotspot analysis for all vertebrate mortality across the study combined for 2020, 2021, and 2022. Warmer colors represent hotspots, or areas that had a clustering of high mortalities, where cooler colors represent coldspots, or areas that were less likely than average to have mortality clusters. The yellow represents no significant spatial clustering in mortality found.



(a) (b) (c)

Figure 3.5. Getis-Ord Gi* hotspot analysis for all vertebrate mortality across the study area for 2020 (a), 2021 (b), and for 2022 (c). Warmer colors represent hotspots, or areas that had a clustering of high mortalities, where cooler colors represent coldspots, or areas that were less likely than average to have mortality clusters. The yellow represents no significant spatial clustering in mortality found.

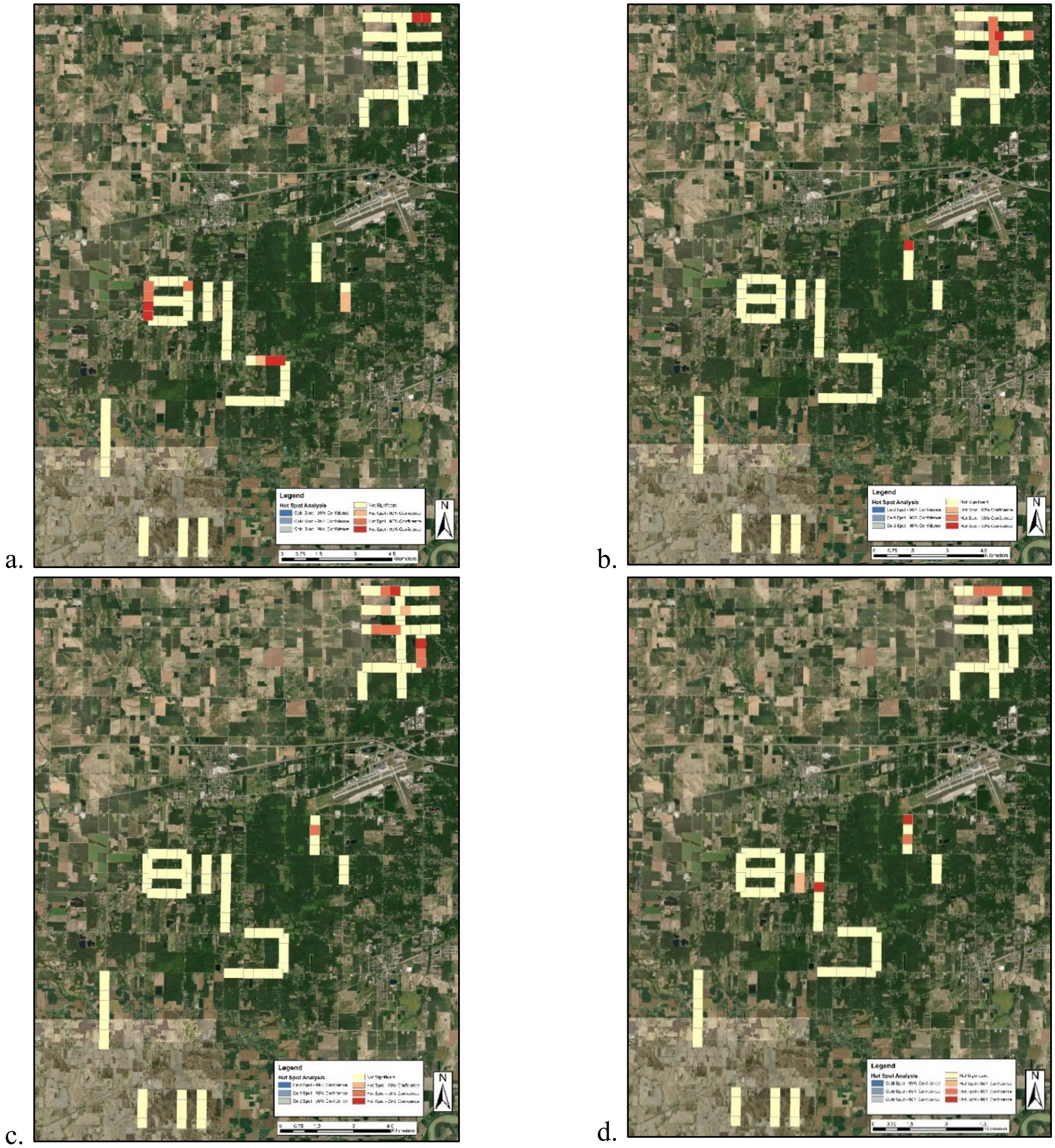


Figure 3.6. Getis-Ord Gi* hotspot analysis for vertebrate mortality across the study area by taxon combined for all years for mammals (a), reptiles (b), amphibians (c), and birds (d). Warmer colors represent hotspots, or areas that had a clustering of high mortalities, where cooler colors represent coldspots, or areas that were less likely than average to have mortality clusters. The yellow represents no significant spatial clustering in mortality found.

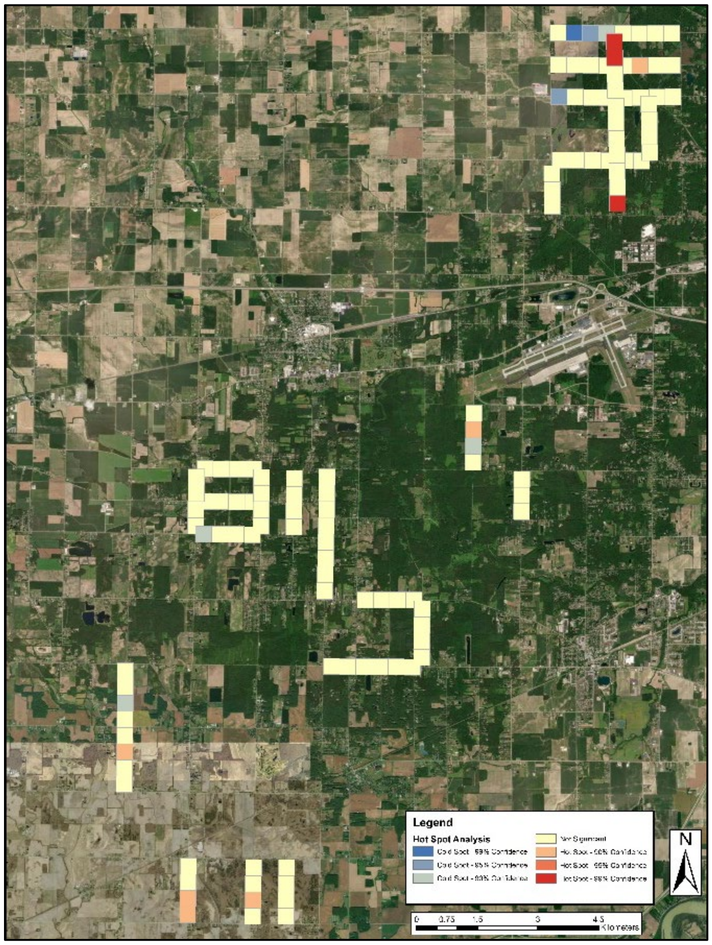


Figure 3.7. Getis-Ord Gi* hotspot analysis for bat acoustic monitoring data combined for 2021 and 2022. Warmer colors represent hotspots, or areas that had a clustering of bat calls, where cooler colors represent coldspots, or areas that were less likely than average to have bat calls clusters. The yellow represents no significant spatial clustering in mortality found.

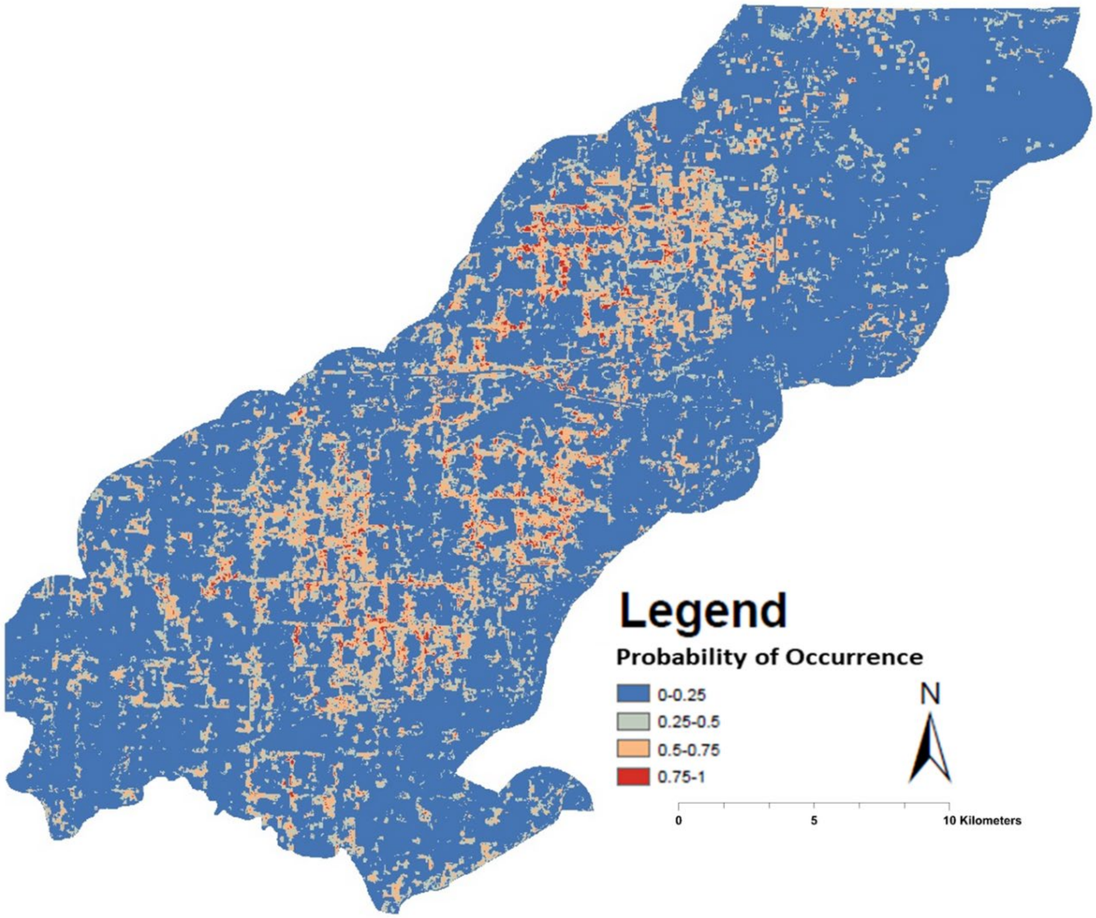


Figure 4.1. Continuous Maxent model results mapped as the probability of vertebrate mortality occurrence in the Oak Openings Region study area of Northwest Ohio. Each color shows the probability of mortality occurrences from low probabilities (in blue) to high probabilities (in red), ranging from 0 to 1.

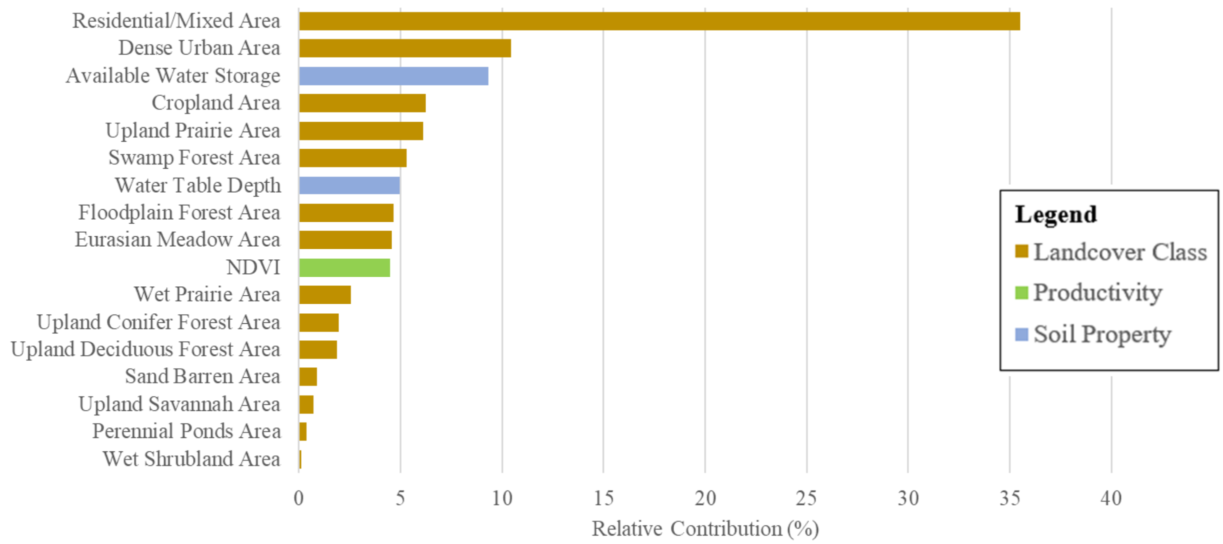


Figure 4.2. Estimates of the relative percent contribution of the continuous predictor variables to the best Maxent model for the probability of vertebrate mortality occurrence in the Oak Openings Region study area. Variables are classified by type including landcover class (brown), productivity (green), and soil property (blue).

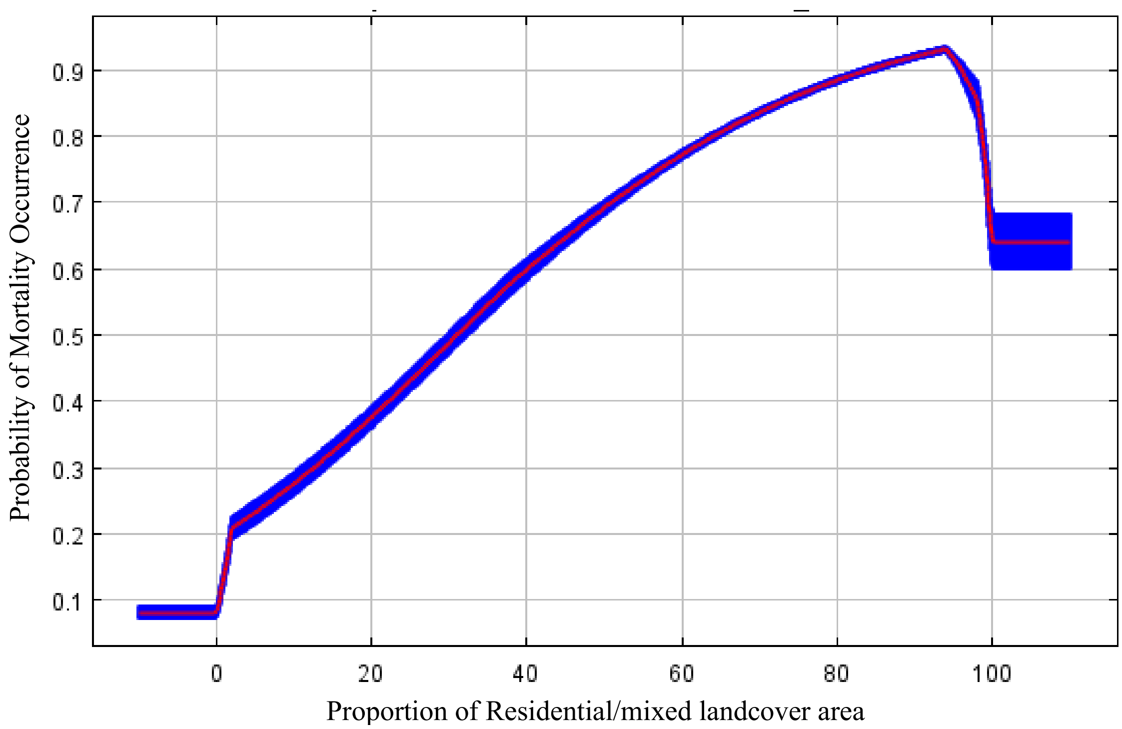


Figure 4.3. Response curve of the predicted probability of vertebrate mortality occurrence in the Oak Openings Region study area to the proportion of residential/mixed landcover area. The x-axis represents the percentage of a residential/mixed land cover type from 0% to 100%. The y-axis represents the probability of mortality occurrence. The curve shows the mean response of the 10 replicate Maxent runs (red) and the mean +/- one standard deviation (blue).

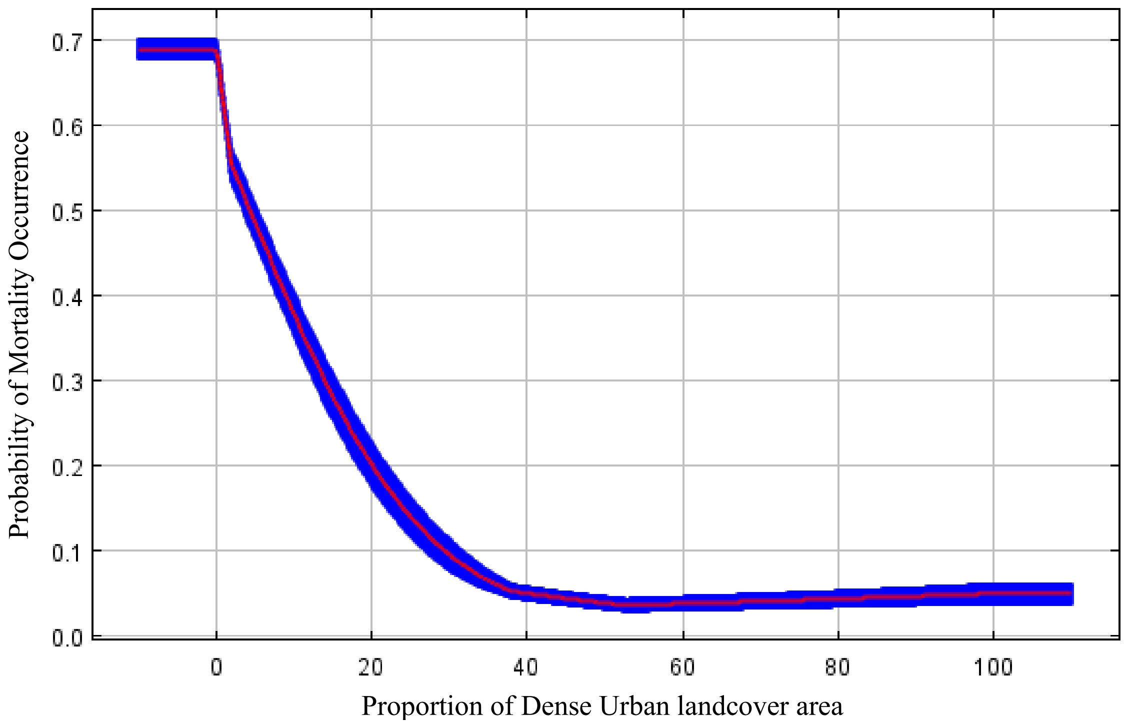


Figure 4.4. Response curve of the predicted probability of vertebrate mortality occurrence in the Oak Openings Region study area to the proportion of dense urban landcover area. The x-axis represents values for the percentage of a residential/mixed land cover type from 0% to 100%. The y-axis represents the probability of mortality occurrence. The curve shows the mean response of the 10 replicate Maxent runs (red) and the mean +/- one standard deviation (blue).

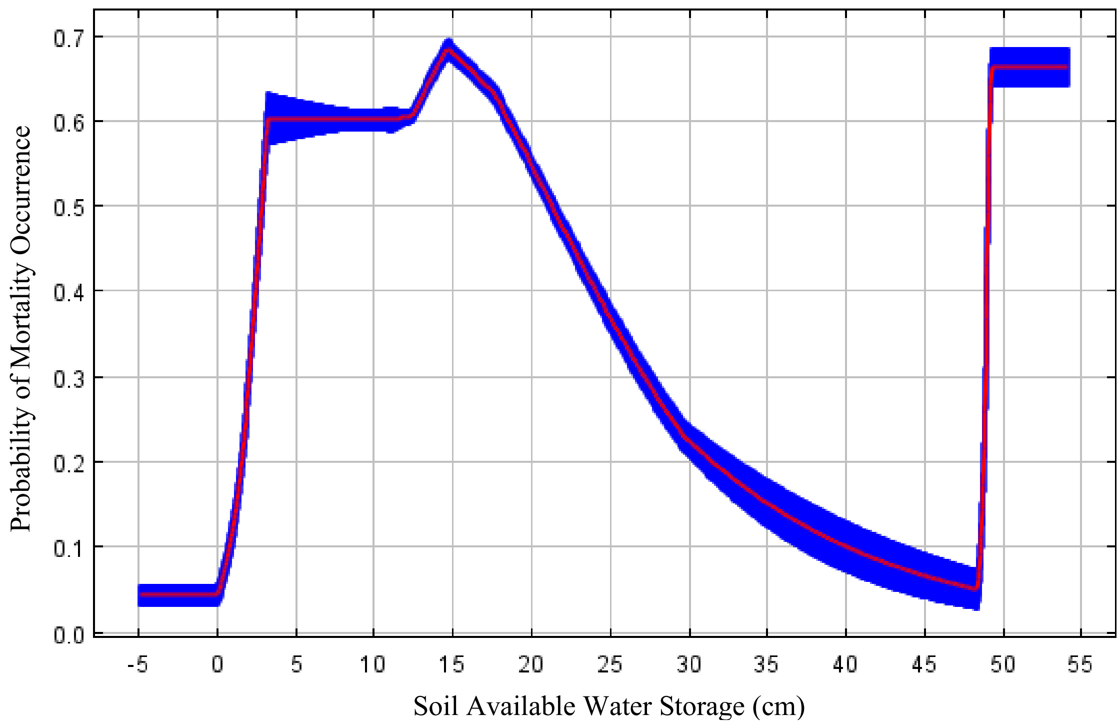


Figure 4.5. Response curve of the predicted probability of vertebrate mortality in the Oak Openings Region study area to the available water storage. The x-axis represents values for the available water storage in centimeters from 0-50. The y-axis represents the probability of mortality occurrence. The curve shows the mean response of the 10 replicate Maxent runs (red) and the mean +/- one standard deviation (blue).

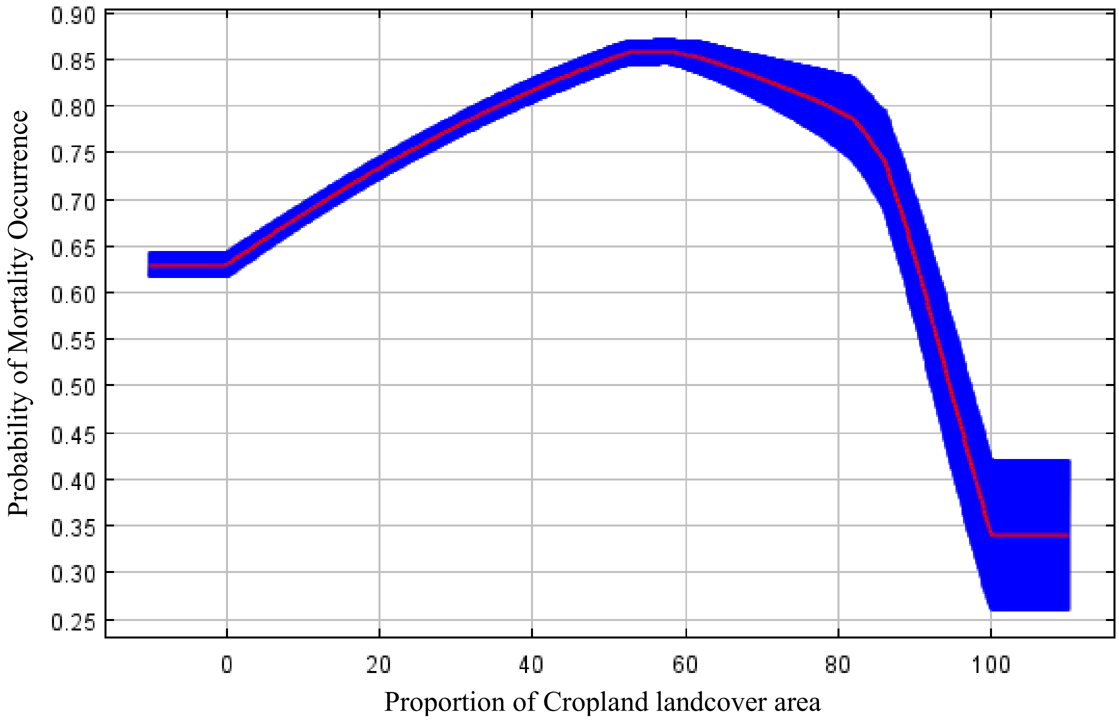


Figure 4.6. Response curve of the predicted probability of vertebrate mortality occurrence in the Oak Openings Region study area to the proportion of cropland landcover area. The x-axis represents values for the percentage of a residential/mixed land cover type from 0% to 100%. The y-axis represents the probability of mortality occurrence. The curve shows the mean response of the 10 replicate Maxent runs (red) and the mean +/- one standard deviation (blue).

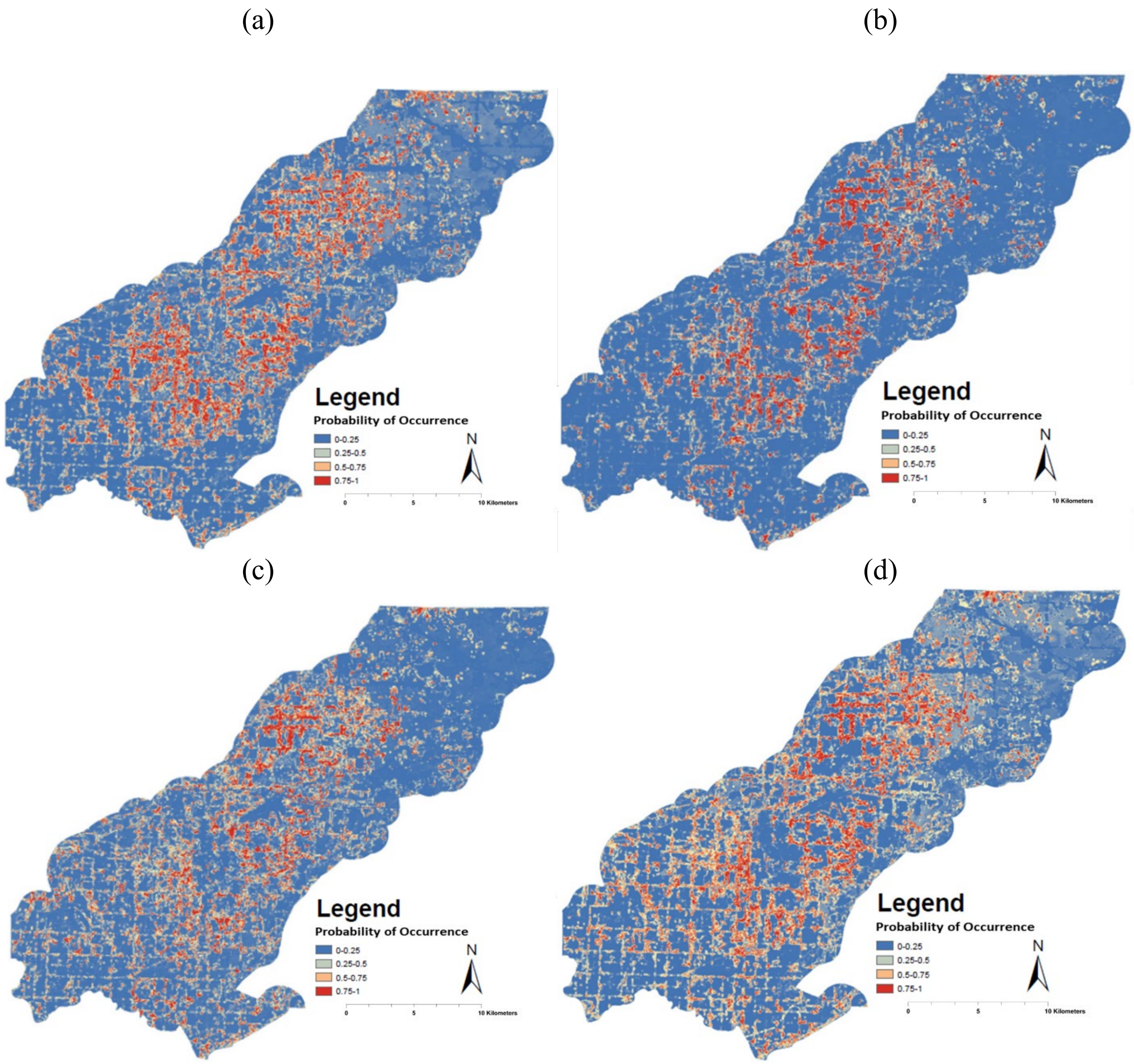


Figure 4.7. Continuous Maxent model results mapped as the probability of mortality occurrence in the Oak Openings Region study area for mammals (a), reptiles(b), amphibians(c), and birds (d). Each color shows the probability of mortality occurrences from low probabilities (in blue) to high probabilities (in red), ranging from zero to one.

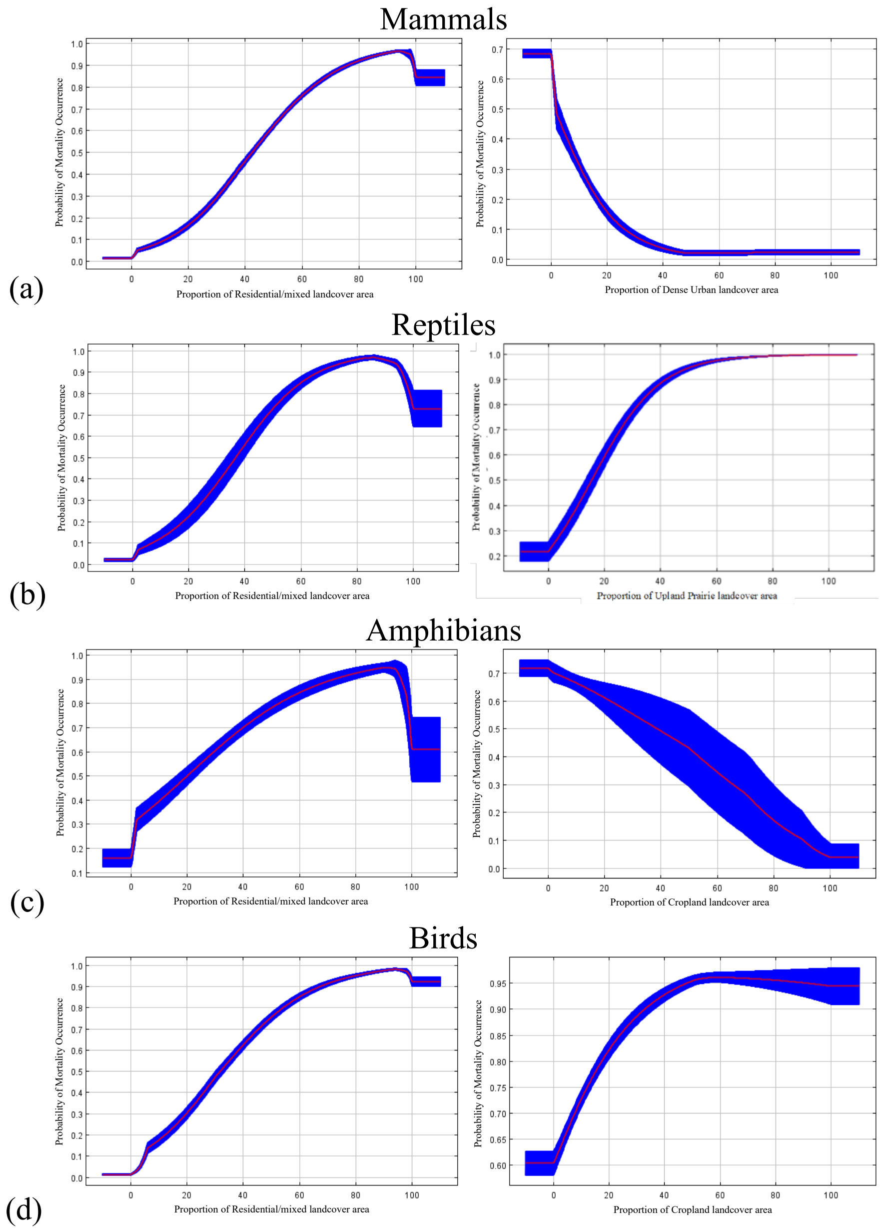


Figure 4.8. Response curves for the top two influential variables for mortality occurrence probability in the Oak Openings Region study area for mammals (a), reptiles (b), amphibians (c), and birds (d). The x-axis represents values for the percentage of a given land cover type from 0% to 100%. The y-axis represents the probability of mortality occurrence. The curve shows the mean response of the 10 replicate Maxent runs (red) and the mean +/- one standard deviation (blue).

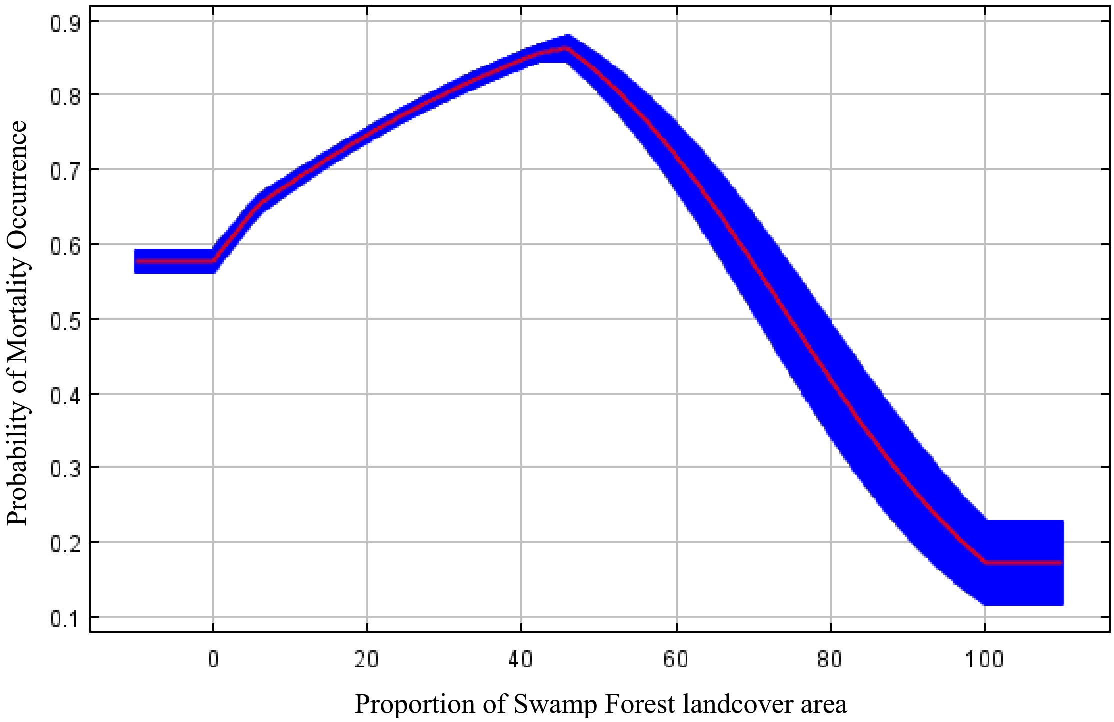
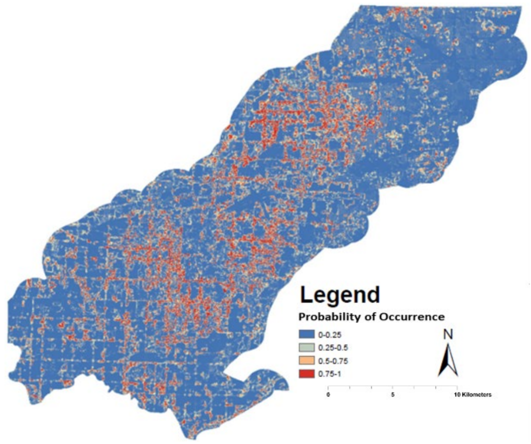
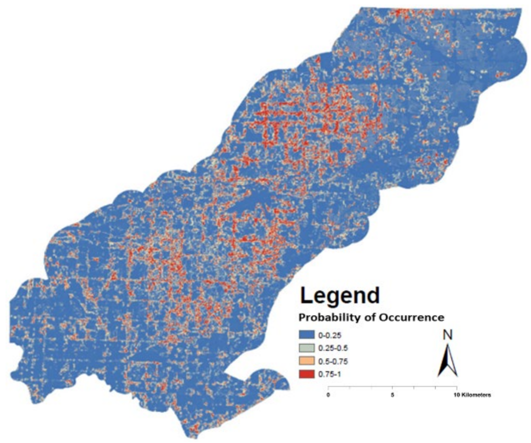


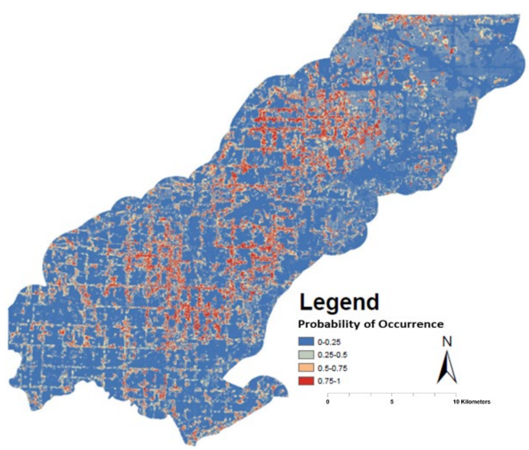
Figure 4.9. Response curve of the predicted probability of vertebrate mortality occurrence in the Oak Openings Region study area to the proportion of swamp forest landcover area. The x-axis represents values for the percentage of a residential/mixed land cover type from 0% to 100%. The y-axis represents the probability of mortality occurrence. The curve shows the mean response of the 10 replicate Maxent runs (red) and the mean +/- one standard deviation (blue).



(a)



(b)



(c)

Figure 4.10. Continuous Maxent model results mapped as the probability of vertebrate mortality occurrence in the Oak Openings Region study area for 2020 (a), 2021 (b), and 2022 (c). Each color shows the probability of mortality occurrences from low probabilities (in blue) to high probabilities (in red), ranging from zero to one.