MONITORING AND PREDICTING TRAFFIC INDUCED VERTEBRATE
MORTALITY NEAR WETLANDS

Professor J. Andrew DeWoody

and

Jamie M. Nogle

(Graduate Research Assistant)

and

Melissa Hoover

(Undergraduate Research Assistant)

and

Professor Barny Dunning

Department of Forestry & Natural Resources

Purdue University

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ABSTRACT

Animal-vehicle collisions are undesirable to the general public, to drivers, to insurance providers, to biologists, and presumably to the animals themselves. However, traffic-induced mortality (“roadkill”) is difficult to mitigate in large part because scientists lack the empirical data required to understand the patterns and processes associated with roadkill. Roadkill is not randomly distributed in space or in time, but what are the primary determinants of roadkill? And do they differ across organismal groups? We monitored vertebrate roadkill at 6 wetland and 6 upland sites in Indiana twice a week for a period of 20 months to determine whether roadkill occurs predominantly near one habitat compared to the other. We documented 14,439 vertebrate carcasses that were mostly distributed near wetlands. A significant fraction of the roadkill was not identifiable based on morphology alone, so we used DNA barcoding as a key element of species assignment. A large proportion of the carcasses (88%) were amphibians, a taxonomic group that has declined precipitously in recent years. Overall, these roadkill data were used along with road and habitat characteristics to develop analytical models that, in the absence of field monitoring, should be useful for predicting sites where roadkill is expected to be substantial. In the case of future highway construction, our models have the potential to help reduce the overall levels of roadkill, and that has the added benefit of translating into fewer human injuries and monetary losses caused by human-wildlife collisions.

Keywords: roadkill, amphibians, DNA barcoding, predictive model, human-wildlife collisions

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TECHNICAL SUMMARY

Introduction

Traffic-induced mortality (“roadkill”) clearly has negative effects on wildlife, where it may be the highest source of mortality of some species. However, roadkill also negatively affects drivers who may suffer from the direct effects from animal impact or from indirect effects (such as loss of vehicle control) due to attempted avoidance of wildlife. Direct effects with large animals such as moose are most obvious, but indirect effects of roadkill can also be severe. For example, some authors have noted that “road surfaces become dangerously slick at certain localities due to accumulated animal remains during peak periods of road crossing by frogs” (Langen et al. 2007). Two points related to this quote are worth considering. First, dangerously slick roads are a threat to human safety, but may also be a threat to wildlife population viability if mortality exceeds recruitment. Second, *where* are the “certain localities” that become dangerously slick and *when* is roadkill most problematic?

The primary objectives of this study were 1) to quantify traffic-induced vertebrate mortality across species, habitats, seasons, and years; and 2) to develop analytical approaches whereby northern Indiana roadkill can be predicted in the absence of field monitoring. To address these objectives, we surveyed 12 study sites evenly split between wetland and upland sites. On the basis of Ashley and Robinson (1996) and Glista et al. (2006), we expected to find more roadkill at the wetland sites versus upland sites, especially with regard to amphibian and reptile (“herp”) mortality. We also predicted that reptile and amphibian mortality would be elevated during the warmer, wetter months based on the fact that most herps are active and breeding during these months and therefore are more likely to encounter roadways

during these movements (Forman and Sperling 2003). In contrast, we predicted that avian and mammalian roadkill would be more uniform across both sites and seasons.

We utilized genetic barcoding to identify unknown carcasses encountered during surveys. Previous studies have been performed to test whether DNA can be successfully extracted from roadkilled carcasses (Doyon et al. 2003), but to our knowledge DNA barcoding has never been used to characterize species composition of unknown roadkill samples although this could be important. For example, Glista et al. (2008) encountered 9,809 amphibian mortalities with 7,602 (77.5%) being listed as “unknown ranids”. By virtue of DNA barcoding, we subsampled these unidentifiable carcasses and assigned them to the appropriate taxonomic category using gene sequences.

The final portion of this project was the development of a predictive GIS model based on the empirical roadkill data we collected. The motivation behind this model was to provide a mechanism to predict roadkill hotspots in the absence of monitoring, which would aid in the identification of areas where traffic-induced vertebrate mortality should be high. Several statistical models, including binary logistic regression and a negative binomial linear model, were implemented and compared to maximize model efficiency (Gomes et al. 2009).

Findings

Overall, the major findings of this study were that 1) significantly more roadkill occurs near wetlands versus uplands; 2) the occurrence of amphibian roadkill dwarfs that of every other vertebrate group; 3) roadkill levels varies annually and seasonally (it is highest in the summer months); 4) the identification of roadkill carcasses is difficult and can be greatly assisted by DNA barcoding; and 5) specific habitat variables are valuable with regard to predicting where roadkill hotspots will occur.

Implementation

David Glista and Joshua Mott (INDOT Environmental Section) will implement this research in their future evaluations of environmental factors that influence new road placement and roadkill mitigation on existing highways. The findings reported within this document will also be published in the primary scientific literature, and thus we hope that others may also implement our findings.

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<p><i>Summary of Implementation Plan: This project provides raw data on vehicle-induced mortality of different species in different habitats at different points in time. These data are directly comparable to those of an early JTRP project and collectively, this report provides the data and the statistical models necessary to help INDOT personnel predict the occurrence of roadkill hotspots in northern Indiana. This should lead to implementation during construction of new roadways as well as provide opportunities for mitigation during roadway maintenance/construction.</i></p>	
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INTRODUCTION

Collisions with automotive traffic can be a major source of mortality in animal populations (Romin & Bissonette 1996; Trombulak & Frissell 2000; Gibbs & Shriver 2002). This is particularly troubling when the species impacted are of conservation concern. Animals are killed by traffic for a number of reasons, including the simple dispersal of juveniles for inbreeding avoidance (foxes), expansive home range size (bobcats), immigration to breeding ponds (salamanders), or juvenile emigration from breeding ponds (frogs). Roadkill is a significant source of mortality in many vertebrate populations; in extreme cases, roadkill is the primary cause of mortality (Fitzgibbon 2001; NCHRP 2002; Marchand & Litvaitis 2004). For example, roadkill mortality is especially important in carnivores that have no natural predators (Riley et al. 2006, Seiler 2005). In those cases, disease and senescence are the only other sources of mortality and they can be exceeded by roadkill. Roadkill can also be a major concern for many threatened or endangered species (e.g., the American badger in Indiana). If scientists and engineers can devise reasonable methods to minimize roadkill, then we have an environmental and ethical obligation to do so.

As if the biological/environmental motivation to reduce roadkill were insufficient, there are safety problems on the human side. For example, drivers and passengers may be injured or killed during impact with large ungulates like deer, when they lose control of their vehicle while trying to avoid mid-sized animals like coyotes, or when they lose control of their vehicle because of roadkill (e.g., “slicks” caused by thousands of dead frogs). Dangerously slick roads usually result from amphibian roadkill, including frogs, toads, salamanders, and newts (e.g., Ashley and Robinson 1996). Because the amphibian life cycle is tied to the water required for breeding, it seems obvious that amphibian roadkill might be elevated near standing water or wetlands.

Serendipitous findings support this contention (Ashley and Robinson 1996; Glista et al. 2008), but to our knowledge there has been no systematic study that quantified roadkill adjacent to wet versus dry habitat. A comparative study of uplands and wetlands could reveal differences that help with better transportation planning decisions as well as wildlife mitigation.

Wetlands are a valuable asset to both humans and wildlife. They serve as hydrology modifiers, contaminant sinks, wildlife habitat, and areas of recreation/aesthetic value (Forman et al. 2003). Wetlands only constitute 5% of the total United States land base, but ~30% of all rare and endangered species utilize wetland habitat. Wetlands possess the highest abundance and diversity of animals per surface area unit versus any other habitat type (Forman et al. 2003). The more we know about these areas and how neighboring roads impact them, the better chance we have of protecting them, their inhabitants, and the drivers who traverse them (Semlitsch & Bodie 1998).

As humans expanded across North America, they created simple dirt roads. Over time, these roads became more advanced and extensive to provide access to natural resources and neighboring communities. Today there are approximately 6.3 million km (3.9 million miles) of public roads in the United States (Forman et al. 2003). Roads and other infrastructure have greatly reduced the habitat available to many wildlife species (Vos and Chardon 1998). Roads impose obvious, direct effects on wildlife populations (e.g. habitat loss and vehicular mortality), but they also have indirect impacts such as habitat degradation, soil erosion, and barrier effects (Sherwood et al. 2002).

Many studies have shown that roads can disrupt animal movement patterns (Dodd & Cade 1998, Forman et al. 2003, Langevelde et al. 2007). This is especially damaging to species that utilize wetlands because as Forman (2003) stated "...land-use patterns around wetlands and the

manner in which roads are situated within landscapes may be just as important as the actual size of wetlands.” In addition to the obvious barrier effects caused by roads, there are also problems associated with the road effect zone. The road effect zone is defined as the area that receives significant ecological effects from the roadway. Although roads only account for approximately 1% of the total land base of the United States (Forman 2000), the ecological impacts from these roads are estimated to affect 20% of the land (Forman and Deblinger 2000). With less habitat available and restricted movement patterns, there is an increase in the occurrence of human-wildlife interactions. When these interactions occur on the roadway, the result is often detrimental to both human and animal.

Human-Wildlife Collisions

Road ecology has focused primarily on charismatic megafauna, such as deer, bear, wolves and elk (Thurber et al. 1994, McClellan and Shackleton 1988). This is partly because these species generate the most damage to humans during collisions via cost and injuries. More than one million deer-vehicle collisions occur annually in the United States, causing \$1.1 billion in damages and 29,000 human injuries (Conover 1995). However, large animals are not the only groups of concern. Smaller animals such as reptiles and amphibians (“herps”) are significantly impacted by vehicular mortality and can cause injury to humans who try to avoid impact or lose control of their vehicle while driving over a “slick” caused by thousands of dead amphibians (Ashley and Robinson 1996). In addition to the risk of human injury and monetary cost of human-wildlife collisions, some herp populations are experiencing severe negative effects from the threat of roadkill which include additive mortality (Roe et al. 2006), changes in genetic structure (Reh & Seitz 1990), and altered growth rates in long-lived species such as turtles (Dodd and Dreslik 2008).

Roadkill & Herps

Numerous studies have shown that amphibian populations are declining worldwide, in dramatic fashion (Blaustein et al. 1994, Kuzman 1994, Wyman 1990). The major contributors to amphibian decline include: increased ultraviolet radiation, predation by invasive fish species, habitat modification/loss, environmental toxicants, diseases, and climate change (Table 1). Of these, habitat loss and modification are commonly identified as the greatest contributors to amphibian declines (Blaustein et al. 1994, Langevelde et al. 2009).

Roads are a well-documented cause of habitat loss/modification and have been shown to negatively impact wildlife populations (Gibbs & Shriver 2005, Hels and Buchwald 2001, Steen and Gibbs 2004). Gibbs and Shriver (2002) found that roads are a significant contributor to habitat fragmentation for turtles and could be contributing to their regional declines and skewed sex ratios. For example, female turtles are more vulnerable to road mortality because they commonly move throughout the landscape during migrations to nesting areas, whereas males traverse fewer roads as they never visit the nesting area (Steen and Gibbs 2004). Sillero (2008) found the same trend with female anurans in Spain who are more vagile than males during the breeding season. Beyond the impacts on one sex of a single species, roads can impact entire ecological communities. Findlay and Houlihan (1997) found that species richness of herps, birds and plants was negatively correlated with road density near wetlands. Furthermore, Trombulak and Frissell (2000) discovered that the simple presence of roads decreased native species composition (and native population sizes) while increasing the presence of invasive species and interrupted hydrologic processes by fragmenting the ecosystem. Also, roads can serve as movement corridors for invasive species such as the cane toad, *Anaxyrus marinus* (Seabrook and Dettman 1996).

When roads bisect or run adjacent to prime habitat, such as wetlands, the results are often dramatic. During a 16-month study, Glista et al. (2008) documented 10,515 road mortalities along 12 kilometers of roadway in northern Indiana. Amphibians and reptiles constituted 95% of the total mortalities, and most occurred where Lindberg Road bisects Celery Bog in Tippecanoe County, Indiana. Ashley and Robinson (1996) found similar results when they encountered 32,000 dead animals over 4 years on a 3.6 km section of road that bisects the Big Creek wetland in Ontario. Almost 94% of the recorded mortalities were amphibians, mostly dispersing juvenile Northern leopard frogs (*Lithobates pipiens*). Carr and Fahrig (2001) found that more vagile species, such as the Northern leopard frog, are more vulnerable to vehicular mortality versus less vagile species like the Green frog (*Lithobates clamitans*). This particular example is especially problematic in Indiana, where the Northern leopard frog is formally listed by the state as a species of special conservation concern. Northern leopard frog populations have declined precipitously in the Midwest, perhaps due in part to roadkill but also due to the loss of wetland habitat, environmental toxicants, and their frequent use as dissection specimens (University of Wisconsin Sea Grant Institute 2001).

Most amphibian studies are conducted at the breeding sites and therefore little is known about their movement patterns (Alford and Richards 1999). Extensive monitoring and analysis of roadkill near wetlands should help scientists better predict what conditions and habitat variables result in elevated amphibian mortality and potentially decrease the impacts of traffic-induced vertebrate mortality on local wildlife populations (Trimm et al. 2007, Paton et al. 2000, Paton and Crouch 2002).

PROBLEM STATEMENT

Roadkill is an obvious problem in that it is detrimental to both humans and animals; less obvious is where and when roadkill is most likely to occur. Animals are not randomly dispersed across the landscape, as different species prefer different habitats at different times of the year (Andrews et al. 2008, Beaudry 2008, Pope et al. 2000). This fact alone means that roadkill across sites should not fit a uniform distribution, but an added complexity is that some species are highly mobile whereas others are relatively sedentary. *The problem is the paucity of roadkill data on most vertebrate species and how mortality rates differ across habitats, seasons, and years.*

Recent research funded by JTRP surveyed 13 stretches of Indiana roadways for vertebrate roadkill. Those data (>11,000 mortality events) suggest that roadkill might be concentrated near wetlands (Glista et al. 2008). If verified, this could have environmental implications for the design, routing, and maintenance of future highways. In the Glista et al. (2008) study, only 1 of the 13 study sites bisected a wetland, but roadkill at that site was extensive (>8000 mortalities). Another of the 13 study sites was adjacent to a wetland, and roadkill was also high there (>1700 mortalities). The remaining 11 study sites, none located near wetlands, accounted for fewer than 1350 mortalities. Clearly, systematic studies of roadkill in wetland and upland habitat are necessary to validate this trend.

STUDY OBJECTIVES

The primary objectives of this study were 1) to quantify traffic-induced vertebrate mortality across species, habitats, seasons, and years; and 2) to develop analytical approaches whereby roadkill can be predicted in the absence of field monitoring. Previous studies have found high numbers of mortality near wetlands (Ashley and Robinson 1996, Steen and Gibbs 2004), but these studies focused on wetland areas only. Based on these previous studies and on Glista et al.

(2008), we expected to find more roadkill at the wetland sites versus upland sites. In particular, amphibian roadkill was expected to be dramatically higher due to their annual immigrations to wetlands (to breed) and annual emigrations from wetlands (juvenile dispersal). We also predicted that herp mortality would be elevated during the warmer, wetter months based on the fact that most species are active and breeding during these months and therefore are more likely to encounter roadways during these movements (Forman and Sperling 2003; Glista et al. 2008). In contrast to herps, avian and mammalian roadkill was expected to be more uniform across the year and across sites.

METHODS

Survey Routes

One approach to determining if roadkill is normally elevated near wetlands is to choose a series of routes that are adjacent to (or bisect) wetlands and intensively monitor them. Those data could then be compared to routes of the same length that are similar in many respects (e.g., the degree of “urbanization”) but are upland in nature. To this end, we evaluated potential study sites using GIS data, topographic maps and information gathered from research scientists at Purdue University and the Indiana Department of Transportation. Site selection was limited to state and federal roads that were close enough in proximity that each could be surveyed twice per week. Surveyor safety was another major consideration, and we visited all possible sites for personnel safety evaluations (e.g. access to shoulders, visibility, etc) prior to final site selection. Ultimately, a total of six wetland and six upland sites were selected (Figure 1). A total of 12 sites were chosen to provide replication while considering the logistical constraints imposed by a single field vehicle and a small survey crew. In order to provide a spatial scale for future

examination of habitat variables, all survey routes were divided into 100 meter sections and are approximately one kilometer in length (Table 2). To represent wetland and upland habitat equitably, the total length of each habitat treatment type was equal (wetlands: 6.5 km; uplands: 6.5 km).

Roadkill Sampling Protocol

In order to monitor vertebrate mortalities extensively, we surveyed all 12 study sites biweekly on a continuous basis from 11 February 2008 until 2 October 2009. We surveyed six sites on Monday and Thursday with the other 6 sites being surveyed on Tuesday and Friday. In order to increase carcass detection rates, we drove routes at slow speeds (<25 kilometers/hour) (Langevelde 2009). We identified all vertebrate carcasses located on the roadway and shoulders to species and sex whenever possible, and the following data were recorded for all carcasses: date, site name, section number, and location in the roadway (e.g. northbound lane, southbound lane, median, etc). Any unknown carcasses were identified to the highest taxonomic category possible (e.g., “mammal” or “ranid”). Approximately ~3% of the unknown specimens (including a minimum of one sample per taxonomic group) were analyzed in the lab and identified via molecular techniques in an effort to more precisely assign them to a species.

Genetic Analysis

One problem with evaluating roadkill is that it is not easy to accurately identify roadkilled animals to species—even for experienced biologists. DNA barcoding has considerable utility in this regard. DNA barcoding utilizes short, standardized gene sequences (i.e. CO1 or 16S rDNA) to identify individuals to species (Hajibabaei et al. 2007). For this study we utilized genetic barcoding to classify unidentifiable specimens encountered during surveys. By positively

identifying a proportion of unknown carcasses, we more firmly established the species composition at each site.

Previous studies have been performed to test whether DNA can be successfully extracted from roadkilled carcasses (Doyon et al. 2003), but to our knowledge DNA barcoding has never been used to characterize species composition of unknown roadkill samples. For example, Glista et al. (2008) encountered 9,809 amphibian mortalities with 7,602 (77.5%) listed as “unknown ranids”. By virtue of DNA barcoding, we subsampled these unidentifiable carcasses and assigned them to taxonomic groups (e.g. species) using gene sequences.

Tissue was collected from several hundred unknown carcasses, and a standard phenol-chloroform extraction was used to isolate DNA from each sample (Sambrook and Russell 2001). The primer pair HCO1-2198 (5'TAAACTTCAGGGTGACCAAAAAATCA-3') and LCO1-1498 (5'GGTCAACAAATCATAAAGATATTGG-3') was selected to amplify a 658 base pair segment of the mitochondrial cytochrome *c* oxidase I (CO1) gene (Herbert et al. 2002). Each polymerase chain reaction (PCR) consisted of 20 μ L and contained 2 μ L of 10x buffer (100mM Tris-HCl, pH 8.9, 500 mM KCl, and 0.5 mg/mL BSA), 2.0 mM of MgCl₂, 1.0 U of Taq DNA polymerase, 0.2 mM dNTPs, 9.7 μ L of distilled water, 0.3 μ M each primer, and 2 μ L of DNA template. The thermal profile included an initial denaturation step of 94°C for 1 min, followed by five cycles of 1 min at 94°C, 1.5 min of 45°C, and 1.5 min of 72°C, followed by 35 cycles of 1 min at 94°C, 1.5 min at 52°C, and 1 min at 72°C and a final extension step of 5 min at 72°C. Negative and positive control PCRs were used during every run to monitor for contamination and PCR performance. All PCR products were cleaned using a standard sodium acetate-ethanol precipitation and were sequenced bi-directionally using the same primers that were used during

the PCR amplification. Sequencing reactions were performed using the BigDye version 3.1 (Applied Biosystems) following the manufacturer's recommended protocol.

In order to identify specimens to the species-level, DNA sequences from unknown carcasses were compared to "positive controls". The positive control sequences were generated from carcasses that were readily identified in the field based on morphology. Nevertheless, tissue was collected from them and they were DNA barcoded to provide a set of standards by which unknown samples could be identified. In addition to using sequences from these positive control carcasses, we also utilized sequences previously published in the GenBank repository (<http://www.ncbi.nlm.nih.gov>). We utilized Basic Local Alignment Search Tool (BLAST) for all sequences. A BLAST search involves using a specified algorithm to compare nucleotide sequences from a given sample to an extensive library of published sequences (Altschul et al. 1990). This method allows researchers to match an empirical sample to a given species (or its nearest relative) represented in the library.

Agricultural Practices & Mortality

In order to better understand the potential impacts of agriculture (i.e. crop type and harvest) on vertebrate road mortality, we examined pre- and post-harvest roadkill data at five of our upland sites (Table 5). We predicted that 1) roadkill would increase post-harvest for seed-eating and/or migrating bird species; 2) that roadkill will decrease post-harvest for mammals; and 3) that the incidence of roadkill would be dependent on individual crop type (i.e. corn versus soybean). We predicted that mortality would increase for birds because of the increased food availability released during harvest. However, harvesting limits the amount of ground cover available and thus we hypothesized this would directly decrease mammalian movements in the

cleared fields due to an increase risk of predation; this should result in lower mortalities on the adjacent roadways.

Habitat Analysis

To determine what habitat variables covaried with mortality, we used an integrative approach with ArcGIS 9.3 to examine mortality data and appropriate GIS layers, such as roads and land cover to locate mortality “hotspots” (Clevenger et al. 2003, Ramp et al. 2005). We also documented the position of all culverts and any pre-existing mitigation structures within the survey route.

Aerial photographs were downloaded for the entire study area from the Indiana Spatial Data Service (<http://www.indiana.edu/~gisdata/>) and were used to digitize the habitat type immediately adjacent to the survey routes and create appropriate buffers for analysis. In order to ensure that our study would be comparable to Glista et al. (2008), we used similar GIS methods and used the same 8 land cover classes to classify and digitize habitat adjacent to all 12 survey routes. Land cover classes were roads, grass/shrub ditches (ditches), agriculture/pasture (ag), forest/woodlot (forest), urban/recreational grasses (urban grasses), urban/residential (residential), water/wetlands (water), and prairie/grassland (grassland). We implemented a 100-meter buffer, the industry standard for roadside management, around each survey transect (Figures 4-15). We used the Editor Tool bar to manually digitize the land cover classes within the 100-meter buffer. Once all survey routes were digitized, we used the CalculateArea tool in ArcGIS to determine the proportion of all land cover classes at each survey route (see Appendix 1). This information was used to determine the dominant land cover for each 100-meter section and the area of each land cover class was used to build the predictive models.

In addition to habitat, we also investigated several intrinsic factors (sex, life history stage, taxa, and ecology), extrinsic factors (mean monthly temperature and precipitation), and anthropogenic factors (traffic volume) with regard to their influence on roadkill (Shepard et al. 2008). We downloaded temperature and precipitation data from the Indiana State Climate Office (<http://climate.agry.purdue.edu/climate/index.asp>) and traffic volume data from the Indiana Department of Transportation traffic count database (<http://dotmaps.indot.in.gov/apps/trafficcounts/>). We then used several standard techniques to identify potential mortality hotspots based on the roadkill data collected during this study. Those include binary logistic regression and a sophisticated linear model which can be used to determine which factors have the greatest influence on roadkill. Subsequent predictive models were constructed from these findings (Gomes et al. 2009).

Predictive Roadkill Models

In order to determine what factors were most significant for predicting vertebrate roadkill, we generated statistical models. These included models for all taxonomic groups evaluated simultaneously as well as separate models for each taxonomic group. In order to account for the numerous normality violations commonly associated with roadkill data (e.g. non-uniformly distributed data), we utilized a linear model based on the negative binomial distribution. Our negative binomial linear model (NBL) is based on count data and provides the probability of roadkill as a function of a time (i.e., the roadkill rate). During model design we also accounted for the presence of repeated measures during the study by using a “PROC GLIMMIX” procedure in SAS software version 9.2 (SAS Institute Inc., 2008). This procedure allows the testing of measures repeated across time and allows for violations of the equal variance assumption which was expected for studying roadkill at wetland and non-wetland sites.

Additionally, we utilized binary logistic regression (BLR) for roadkill hotspot modeling (see Gomes et al. 2009). BLR models are based on a response variable, the presence/absence of vehicle-induced mortalities within each 100-meter survey section. Any section with 1 or more carcasses recording during each season was recorded as “present” whereas any section lacking a carcass during that same season was listed as “absent”. This model is different than the NBL model because it does not consider absolute numbers of mortalities, but simply the presence or absence of mortalities in a particular 100-meter section.

RESULTS

Roadkill Data

During this 20-month study we encountered 14,439 vertebrate mortalities along ~13 kilometers of roadway in northern Indiana (Table 3). Overall, we encountered 110 different vertebrate species: 13 amphibians, 10 reptiles, 59 birds, and 28 mammals. Amphibians represented ~88% of the total mortalities (12,641) followed by mammals (849), birds (523), and then reptiles (426). Amphibian mortality peaked from June through October (Figure 2a) whereas birds, mammals, and reptiles peaks were less obvious (Figure 2b). These quantitative results are quite similar to previous studies that have examined roadkill near wetlands (Glista et al. 2008, Ashley and Robinson 1996).

The most common species encountered during our roadkill surveys were American toad (*Anaxyrus americanus*, n = 3915), American bullfrog (*Lithobates catesbeianus*, n = 2,297), midland painted turtle (*Chrysemys picta*, n = 193), midland brown snake (*Storeria dekayi wrightorum*, n = 80), Virginia opossum (*Didelphis virginiana*, n = 133), prairie vole (*Microtus ochrogaster*, n = 120), House Sparrow (*Passer domesticus*, n = 71), and American Robin (*Turdus*

migratorius, n = 47). The survey route with the highest level of mortality per unit distance was Prophetstown (n = 3,051 mortalities; 97.2% amphibians, 1.3% reptiles, 0.75% mammals, 0.75% birds). At this survey site, Indiana SR 225 bisects a small wetland near Battleground (Figure 10).

Our dataset (Table 3) clearly indicates that traffic-induced vertebrate mortality is significantly elevated at wetland sites ($\chi^2=3506.24$; p -value <0.001 ; 1 df). When broken down by taxa, we see that 457 mammalian carcasses were identified at wetland sites compared to 392 at upland sites; this is a significant difference ($\chi^2=5.81$; $p=0.02$; 1 df). Similarly, there were 299 avian mortalities at wetland sites compared to 224 at upland sites; this is also a significant difference ($\chi^2=10.76$; $p<0.01$; 1 df). We documented 411 reptile carcasses at wetland sites versus only 15 at upland sites ($\chi^2=368.11$; $p<0.001$; 1 df). Finally, amphibian roadkill is also statistically greater at wetland sites; 9601 carcasses were documented at wetland sites versus 3034 at upland sites ($\chi^2=3417.79$; $p<0.0001$; 1 df).

Genetic Analysis

The purpose of utilizing genetic barcodes in this project was to accurately identify individuals killed on the roadway. A large proportion of the current dataset (31%; 4533 individuals) was labeled as “unknown” which indicates that we were unable to identify the individuals to the species level (Table 4 to 7). There were a total of 152 birds, 77 mammals, 14 reptiles, and 4290 amphibians labeled as “unknown”. We selected a subset of the unknown individuals (~3%) for genetic analysis.

By using positive controls (i.e. individuals that we positively identified to the species level), we validated the genetic barcoding methodology as a successful tool for identifying roadkill across all taxa. For example, the neighbor-joining tree shown in Figure 15 shows that the

positive controls (e.g. *Lithobates pipiens*) selected for this study match previously published sequences from the GenBank repository (*Lithobates pipiens* EF 525888). Further, “unknown” individuals that could not be assigned to a species normally were assigned to the correct taxonomic category; in other words, unknown individuals had a CO1 sequence more similar to other individuals from the same taxonomic group. This is exemplified by the unknown bird in Fig. 15 that matches another bird species, *Turdus migratorius* (AY666254), better than more distantly related groups such as mammals.

As expected when attempting to sequence DNA obtained from roadkill carcasses, we experienced a low sequencing success rate. We performed DNA extractions on 650 tissue samples and from those, generated only 200 DNA sequences (~30%). Many of the tissue samples failed to yield DNA, whereas others did (according to both spectrometry and agarose gel electrophoresis) but the PCR failed to amplify the CO1 gene. In general, we found that amphibian tissue seemed to degrade much more rapidly than mammalian or avian tissue but this may have been confounded by the small size of amphibian carcasses and the fact that most of them were collected during the hot months of summer.

Ultimately, we generated sequences for approximately 200 unknown roadkill carcasses which were 1) BLASTed against all published sequences in GenBank and 2) compared to our own reference samples collected during the study. Due to the conserved nature of the CO1 gene (and perhaps also due to the homogenous nature of some amphibian mtDNA regions; Bos et al. 2008), our barcoding approach did not provide sufficient resolution to consistently discriminate between closely related anuran species (e.g. *Lithobates catesbeianus* vs. *Lithobates clamitans* and *Anaxyrus americanus* vs. *Anaxyrus fowleri*). This is evident in the BLAST tables (Tables 10 to 13). Most samples BLASTed back to the suspected genus, but several samples (~10%)

yielded DNA sequences that best matched a congeneric species. These likely represent samples that were misidentified in the field.

Agricultural Practices & Mortality

Avian mortality was significantly different ($\chi^2=28.0$, 1 df, $p < 0.001$) pre-harvest ($n = 28$) versus post-harvest ($n = 0$). Likewise herpetofauna mortality was markedly different ($\chi^2=28.0$, 1 df, $p < 0.001$) between pre-harvest mortality ($n = 524$) and post-harvest mortality ($n = 2$).

Mammalian mortality also differed with regard to harvest, with 47 mortalities recorded during pre-harvest and 27 post-harvest ($\chi^2=5.405$, 1 df, $p\text{-value} = 0.0201$).

Weather

To determine if weather patterns were significant indicators of roadkill, we downloaded temperature and precipitation data from the Indiana State Climate Office (<http://climate.agry.purdue.edu/climate/index.asp>) and incorporated mean daily temperature and mean daily precipitation into the analyses. As expected in temperate climates such as Indiana, Figure 2c illustrates temperature peaks in July and August and extreme lows in December through February. This pattern corresponds very closely to roadkill numbers throughout both study years (highest from May through October). However, precipitation trends did not follow such an obvious bimodal pattern. In order to test the significance of daily mean temperature and daily mean precipitation on vertebrate road mortality, we incorporated both of these factors into the statistical models which will be discussed below.

Habitat Analysis

Our specific GIS habitat analysis considered the importance of various factors (e.g. land cover class immediately adjacent to the road) for identifying areas with potentially high levels of traffic-induced vertebrate mortality. Simply by examining the spatial distribution of roadkill

across survey routes (Figure 16 to 18), it is evident that certain factors could be important predictors of elevated vertebrate mortality. For example, we have recorded high levels of mortality in sections with water/wetland on both sides of the road (Figure 17) and when water/wetland is on one side of the road and forest/woodlot on the other (Figure 18). In order to better understand the importance of various habitat types, we examined road mortality by 100-meter sections within the 12 survey routes and manually digitized the land cover types (Figures 4 to 15). We classified the habitat on both sides of the road by its dominant land cover classification using a 100-meter buffer based on the road transect (see Appendix 1). After each section was assigned a dominant habitat type on each side of the roadway, this information was fed into the statistical models and used to predict roadkill patterns. Different habitat combinations proved to be more important in some models and not significant in other models and will be explained in further detail in the section below.

Predictive Roadkill Models

Overall models

First, we fitted a negative binomial distribution to a linear model (the NBL) and accounted for repeated measures to predict roadkill patterns when all four taxonomic groups were pooled. There were many land cover classifications that were significant predictors of overall roadkill. Based on the Akaike Information Criterion (AIC) model selection procedure, we determined that the total NBL model (which considers ground cover, average temperature, average precipitation, and traffic volume) was most appropriate because it had the smallest AIC value (Table 14). We used this NBL model to consider each taxonomic group independently (i.e., mammals, birds, reptiles, and amphibians) and then to consider all vertebrate roadkill simultaneously.

Significant ground cover predictors that occurred on opposite sides of the road are represented by a colon separating the two land cover classes (e.g., Ag:Forest means Ag on one side of the road and Forest on the other). The significant predictors include: Road:Residential ($p<0.0001$), Ag:Ag ($p<0.0001$), Ag:Forest ($p<0.0001$), Ag:Urban Grasses ($p<0.0001$), Ag:Water ($p<0.0001$), Ag:Grassland ($p<0.0001$), Forest:Forest ($p<0.0001$), Forest:Urban Grasses ($p<0.0001$), Forest:Residential ($p<0.0001$), Forest:Water ($p<0.0001$), Urban Grasses:Urban Grasses ($p<0.0001$), Urban Grasses:Residential ($p=0.002$), Grassland:Urban Grasses ($p=0.0104$), Water:Residential ($p<0.0001$), Water:Water ($p<0.0001$), and Water:Grassland ($p<0.0001$). In addition to the previous land cover variables, the best model also included average temperature ($p<0.0001$), average precipitation ($p<0.0001$), and traffic volume ($p<0.0001$) as significant predictors (Table 8). Due to the expected spatial correlation between road sections, we obtained covariance parameter estimates which indicated that the sections at each survey site were spatially correlated ($p<0.001$).

The best BLR model (according to AIC; Table 15) also identified ground cover, average temperature, average precipitation, and traffic volume as significant predictors of roadkill. Thus, this comprehensive BLR model was used to consider each taxonomic group independently (i.e., mammals, birds, and herps) and then to consider vertebrate roadkill simultaneously .

The BLR analysis revealed the following habitat classifications as significant for predicting roadkill : Ag:Ag ($p=0.0032$), Ag:Forest ($p=0.0005$), Ag:Water ($p=0.0218$), Ag:Grassland ($p=0.0034$), Forest:Forest ($p=0.0001$), Forest:Water ($p=0.0154$), Grassland:Forest ($p=0.0069$), Urban Grasses:Urban Grasses ($p=0.0336$), Grassland:Urban Grasses ($p=0.0055$), and Water:Water ($p=0.0002$). In addition to these variables the average temperature, average precipitation, and traffic were also significant predictors of roadkill ($p<0.0001$). Key factors that

were important in both the NBL and BLR models and included: Ag:Ag, Ag: Forest, Ag:Water, Ag:Grassland, Forest:Forest, Urban Grasses:Urban Grasses, Grassland:Urban Grasses, Water:Water, average temperature, average precipitation, and traffic volume. Thus, these are expected to be the best (i.e., most robust) predictors of roadkill.

Amphibian Models

Based on the NBL model the following ground cover factors were significant predictors of amphibian roadkill: Road:Residential ($p<0.0001$), Ag:Ag ($p<0.0001$), Ag:Forest ($p<0.0001$), Ag:Grassland ($p<0.0001$), Ag:Urban Grasses ($p<0.0001$), Ag:Water ($p<0.0001$), Forest:Forest ($p<0.0001$), Forest:Urban Grasses ($p=0.0003$), Forest:Residential ($p<0.0001$), Forest:Water ($p<0.0001$), Urban Grasses:Urban Grasses ($p<0.0001$), Urban Grasses:Residential ($p<0.0001$), Grassland:Urban Grasses ($p=0.0003$), and Water:Water ($p<0.0001$). In addition, average temperature, average precipitation, and traffic volume (all $p<0.0001$) were significant predictors of roadkill. The BLR model identified many of the same land cover combinations as significant, but there was not complete overlap between the BLR and NBL results; significant land cover classes identified by the BLR include: Road:Residential ($p=0.0143$), Ag:Ag ($p=0.0001$), Ag:Forest ($p<0.0001$), Ag:Urban grasses ($p=0.0199$), Ag:Water ($p=0.0255$), Ag:Grassland ($p<0.0001$), Forest:Forest ($p=0.0038$), Forest:Residential ($p=0.0387$), Forest:Water ($p=0.0002$), Grassland:Forest ($p<0.0001$), Urban Grasses:Urban Grasses ($p=0.0004$), Grassland:Urban Grasses ($p=0.0005$), and Water:Water ($p<0.0001$). In addition, average temperature and average precipitation (both $p<0.0001$) were significant predictors of roadkill.

Reptile Models

The best NBL model identified several ground cover combinations as significant predictors of reptile roadkill: Ag:Forest ($p=0.0274$), Ag:Water ($p<0.0001$), Forest:Forest ($p=0.0001$),

Forest:Urban Grasses ($p=0.0003$), Forest:Water ($p<0.0001$), Urban Grasses:Urban Grasses ($p=0.002$), Urban Grasses:Residential ($p=0.0260$), and Water:Water ($p<0.0001$). Similar to amphibians, average temperature, precipitation (both $p<0.0001$), and traffic volume ($p=0.0029$) were significant indicators of reptile roadkill. The best BLR model identified the following factors as significant predictors of reptile roadkill: Ag:Forest ($p=0.0451$), Ag:Urban Grasses ($p=0.0468$), Ag:Water ($p=0.0004$), Forest:Forest ($p=0.0013$), Forest:Urban Grasses ($p=0.0185$), Forest:Water ($p=0.0004$), Urban Grasses:Urban Grasses ($p=0.0002$), Water:Water ($p<0.0001$), average temperature, precipitation (both $p<0.0001$), and traffic volume ($p=0.0068$).

Bird Models

The best models identified very few factors that were significant predictors of avian road mortalities. The NBL model identified: Ag:Ag ($p=0.0233$), Water:Water ($p=0.0130$), average temperature ($p<0.0001$), and traffic ($p<0.0001$) as significant variables. The BLR model was unable to label any specific land cover classification as significant, although the model did show that ground cover was a significant predictor of bird mortality ($p=0.0104$) as well as average temperature ($p<0.0001$) and traffic ($p=0.0002$).

Mammal Models

The NBL model revealed 9 significant variables that predicted mammalian road mortalities: Ag:Forest ($p=0.0044$), Forest:Forest ($p<0.0001$), Forest:Urban Grasses ($p=0.0008$), Forest:Residential ($p=0.0277$), Forest:Water ($p<0.0001$), Water:Grassland ($p=0.0218$), average temperature ($p<0.001$), average precipitation ($p=0.0038$), and traffic volume ($p<0.0001$). The BLR model identified 5 such factors, each also significant in the NBL model: Ag:Forest ($p=0.0371$), Forest:Forest ($p<0.0001$), Forest:Water ($p=0.0050$), average temperature ($p<0.0001$), and traffic volume ($p<0.0001$).

DISCUSSION

Over the past two decades, a number of road mortality studies have been published in the scientific literature (Marsh et al. 2007, Sillero 2008, Steen & Gibbs 2004). Of these, many have focused specifically on large herbivores, carnivores and other charismatic species (Millions & Swanson 2007, Thurber et al. 1996, McClellan & Shackleton 1988). Others have focused on a single survey site (Coleman et al. 2008, Ashley & Robinson 1996). However, this study is (to our knowledge) the most comprehensive roadkill study yet undertaken. We did so to characterize more accurately spatial and temporal patterns of vertebrate roadkill in a fragmented agricultural landscape that is typical of much of the Midwestern United States.

Wetlands and Uplands

A review of the primary roadkill literature suggested that vertebrate mortality may be greatly elevated on roads adjacent to or bisecting wetlands (Ashley & Robinson 1996, Dodd et al. 2004, Glista et al. 2008). One primary aim of our study was to directly characterize roadkill at both upland and wetland sites in an effort to test this hypothesis. Our data are unequivocal; overall roadkill was statistically greater at wetland sites. Furthermore, roadkill near wetlands was higher for mammals, birds, reptiles, and amphibians—a consistent pattern across terrestrial vertebrates. These findings indicate that from a wildlife perspective alone (not to mention the additional engineering hurdles), new road construction near wetlands is undesirable. With regard to existing roads, it seems clear that mitigation methods (e.g., culverts, drift fences, etc) should be most effective if employed near wetlands.

Clearly, wetlands are hotspots for roadkill. Below, we discuss other habitat attributes associated with elevated roadkill in mammals, birds, and herps.

Mammals

Overall, the most important factor for predicting mammalian roadkill was the presence of the habitat variable forest/woodlot. In both the NBL and BLR models all significant land cover classifications contained forest/woodlot on one side of the roadway. In addition to forest/woodlot habitat, the average temperature and traffic volume were also significant predictors of mammalian mortality in both models; however average precipitation was only significant in the NBL model. Many mammal species rely heavily on forested habitat for shelter and food, and we see this pattern in mammal mortality based on their common association with these forested areas.

Birds

The NBL model identified two significant land cover classes associated with avian mortality: Ag:Ag ($p=0.0233$) and Water:Water ($p=0.0130$). One possible explanation for this pattern is that we often observed birds (especially Chimney Swifts) diving for the abundant insects usually present near water/wetland habitat. This feeding behavior increases the risk of traffic-induced mortality and is to be expected when important resources are located near roadways. Mumme et al. (2000) found that young Florida Scrub-Jays were often forced to occupy territories in less-favorable habitat near roadways. These younger individuals experienced significantly higher levels of road mortality than older individuals that were able to maintain territories further away from the road. The habitat factor Ag:Ag was identified by the model as having a negative effect on predicting roadkill ($B = -0.6329$) and although this habitat configuration is utilized by some bird species encountered during this study, it is not a commonly used habitat type for this taxonomic group as a whole.

Reptiles

The NBL model and the BLR model both identified land cover classifications that included water and forest as being significant predictors of reptile road mortality. We would expect water/wetland to be a significant factor for reptile road mortality since a large proportion of the reptile data set (~62%) consists of aquatic species such as *Trachemys scripta elegans*, *Chrysemys picta*, and *Chelydra serpentina*. The remainder of the data set consisted of snake species that are commonly associated with diverse habitats including wetlands, grasslands, meadows, and dense forest (Minton 2001). These habitat requirements are partially encompassed in the two significant land cover classes listed above.

Amphibians

Amphibians dominated this dataset (~88%), as in at least three previous studies that examined vertebrate road mortality (Ashley & Robinson 1996, Dodd et al. 2004, Glista et al. 2008). Amphibian mortalities in our study were most common when water/wetlands were located on both sides of the survey route (Figure 17), or were spatially aggregated at features such as water/wetland and forest (Figure 18). Our models also identify specific habitat configurations that are important indicators of elevated amphibian mortality. For example, these important cover types include forest habitat on one side of the road opposite wetland/water was present at several survey sections that demonstrated high levels of amphibian mortality (e.g. SR 26 Wet – Figure 18). This pattern can be explained by the fact that many amphibian species (especially salamanders) breed at wetland sites and spend the remainder of the year in upland forest (Marsh et al. 2007). These results confirm that idiosyncrasies associated with the biology of specific taxa can be crucial for effectively predicting and mitigating roadkill.

Agricultural Practices & Mortality

Vertebrate mortality across 5 of the surveyed sites was consistently higher pre-harvest than post-harvest (Table 16). This seasonal variation in roadkill is probably due to two factors, food and cover. Pre-harvest, agricultural fields offer cover from predators and (for many wildlife species) a food source. Post-harvest, these wildlife benefits are removed and thus animal use of agricultural fields is greatly reduced.

Site-specific insights

One important aspect of this study came from the trends seen at the Prophetstown site and at the SR 26 Ag survey route. The SR 26 Ag site had a large number of amphibian mortalities ($n = 2,114$) despite the fact that the nearest body of water was located >700 meters from the survey route. These data suggest that amphibians are more vagile than previously supposed, and/or more adapted to the agricultural landscape common in northern Indiana (e.g. breeding in small, wet areas throughout ag fields). This interesting site will be discussed in further detail below.

State Road 26 Ag

This survey route is a classic example of the agriculturally dominated landscape of northern Indiana. The entire site is dominated by corn and soybean fields on both sides of the road and there is one single-family residence on the north side of the road and an abandoned homestead with only a few mature trees and overgrown grass on the south side. The mortality levels recorded at this site were rather surprising based on the fact that this site was selected as a control (upland) site and yet most of the mortalities at this site (~96%) turned out to be amphibians. A large proportion of these were American toads, which are often found in agricultural settings because of their increased desiccation tolerance. However, many other amphibian species were also present at this site (e.g. Northern leopard frog, American bullfrog, and Cope's and Grey Tree frog). The highest levels of mortality at the State Road 26 Ag site

were seen in a section of roadway where an abandoned homestead was located (Figure 7). This particular section accounted for 470 of the 2,187 mortalities at this site, significantly more than the 182 carcasses per 100-meter road section expected under a uniform spatial distribution of roadkill across all 12 sections. Thus, it is possible that amphibians are utilizing this small “island” of non-agricultural habitat as “stepping stones”. If so that could explain why so many individuals were killed on the adjacent roadway. This idea of individuals utilizing small “islands” of preferred habitat to traverse inhospitable habitat (e.g. agricultural fields) has been shown in the painted turtle (*Chrysemys picta*) as well (Bowne et al. 2006).

During the breeding season, a few anuran calls were heard at the State Road 26 Ag site, but they were apparently >100 meters from the roadway and there were no signs of breeding near the road nor were there wet ditches along the roadway that would support breeding activity. The high level of mortality at this site was largely responsible for the land cover classification of Ag:Grassland being a significant predictor of both amphibian mortality and overall mortality (Table 8 and 9).

These findings are interesting because most amphibians require aquatic habitats for breeding and are classified as one of the least vagile vertebrate groups (Carr and Fahrig 2006). Since the nearest aquatic habitat was over 700 meters from the survey route, there were only a few possible explanations to the high levels of mortality documented at this site. One explanation is that amphibians are breeding in surrounding habitats (e.g. the wetland area >700 meters away from the survey route) and moving through a large area of inhospitable habitat in search of resources. This finding illustrates the importance of incorporating ecologically-relevant buffer zones around roadways for habitat analysis. We implemented a 100-meter buffer to determine land cover classes adjacent to road survey routes, but of course size buffer zone may not be ideal

for all taxa. We chose the 100-meter buffer because it is biologically reasonable for most terrestrial vertebrates. Furthermore, a 100-meter buffer is an industry standard and thus our models would have the greatest utility for local and state agencies. Future projects will examine various buffer sizes and their impacts on species-specific models.

Another explanation for the high levels of amphibian roadkill at the upland State Road 26 Ag site is that amphibians may be forced to breed in small pools formed in flooded agricultural fields (and in nearby roadside ditches) because of a lack of primary breeding habitat. Or perhaps this abandoned home site serves as amphibian refugia due to the lack of traditional predators such as Great Blue Herons. Of course, these alternatives are not mutually exclusive and the elevated mortality levels at this site could be a combination of these processes. Regardless of the cause, the roadkill trends documented at this site provide evidence for the importance of considering upland areas as sources of amphibian recruitment (and in the context of the current study, sources of roadkill).

Prophetstown

This survey route is located near Battleground, Indiana (Tippecanoe County) and bisects Prophetstown State Park (Figure 10). A majority of the survey route bisects reconstructed prairie habitat, but part of the route bisects shallow marsh habitat. This marshy area is seasonally wet and several species of frogs and toads were heard calling during the breeding season. This site had one of the highest levels of overall mortality ($n = 3,051$) and amphibians represented 97% of the total kills. This site was different than the other 5 wetland sites because the mortalities consisted mainly of Northern Leopard frogs (*Lithobates pipiens*) and carcasses designated “unknown *Lithobates*”. During July 2008, we documented a massive migration of adult and metamorph Northern Leopard frogs at this site and on a single survey day (09 July 2008) we

encountered over 800 Northern Leopard frogs and unknown ranids within 200 linear meters of survey route. Many of the resultant carcasses were identified as dispersing juvenile Northern Leopard frogs. This level of mortality, when accompanied by all the other factors that are accelerating worldwide amphibian declines (Table 1), does not bode well for this species of state conservation concern. In this instance, road mortality could be considered additive mortality that effectively reduces recruitment into the breeding adult population. Note, however, that this mass migration event was not documented in the summer of 2009. This emphasizes the importance of extensive, long-term sampling in order to understand the temporal variance in roadkill (and in associated animal breeding patterns) in order to accurately predict roadkill hotspots.

DNA barcoding

To our knowledge, the DNA barcoding approach utilized herein has never before been systematically applied to roadkill. We expected the success rate (30%) to be higher than it was, and the discrepancy between our expectations (>50%) and reality was largely due to the rapid degradation of amphibian DNA. This is somewhat but not entirely surprising. Avian DNA can be successfully genotyped from feathers shed weeks or months earlier (Rudnick et al. 2005, 2009), but such feathers are not typically subject to repeated crushing and the baking hot temperatures encountered on asphalt in the summer. Nevertheless, our DNA barcoding efforts yielded some significant biological insights.

Barcoding revealed that several specimens had been incorrectly identified in the field (Table 10 and 12). For example, seven salamanders were incorrectly identified in the field as desiccated frog carcasses. Overall, however, there were very few known misidentifications (~10%). In other words, most specimens were correctly categorized in the field. Thus, unrecognized misidentifications (from those samples that were not barcoded) should be rare. Thus, their

impact on our dataset should be minimal because our goal was to understand better the ultimate factors that result in elevated roadkill; those factors are similar in taxonomic groups such as amphibians (see above).

Our study also revealed some interesting twists of DNA barcoding. Six presumptive amphibian samples BLASTed back to a plant fungus (*Phytophthora* sp.). This suggests that the universal primer set that we employed amplified fungal DNA instead of the target amphibian DNA; presumably a fungus had infected the carcass prior to our tissue collection. All six of these samples were collected during July and August which are prime months for fungal growth due to high temperatures and an elevated rate of decomposition (especially with amphibians). We do not know if the fungal DNA we amplified represents a unique species (we have not attempted to culture it), but it seems unlikely to us that the same species utilizes domestic crops and amphibian carcasses as hosts.

The limitations of barcoding were related to 1) the rapid degradation of DNA in roadkilled carcasses and 2) our attempt to use “universal” barcoding primers to amplify DNA from all terrestrial vertebrates. The former was a function of our sampling scheme (and the limitations associated with a small field crew), the latter was associated with our broad efforts to characterize all vertebrate roadkill. In a more focused study of a specific species or taxonomic group, one could imagine sampling at shorter time intervals in an effort to avoid DNA degradation. Similarly, a study could more readily focus on a species of special concern if more exclusive PCR primers were utilized. For example, if anurans were of particular concern, then a different set of primers (e.g., those that discriminate among frogs and toads) could be used to the exclusion of other vertebrates.

Overall, barcoding was a useful tool for identifying unknown roadkill carcasses. This method revealed that some samples were misidentified in the field. It also provided evidence that a fungus exploits amphibian carcasses, which could be of interest to those studying amphibian pathogens. Finally, DNA barcoding could be helpful when researchers are interested in roadkill but lack the necessary skills to accurately identify carcasses to the species or genus level (or simply want to confirm species proportions recorded in the field).

Predictive Roadkill Models

A primary goal of this project was to provide analytical models that managers and road planners could use to help build “wildlife-friendly” roadways and, where necessary, to mitigate roadkill effectively. Our NBL and BLR models should help in this regard. For example, in both models Water:Water ($p < 0.0001$) and average temperature ($p < 0.0001$) were important predictors of amphibian mortality. Thus, the two models suggest both where and when amphibian roadkill is likely to occur and stresses the importance of open water and wetland habitats for predicting these trends. If road planners are interested in building transportation infrastructure that minimizes the probability of traffic-induced amphibian mortality, they should limit, or ideally eliminate, road construction immediately adjacent to or through wetland habitat. Likewise, if planners or wildlife managers are interested in mitigating amphibian roadkill on already existing roads, they should focus on roads that have water/wetland habitat on both sides of the roadway. Both the NBL and BLR models indicated other land cover classifications that are also important for predicting amphibian roadkill (e.g. Forest:Water) and these should be considered when developing road construction and management plans.

For the complex, nonlinear function used under the “PROC GLIMMIX” procedure in SAS, there is not a formal statistical procedure that determines how much of the variance was

explained by each predictor variable. However, we used AIC model selection and the chi-square goodness-of-fit statistic (Tables 14 and 15) to illustrate that four primary factors (ground cover, average temperature, average precipitation, and traffic volume) are all significant predictors of traffic-induced vertebrate mortality. Thus, each is an important component of the predictive models..

A benefit of this research is that we developed models for all major taxonomic groups, as well as for all vertebrate mortality, which makes this a well-rounded and versatile toolset for minimizing vertebrate roadkill across several habitat types. The results of this study would allow for targeted mitigation measures which are often ineffective if poorly planned or improperly placed (Woltz et al. 2008, Dodd et al. 2004).

MANAGEMENT CONSIDERATIONS

Overall, the major findings of this study were that 1) significantly more roadkill occurs near wetlands versus uplands; 2) the occurrence of amphibian roadkill dwarfs that of every other vertebrate group; 3) roadkill levels vary annually and seasonally (it is highest in the summer months); 4) the identification of roadkill carcasses is difficult and can be assisted by DNA barcoding; and 5) specific habitat variables can be used to predict when and where roadkill hotspots will occur. The results obtained from this study can be used to fit the management needs of a particular taxa or roadkill in general. Collectively, these findings can contribute to wildlife and wetland conservation and potential decrease human injury and monetary loss due to human-wildlife collisions not only in Indiana but hopefully throughout the Midwest and other regions of the world.

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Table 1. Key determinants of worldwide amphibian decline.

Factor	Sources
Increased ultraviolet radiation	Anzalo et al. 1998 Fite et al. 1998 Kiesecker et al. 2001
Predation by invasive fish	Gamradt and Kats 1996 Smith et al. 1999 Walston and Mullin 2007
Habitat modification/loss	Hecnar and M'Closkey 1996 Demaynadier and Hunter 1998 Dodd and Dreslik 2008 Seabrook and Dettmann 1996
Toxicants	Pickford and Morris 2003 Rohr et al. 2006 Solomon et al. 2008 Ortiz et al. 2004
Disease	Dodd 1988 Laurance et al. 1996 Lips et al. 2006 Johnson et al. 2006
Climate Change	D'Amen and Bombi 2009 McCallum et al. 2009 McMenamin et al. 2008 Pounds and Crump 1994

Table 2. Site descriptions and road characteristics for all 12 vertebrate road mortality study sites. Traffic volume data was downloaded from INDOT and is represented as Annual Average Daily Traffic (AADT) for 2006 and 2007).

Site Name	Survey Route	Distance (km)	Traffic Volume (AADT)	Site Description	Road Characteristics
Lindberg Road	0.35 miles east of McCormick Road to 0.15 miles west of Northwestern Avenue	1.2	6287	Wetland bisected by road and surrounded by golf course and housing developments	Straight, 2-lane paved road; center turning lane, paved shoulder throughout wetland portion; 30 mph
Prophetstown	SR 225 immediately after Battleground to the Prophetstown SP office	1.1	903	Wetland bisected by road and surrounded by prairie habitat	Straight, 2-lane paved road; small hill and bridge over main road of Prophetstown SP; 55 mph
SR 26 Ag	SR 26/ County Line Road (Tippecanoe & Warren) to SR 26/Armstrong Chapel Road	1.2	1565	Entirely agricultural fields	Straight, 2-lane paved road; some roadside ditches; 55 mph
SR 26 Mix	0.26 miles west of SR 26/Jackson Highway to SR 26/CR 500 W	1.0	2278	Small patch of mixed hardwoods on north side of the road with agricultural fields on both sides on the road	Straight, 2 lane paved road; some roadside ditches; 55 mph
SR 26 Wetland	0.5 miles west of SR 26/CR 750 W to small gravel pull-off on north side of road	1.0	1666	Wetland surrounded by mixed hardwoods and some agricultural fields	Straight, semi-hilly, 2-lane paved road; 55 mph
SR 38 Mix	SR 38/CR680 to SR 38/North Hamilton Road	1.1	2338	Small patch of mixed hardwoods on south side of road with agricultural fields on both sides of the road	Straight, 2-lane paved road; some roadside ditches; 55 mph
US 231N Dynamic	0.41 miles north of US 52/US 231 to US 231/CR 500 N	1.1	1646	Wetland on west side of road with agricultural fields on both sides of the road; also small creek with wooded riparian habitat	Straight, 2-lane paved road with large paved shoulder on both sides of road; some roadside ditches; 55 mph
US 231N Wetland	0.77 miles north of US 231/CR 900 N to small gravel pull-off on east side of road	1.0	1505	Wetland and prairie habitat on west side of road surrounded by agricultural fields	Straight, 2-lane paved road with large paved shoulder on both sides of road; some roadside ditches; 55 mph
US 231S Ag	0.17 miles south of US 231/CR 800 S to large gravel pull-off on east side of road	1.0	7770	Entirely agricultural fields	Straight 2-lane paved road with small paved shoulder on west side of road; some roadside ditches
US 52 Ag	US 52/S County Line Road East to US 52/1000	1.1	5469	Entirely agricultural fields	Straight, 4-lane paved road with grassy median; some roadside ditches; 55 mph
US 52 Mix	2.95 miles south of US52/CR 1200 S to 0.14 miles south of US 52/Colfax Mason Road	1.1	5340	Large patch of mixed hardwoods on east side of the road with agricultural fields on both sides of the road	Straight, 4-lane paved road with grassy median; some roadside ditches; 55 mph
US 52 Wetland	0.55 miles south of US52/CR 800N at beginning of the Sugar Creek bridge to US 52/CR 700N	1.1	5237	Wetland on east side of the road with large pond on west side of road; small creek with wooded, riparian habitat	Straight, semi-hilly, 4-lane paved road with grassy median; some roadside ditches; 55 mph

Table 3. Total vertebrate mortalities by taxonomic group across all 12 study sites during the entire 20-month study season.

Route	Mammals	Birds	Amphibians	Reptiles	Total Mortalities
Lindberg	102	75	2602	154	2933
Prophetstown	23	23	2966	39	3051
SR 26 Wetland	78	54	1742	95	1969
US 231N Dynamic	63	29	1431	75	1598
US 231N Wetland	26	36	545	16	623
US 52 Wetland	165	82	321	32	600
SR 26 Ag	60	11	2114	2	2187
SR 26 Mix	35	14	91	4	144
SR 38 Mix	30	33	253	3	319
US 231S Ag	108	78	141	1	328
US 52 Ag	85	25	191	0	301
US 52 Mix	74	63	244	5	386
Grand Total	849	523	12641	426	14439

Table 4. Total amphibian road mortalities across all 12 study sites during the entire 20-month study season.

Scientific Name	Common Name	Total
<i>Acris crepitans</i>	Cricket Frog	60
<i>Ambystoma maculatum</i>	Spotted Salamander	1
<i>Ambystoma texanum</i>	Small-mouthed Salamander	6
<i>Ambystoma tigrinum</i>	Tiger Salamander	97
<i>Anaxyrus americanus</i>	American Toad	3915
<i>Anaxyrus fowleri</i>	Fowler's Toad	1
<i>Hyla spp.</i>	Tree Frog	167
<i>Pseudacris crucifer</i>	Spring Peeper	24
<i>Pseudacris triseriata</i>	Chorus Frog	4
<i>Lithobates catesbeianus</i>	American Bullfrog	2297
<i>Lithobates clamitans</i>	Green Frog	17
<i>Lithobates palustris</i>	Pickerel Frog	82
<i>Lithobates pipiens</i>	Northern Leopard Frog	1680
	<i>Lithobates</i> sp.	4104
	unk frog	182
	unk salamander	3
	unk toad	1
Total		12641

Table 5. Total reptile road mortalities across all 12 study sites during the entire 20-month study season.

Scientific Name	Common Name	Total
<i>Chrysemys picta</i>	Midland Painted Turtle	193
<i>Chelydra serpentina</i>	Snapping Turtle	40
<i>Coluber constrictor foxii</i>	Blue Racer	12
<i>Pantherophis obsoleta spiloides</i>	Gray Rat Snake	2
<i>Pantherophis vulpina</i>	Western Fox Snake	31
<i>Nerodia sipedon</i>	Northern Water Snake	9
<i>Storeria dekayi wrightorum</i>	Midland Brown Snake	80
<i>Terrapene carolina carolina</i>	Eastern Box Turtle	2
<i>Thamnophis sirtalis</i>	Common Garter Snake	12
<i>Trachemys scripta elegans</i>	Red-eared Slider	31
	unk snake	10
	unk turtle	4
Total		426

Table 6. Total mammal road mortalities across all 12 study sites during the entire 20-month study season.

Scientific Name	Common Name	Total
<i>Blarina brevicauda</i>	Northern Short-tailed Shrew	31
<i>Castor canadensis</i>	North American Beaver	1
<i>Didelphis virginiana</i>	Virginia Opossum	133
<i>Felis catus</i>	Domestic Cat	12
<i>Lasiurus borealis</i>	Eastern Red Bat	2
<i>Marmota monax</i>	Woodchuck	1
<i>Mephitis mephitis</i>	Striped Skunk	5
<i>Microtus ochrogaster</i>	Prairie Vole	120
<i>Microtus pennsylvanicus</i>	Meadow Vole	38
<i>Mus musculus</i>	House Mouse	34
<i>Mustela nivalis</i>	Least Weasel	2
<i>Mustela vison</i>	Mink	13
<i>Odocoileus virginianus</i>	White-tailed Deer	21
<i>Ondatra zibethicus</i>	Muskrat	14
<i>Peryomyscus leucopus</i>	White-footed Mouse	86
<i>Peromyscus maniculatus</i>	Deer Mouse	12
<i>Procyon lotor</i>	Raccoon	104
<i>Rattus norvegicus</i>	Norway Rat	1
<i>Scalopus aquaticus</i>	Eastern Mole	13
<i>Sciurus carolinensis</i>	Eastern Gray Squirrel	5
<i>Sciurus niger</i>	Eastern Fox Squirrel	53
<i>Sorex cinereus</i>	Masked Shrew	1
<i>Spermophilus tridecemlineatus</i>	13-lined Ground Squirrel	17
<i>Sylvilagus floridanus</i>	Eastern Cottontail	39
<i>Tamiasciurus hudsonicus</i>	Red Squirrel	5
<i>Tamias striatus</i>	Eastern Chipmunk	6
<i>Vulpes vulpes</i>	Red Fox	1
<i>Zapus hudsonius</i>	Meadow Jumping Mouse	2
	unk bat	7
	unk mammal	59
	unk microtus	1
	unk mouse	5
	unk muridae	1
	unk rodent	3
	unk sciuridae	1
Total		849

Table 7. Total bird road mortalities across all 12 study sites during the entire 20-month study season.

Scientific Name	Common Name	Total
<i>Agelaius phoeniceus</i>	Red-winged Blackbird	13
<i>Anas platyrhynchos</i>	Mallard	2
<i>Archilochus colubris</i>	Ruby-throated Hummingbird	6
<i>Bombycilla cedrorum</i>	Cedar Waxwing	1
<i>Branta canadensis</i>	Canada Goose	1
<i>Buteo jamaicensis</i>	Red-tailed Hawk	1
<i>Calcarius lapponicus</i>	Lapland Longspur	2
<i>Cardinalis cardinalis</i>	Northern Cardinal	4
<i>Carduelis tristis</i>	American Goldfinch	17
<i>Carpodacus mexicanus</i>	House Finch	3
<i>Chaetura pelagica</i>	Chimney Swift	18
<i>Charadrius vociferus</i>	Killdeer	7
<i>Coccyzus americanus</i>	Yellow-billed Cuckoo	1
<i>Colaptes auratus</i>	Northern Flicker	2
<i>Colinus virginianus</i>	Northern Bobwhite	1
<i>Contopus virens</i>	Eastern Wood Pewee	1
<i>Corvus brachyrhynchos</i>	American Crow	1
<i>Cyanocitta cristata</i>	Blue Jay	4
<i>Dendroica coronata</i>	Yellow-rumped Warbler	1
<i>Dendroica palmarum</i>	Western Palm Warbler	1
<i>Dendroica petechia</i>	Yellow Warbler	2
<i>Dolichonyx oryzivorus</i>	Bobolink	1
<i>Dumetella carolinensis</i>	Gray Catbird	10
<i>Eremophila alpestris</i>	Horned Lark	3
<i>Falco sparverius</i>	American Kestrel	2
<i>Fulica americana</i>	American Coot	1
<i>Geothlypis trichas</i>	Common Yellowthroat	7
<i>Hirundo rustica</i>	Barn Swallow	6
<i>Icterus spurius</i>	Orchard Oriole	1
<i>Junco hyemalis</i>	Dark-eyed Junco	1
<i>Megascops asio</i>	Eastern Screech Owl	1
<i>Melanerpes erythrocephalus</i>	Red-headed Woodpecker	1
<i>Melospiza georgiana</i>	Swamp Sparrow	1
<i>Melospiza melodia</i>	Song Sparrow	5
<i>Molothrus ater</i>	Brown-headed Cowbird	20
<i>Oporornis philadelphia</i>	Mourning Warbler	1
<i>Otus asio</i>	Eastern Screech Owl	1
<i>Passer domesticus</i>	House Sparrow	71

<i>Passerina cyanea</i>	Indigo Bunting	8
<i>Passerculus sandwichensis</i>	Savannah Sparrow	1
<i>Phasianus colchicus</i>	Ring-necked Pheasant	2
<i>Picoides pubescens</i>	Downy Woodpecker	1
<i>Pipilo erythrophthalmus</i>	Eastern Towhee	2
<i>Poecile atricapilla</i>	Black-capped Chickadee	1
<i>Quiscalus quiscula</i>	Common Grackle	11
<i>Sialia sialis</i>	Eastern Bluebird	2
<i>Spizella passerina</i>	Chipping Sparrow	23
<i>Stelgidopteryx serripennis</i>	Northern Rough-Winged Swallow	16
<i>Sturnella magna</i>	Eastern Meadowlark	4
<i>Sturnus vulgaris</i>	European Starling	9
<i>Tachycineta bicolor</i>	Tree Swallow	2
<i>Thryothorus ludovicianus</i>	Carolina Wren	1
<i>Toxostoma rufum</i>	Brown Thrasher	2
<i>Troglodytes aedon</i>	House Wren	1
<i>Turdus migratorius</i>	American Robin	47
<i>Tyrannus tyrannus</i>	Eastern Kingbird	1
<i>Wilsonia citrina</i>	Hooded Warbler	1
<i>Zenaida macroura</i>	Mourning Dove	13
<i>Zonotrichia albicollis</i>	White-throated Sparrow	1
	unk bird	150
	unk sparrow	2
Total		523

Table 8. Linear roadkill models constructed using a negative binomial distribution and various predictors based on model selection (NBL). All listed variables represent factors that are significant predictors for estimating roadkill (p -value <0.05). Land cover classes are represented by an X:X format under the “variable” column and represent the habitat on opposite sides of the roadway. For example, “forest:water” means a road segment with forested habitat on one side of the road and water on the opposite side.

Model	Chi-square	Variable	Coefficient		P
			B	SE	
<i>Total (all taxa)</i>	1324.33	Intercept	-3.1921	0.2593	<.0001
		road:residential	1.7869	0.4092	<.0001
		ag:ag	0.9596	0.1870	<.0001
		ag:forest	1.0824	0.1959	<.0001
		ag:urban grasses	0.9354	0.1999	<.0001
		ag:water	2.4111	0.3904	<.0001
		ag:grassland	1.9095	0.2521	<.0001
		forest:forest	2.1809	0.2504	<.0001
		forest:urban grasses	1.4277	0.3264	<.0001
		forest:residential	2.4365	0.4322	<.0001
		forest:water	2.6506	0.3889	<.0001
		urban grasses:urban grasses	2.0437	0.2421	<.0001
		urban grasses:residential	1.9242	0.4150	0.0002
		grassland:urban grasses	1.0844	0.2941	0.0104
		water:residential	2.5574	0.9962	<.0001
		water:water	3.0169	0.2165	<.0001
		water:grassland	1.1905	0.4644	0.0105
		grassland:grassland	0	.	.
		Average Temperature	0.09790	0.002921	<.0001
		Average Precipitation	-0.1144	0.01426	<.0001
		Traffic	-0.00011	0.000017	<.0001
		Scale	0.8560	0.04846	.
<i>Amphibians</i>	1291.17	Intercept	-4.5246	0.3325	<.0001
		road:residential	2.4857	0.4918	<.0001
		ag:ag	1.0708	0.2401	<.0001
		ag:forest	1.0536	0.2497	<.0001
		ag:urban grasses	1.0760	0.2544	<.0001
		ag:water	2.5226	0.4682	<.0001
		ag:grassland	2.1764	0.3101	<.0001
		forest:forest	2.2093	0.3097	<.0001
		forest:urban grasses	1.4295	0.3952	0.0003
		forest:residential	2.8636	0.5090	<.0001
		forest:water	2.8744	0.4654	<.0001
		urban grasses:urban grasses	2.5433	0.3008	<.0001
		urban grasses:residential	2.3188	0.4948	<.0001
		grassland:urban grasses	1.3119	0.3580	0.0003
		water:water	3.4071	0.2729	<.0001
		grassland:grassland	0	.	.
		Average Temperature	0.1268	0.004061	<.0001
		Average Precipitation	-0.1602	0.01691	<.0001
		Traffic	-0.00022	0.000021	<.0001
		Scale	1.1101	0.06724	.
<i>Reptiles</i>	1030.93	Intercept	-3.3446	0.6222	<.0001
		ag:forest	1.0655	0.4825	0.0274

		ag:water	3.9809	0.6923	<.0001
		forest:forest	2.1225	0.5511	0.0001
		forest:urban grasses	2.4103	0.6622	0.0003
		forest:water	3.5433	0.7217	<.0001
		urban grasses:urban grasses	2.1935	0.5921	0.0002
		urban grasses:residential	2.0077	0.9006	0.0260
		water:water	3.5913	0.5320	<.0001
		grassland:grassland	0	.	.
		Average Temperature	0.07539	0.008029	<.0001
		Average Precipitation	-0.2632	0.03310	<.0001
		Traffic	-0.00015	0.0000049	0.0029
		Scale	1.7512	0.2809	.
<hr/>					
<i>Mammals</i>	1183.67	Intercept	-2.5171	0.3542	<.0001
		ag:forest	0.8317	0.2913	0.0044
		forest:forest	1.9314	0.3342	<.0001
		forest:urban grasses	1.4230	0.4212	0.0008
		forest:residential	1.1441	0.5188	0.0277
		forest:water	1.9547	0.4861	<.0001
		water:grassland	1.1608	0.5053	0.0218
		grassland:grassland	0	.	.
		Average Temperature	0.03529	0.003509	<.0001
		Average Precipitation	-0.08167	0.01939	<.0001
		Traffic	0.000174	0.000023	<.0001
		Scale	0.7574	0.1041	.
<hr/>					
<i>Birds</i>	1058.46	Intercept	-4.3788	0.4211	<.0001
		ag:ag	-0.6329	0.2785	0.0233
		water:water	0.7858	0.3160	0.0130
		Average Temperature	0.05151	0.004878	<.0001
		Traffic	0.000160	0.000028	<.0001
		Scale	0.8612	.	.

Table 9. Predictive roadkill models created using binary logistic regression (BLR) and various predictors based on model selection. All listed variables represent factors that are significant predictors for estimating roadkill (p -value <0.05). Land cover classes are represented by an X:X format under the “variable” column and represent the habitat on opposite sides of the roadway. For example, “forest:water” means a road segment with forested habitat on one side of the road and water on the opposite side.

Model	Chi-square	Variable	Coefficient		
			B	SE	P
<i>Total (all taxa)</i>	1014.85	Intercept	-4.3034	0.6597	<0.0001
		ag:ag	1.2387	0.4186	0.0032
		ag:forest	1.6000	0.4548	0.0005
		ag:water	3.1064	1.3526	0.0218
		ag:grassland	2.0335	0.6928	0.0034
		forest:forest	2.9334	0.7620	0.0001
		grassland:forest	2.5101	0.9269	0.0069
		urban grasses:urban grasses	1.4261	0.6702	0.0336
		grassland:urban grasses	2.1710	0.7811	0.0055
		water:water	2.2077	0.5948	0.0002
		Average temperature	0.1278	0.007971	<.0001
		Average precipitation	-0.2196	0.04739	<.0001
		Traffic	0.000216	0.000051	<.0001
<i>Amphibians</i>	1037.28	Intercept	-8.0716	0.8484	<.0001
		road:residential	3.8515	1.5704	0.0143
		ag:ag	1.7163	0.4494	0.0001
		ag:forest	2.1625	0.4885	<.0001
		ag:urban grasses	1.1200	0.4801	0.0199
		ag:water	3.4668	1.5500	0.0255
		ag:grassland	3.4546	0.8651	<.0001
		forest:forest	2.1405	0.7381	0.0038
		forest:residential	3.5929	1.7356	0.0387
		forest:water	5.5205	1.4898	0.0002
		grassland:forest	4.4818	1.1235	<.0001
		urban grasses:urban grasses	2.6135	0.7308	0.0004
		grassland: urban grasses	3.4194	0.9718	0.0005
		water:water	3.4045	0.6867	<.0001
		Average Temperature	0.1878	0.01215	<.0001
		Average Precipitation	-0.2151	0.04449	<.0001
<i>Reptiles</i>	926.48	Intercept	-3.7808	0.7118	<.0001
		ag:forest	1.0905	0.5435	0.0451
		ag:urban grasses	1.1042	0.5547	0.0468
		ag:water	3.6566	1.0200	0.0004
		forest:forest	2.1321	0.6590	0.0013
		forest:urban grasses	1.8645	0.7906	0.0185
		forest:water	3.6566	1.0200	0.0004
		urban grasses:urban grasses	2.4878	0.6576	0.0002
		water:water	3.7347	0.6142	<.0001
		Average Temperature	0.09047	0.009758	<.0001
		Average Precipitation	-0.3236	0.04241	<.0001
		Traffic	-0.00014	0.000053	0.0068
<i>Mammals</i>	1046.39	Intercept	-3.7077	0.5025	<.0001
		ag:forest	0.7943	0.3805	0.0371
		forest:forest	2.7766	0.5852	<.0001

		forest:water	2.5751	0.9159	0.0050
		Average Temperature	0.03857	0.004865	<.0001
		Traffic	0.000274	0.000035	<.0001
Birds	1050.19	Intercept	-4.6913	0.5376	<.0001
		Ground Cover	.	.	0.0104
		Average Temperature	0.05976	0.006099	<.0001
		Traffic	0.000138	0.000037	0.0002

Table 10. Top BLAST hits for all amphibian samples that were successfully sequenced at the CO1 gene. Samples that are in bold text resulted in an unexpected BLAST result (e.g. incorrect genus).

Sample	Accession Number	Description	Max Identity	E-value	Score	Comments
Anaxyrus_americanus_S0377	gii156788318 gb EF525769.1	Bufo fowleri voucher MAS-1210-02 CO1	100	0.00	1179	very desiccated tissue
	gii156788230 gb EF525725.1	Bufo americanus voucher MAS-902-01 CO1	100	0.00	1179	
Anaxyrus_americanus_S0466	gii156788466 gb EF525843.1	Pseudacris crucifer voucher HLC-10597 CO1	97.72	0.00	1057	
Bufo_fowleri	gii156788396 gb EF525808.1	Bufo fowleri voucher MAS-048B-01 CO1	95.1	0.00	1085	
Bufo_americanus	gii156788394 gb EF525807.1	Bufo fowleri voucher MAS-138-01 CO1	97.09	0.00	1138	
Hyla_versicolor_S0059	gii156788424 gb EF525822.1	Hyla versicolor voucher MAS216-05 CO1	100	0.00	833	
Hyla_versicolor_S0220	gii156788424 gb EF525822.1	Hyla versicolor voucher MAS216-05 CO1	100	0.00	1035	
Hyla_versicolor_S0372	gii156788424 gb EF525822.1	Hyla versicolor voucher MAS216-05 CO1	100	0.00	1190	
Hyla_versicolor_S0374	gii156788424 gb EF525822.1	Hyla versicolor voucher MAS216-05 CO1	99.84	0.00	1123	
Lithobates_catesbeiana	gii156788490 gb EF525855.1	Rana catesbeiana voucher MAS060-06 CO1	99.24	0.00	1192	
Lithobates_clamitans	gii156788538 gb EF525879.1	Rana clamitans voucher MAS237-05 CO1	98.63	0.00	1177	
Lithobates_clamitans_S0078	gii156788540 gb EF525880.1	Rana clamitans voucher MAS045-05 CO1	99.35	0.00	835	
Lithobates_pipiens_	gii156788556 gb EF525888.1	Rana pipiens voucher MAS006i-05 CO1	97.41	0.00	1151	
unknown_ranid_S0114	gii156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	99.83	0.00	1105	
Pseudacris_crucifer_S0373	gii49066264 gb AY659992.1	Pseudacris crucifer voucher HLC-10597 CO1	99.24	0.00	952	
Pseudacris_triseriata_S0423		No significant BLAST hit				
unknown_frog_S0043	gii8347068 emb AJ238588.1 	Sciuirus vulgaris complete mitochondrial genome	87.34	0.00	725	
unknown_frog_S0206	gii156788466 gb EF525843.1	Pseudacris crucifer voucher HLC-10597 CO1	97.71	0.00	1051	
unknown_frog_S0268	gii156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	99.33	0.00	1074	
unknown_frog_S0268	gii156788492 gb EF525856.1	Rana catesbeiana voucher MAS062-05 CO1	94.94	0.00	929	
unknown_frog_S0356	gii156788466 gb EF525843.1	Pseudacris crucifer voucher HLC-10597 CO1	97.81	0.00	1101	
unknown_frog_S0428	gii49066264 gb AY659992.1 	Ambystoma tigrinum tigrinum mitochondrion, complete genome	99.35	0.00	1112	
unknown_frog_S0484	gii156788424 gb EF525822.1	Hyla versicolor voucher MAS216-05 CO1	99.62	0.00	972	
unknown_frog_S0526	gii156788382 gb EF525801.1 	Bufo fowleri voucher MAS1233-02 CO1	100	0.00	1099	
	gii156788230 gb EF525725.1 	Bufo americanus voucher MAS-902-01 CO1	100	0.00	1099	
unknown_frog_S0527	gii156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	99.66	0.00	1079	
unknown_frog_S0532	gii156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	100	0.00	1136	
	gii156788492 gb EF525856.1	Rana catesbeiana voucher MAS062-05 CO1	94.47	0.00	948	
unknown_frog_S0535	gii156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	99.83	0.00	1088	
	gii156788492 gb EF525856.1	Rana catesbeiana voucher MAS062-05 CO1	95.1	0.00	933	
unknown_frog_S0542	gii156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	99.28	0.00	1009	
	gii156788492 gb EF525856.1	Rana catesbeiana voucher MAS062-05 CO1	94.59	0.00	859	
unknown_frog_S0543	gii156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	99.19	0.00	1110	
	gii156788492 gb EF525856.1	Rana catesbeiana voucher MAS062-05 CO1	94.6	0.00	946	
unknown_frog_S0547	gii156788556 gb EF525888.1	Rana pipiens voucher MAS006i-05 CO1	100	0.00	1026	
unknown_frog_S0551	gii156788424 gb EF525822.1	Hyla versicolor voucher MAS216-05 CO1	100	0.00	1116	
unknown_frog_S0558	gii156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	99.84	0.00	1175	
	gii156788476 gb EF525848.1	Rana catesbeiana voucher GANA737-06 CO1	94.68	0.00	992	
unknown_frog_S0564	gii156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	100	0.00	1013	
	gii156788492 gb EF525856.1	Rana catesbeiana voucher MAS062-05 CO1	94.85	0.00	850	
unknown_frog_S0599	gii156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	99.7	0.00	1205	
	gii156788476 gb EF525848.1	Rana catesbeiana voucher GANA737-06 CO1	94.37	0.00	1011	
unknown_ranid_S0006	gii49066264 gb AY659992.1 	Ambystoma tigrinum tigrinum mitochondrion, complete genome	99.32	0.00	1070	
unknown_ranid_S0009	gii156788556 gb EF525888.1	Rana pipiens voucher MAS006i-05 CO1	99.65	0.00	1050	
unknown_ranid_S0010	gii156788556 gb EF525888.1	Rana pipiens voucher MAS006i-05 CO1	98.9	0.00	976	
unknown_ranid_S0013	gii223470488 gb FJ527882.1 	Mus musculus domesticus isolate 3 CO1	84.75	0.00	643	
unknown_ranid_S0014	gii156788382 gb EF525801.1 	Bufo fowleri voucher MAS1233-02 CO1	99.53	0.00	1155	
	gii156788230 gb EF525725.1 	Bufo americanus voucher MAS-902-01 CO1	99.53	0.00	1155	
unknown_ranid_S0021	gii156788246 gb EF525733.1 	Bufo americanus voucher MAS026-05 CO1	100	0.00	1042	
	gii156788382 gb EF525801.1 	Bufo fowleri voucher MAS1233-02 CO1	99.65	0.00	1031	
unknown_ranid_S0022	gii156788424 gb EF525822.1	Hyla versicolor voucher MAS216-05 CO1	100	0.00	1194	
unknown_ranid_S0023	gii156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	98.68	0.00	1079	

	gij156788492 gb EF525856.1	Rana catesbeiana voucher MAS062-05 CO1	94.05	0.00	924	
unknown_ranid_S0024	gij156788424 gb EF525822.1	Hyla versicolor voucher MAS216-05 CO1	99.82	0.00	1018	
unknown_ranid_S0025	gij156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	99.14	0.00	1046	
	gij156788492 gb EF525856.1	Rana catesbeiana voucher MAS062-05 CO1	94.67	0.00	902	
unknown_ranid_S0027	gij156788424 gb EF525822.1	Hyla versicolor voucher MAS216-05 CO1	99.82	0.00	1026	
unknown_ranid_S0040	gij156788492 gb EF525856.1	Rana catesbeiana voucher MAS062-05 CO1	99.73	0.00	678	
	gij156788540 gb EF525880.1	Rana clamitans voucher MAS045-05 CO1	95.92	0.00	597	
unknown_ranid_S0048	gij156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	99.3	0.00	1037	
	gij156788492 gb EF525856.1	Rana catesbeiana voucher MAS062-05 CO1	94.76	0.00	893	
unknown_ranid_S0050	gij156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	100	0.00	1098	
	gij156788492 gb EF525856.1	Rana catesbeiana voucher MAS062-05 CO1	94.61	0.00	920	
unknown_ranid_S0052	gij156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	99.84	0.00	1171	
	gij156788476 gb EF525848.1	Rana catesbeiana voucher GANA737-06 CO1	94.66	0.00	989	
unknown_ranid_S0055	gij156788492 gb EF525856.1	Rana catesbeiana voucher MAS062-05 CO1	99.66	0.00	1081	
	gij156788538 gb EF525879.1	Rana clamitans voucher MAS237-05 CO1	94.92	0.00	928	
unknown_ranid_S0058	gij156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	99.39	0.00	1192	
unknown_ranid_S0061	gij156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	99.39	0.00	1192	
unknown_ranid_S0062	gij156788318 gb AF069423.1	Chrysemys picta mitochondrion, complete genome	99.84	0.00	1173	very desiccated tissue
	gij156788536 gb EF525769.1	Bufo fowleri voucher MAS-1210-02 CO1	100	0.00	1192	
	gij156788230 gb EF525725.1	Bufo americanus voucher MAS-902-01 CO1	100	0.00	1192	
unknown_ranid_S0065	gij156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	99.83	0.00	1068	
	gij156788510 gb EF525865.1	Rana catesbeiana voucher MAS062-05 CO1	99.83	0.00	1068	
unknown_ranid_S0067	gij156788536 gb EF525888.1	Rana pipiens voucher MAS0061-05 CO1	99.82	0.00	1005	
unknown_ranid_S0068	gij156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	99.82	0.00	1050	
	gij156788492 gb EF525856.1	Rana catesbeiana voucher MAS062-05 CO1	94.92	0.00	894	
unknown_ranid_S0070	gij156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	99.49	0.00	1072	
	gij156788492 gb EF525856.1	Rana catesbeiana voucher MAS062-05 CO1	94.66	0.00	902	
unknown_ranid_S0072	gij156788476 gb EF525848.1	Rana catesbeiana voucher GANA737-06 CO1	99.7	0.00	1203	
	gij156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	94.22	0.00	1003	
unknown_ranid_S0077	gij156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	99.83	0.00	1075	
	gij156788476 gb EF525848.1	Rana catesbeiana voucher GANA737-06 CO1	94.87	0.00	915	
unknown_ranid_S0079	gij156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	99.34	0.00	1094	
	gij156788476 gb EF525848.1	Rana catesbeiana voucher GANA737-06 CO1	94.54	0.00	933	
unknown_ranid_S0080	gij156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	99.37	0.00	1157	
	gij156788492 gb EF525856.1	Rana catesbeiana voucher MAS062-05 CO1	94.65	0.00	987	
unknown_ranid_S0081	gij156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	99.35	0.00	1120	
	gij156788492 gb EF525856.1	Rana catesbeiana voucher MAS062-05 CO1	94.5	0.00	953	
unknown_ranid_S0082	gij156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	100	0.00	974	
	gij156788492 gb EF525856.1	Rana catesbeiana voucher MAS062-05 CO1	94.88	0.00	824	
unknown_ranid_S0082b	gij156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	99.83	0.00	1096	
	gij156788476 gb EF525848.1	Rana catesbeiana voucher GANA737-06 CO1	94.97	0.00	935	
unknown_ranid_S0087	gij156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	99.44	0.00	966	
	gij156788492 gb EF525856.1	Rana catesbeiana voucher MAS062-05 CO1	94.55	0.00	822	
unknown_ranid_S0089	gij156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	99.33	0.00	1074	
	gij156788492 gb EF525856.1	Rana catesbeiana voucher MAS062-05 CO1	94.77	0.00	924	
unknown_ranid_S0097	gij156788382 gb EF525801.1	Bufo fowleri voucher MAS1233-02 CO1	99.49	0.00	1074	
	gij156788378 gb EF525799.1	Bufo americanus voucher MAS-902-01 CO1	99.49	0.00	1074	
unknown_ranid_S0099	gij31158395 gb AY129166.1	Phytophthora capsici CO1	88.73	0.00	86.1	plant fungus
unknown_ranid_S0100	gij156788536 gb EF525888.1	Rana pipiens voucher MAS0061-05 CO1	99.84	0.00	1116	
unknown_ranid_S0102	gij156788332 gb EF525776.1	Bufo fowleri voucher MAS-505-01 CO1	100	0.00	1214	
	gij156788258 gb EF525739.1	Bufo americanus voucher MAS-225-02 CO1	96.64	0.00	1085	
unknown_ranid_S0103	gij156788492 gb EF525856.1	Rana catesbeiana voucher MAS062-05 CO1	99.53	0.00	1158	
	gij156788538 gb EF525879.1	Rana clamitans voucher MAS237-05 CO1	94.65	0.00	989	
unknown_ranid_S0104	gij156788492 gb EF525856.1	Rana catesbeiana voucher MAS062-05 CO1	99.51	0.00	1105	
	gij156788538 gb EF525879.1	Rana clamitans voucher MAS237-05 CO1	94.87	0.00	946	
unknown_ranid_S0105	gij156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	99.51	0.00	1103	
	gij156788476 gb EF525848.1	Rana catesbeiana voucher GANA737-06 CO1	94.4	0.00	931	
unknown_ranid_S0106	gij156788476 gb EF525848.1	Rana catesbeiana voucher GANA737-06 CO1	99.08	0.00	1168	
	gij156788538 gb EF525879.1	Rana clamitans voucher MAS237-05 CO1	94.91	0.00	1016	

unknown_ranid_S0109	gii 156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	100	0.00	1110	
	gii 156788492 gb EF525856.1	Rana catesbeiana voucher MAS062-05 CO1	94.84	0.00	939	
unknown_ranid_S0110	gii 156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	99.83	0.00	1114	
	gii 156788534 gb EF525877.1	Rana catesbeiana voucher MAS062-05 CO1	99.83	0.00	1114	
	gii 156788492 gb EF525856.1	Rana catesbeiana voucher MAS062-05 CO1	94.88	0.00	948	
unknown_ranid_S0119	gii 31158395 gb AY129166.1 	Phytophthora capsici CO1	88.73	0.00	86.1	plant fungus
unknown_ranid_S0123	gii 156788556 gb EF525888.1	Rana pipiens voucher MAS006i-05 CO1	99.64	0.00	1027	
unknown_ranid_S0140	gii 156788466 gb EF525843.1	Pseudacris crucifer voucher HLC-10597 CO1	98.01	0.00	959	
unknown_ranid_S0143	gii 31158395 gb AY129166.1 	Phytophthora capsici CO1	88.73	0.00	86.1	plant fungus
unknown_ranid_S0169	gii 156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	100	0.00	1206	
	gii 156788476 gb EF525848.1	Rana catesbeiana voucher GANA737-06 CO1	94.33	0.00	1002	
unknown_ranid_S0185	gii 156788556 gb EF525888.1	Rana pipiens voucher MAS006i-05 CO1	99.81	0.00	955	
unknown_ranid_S0187	gii 31158395 gb AY129166.1 	Phytophthora capsici CO1	88.73	0.00	86.1	plant fungus
unknown_ranid_S0208	gii 156788260 gb EF525740.1 	Bufo americanus voucher MAS-293-03 CO1	100	0.00	843	
	gii 156788402 gb EF525811.1 	Bufo fowleri voucher MAS-148-01 CO1	99.56	0.00	832	
unknown_ranid_S0209	gii 156788382 gb EF525801.1 	Bufo fowleri voucher MAS1233-02 CO1	100	0.00	918	
	gii 156788230 gb EF525725.1 	Bufo americanus voucher MAS-902-01 CO1	100	0.00	918	
unknown_ranid_S0213	gii 156788224 gb EF525722.1 	Bufo americanus voucher MAS-003-02 CO1	100	0.00	1070	
	gii 156788394 gb EF525807.1 	Bufo fowleri voucher MAS-138-01 CO1	99.83	0.00	1064	
unknown_ranid_S0216	gii 156788556 gb EF525888.1	Rana pipiens voucher MAS006i-05 CO1	99.46	0.00	1016	
unknown_ranid_S0219	gii 156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	99.67	0.00	1101	
	gii 156788492 gb EF525856.1	Rana catesbeiana voucher MAS062-05 CO1	94.68	0.00	935	
unknown_ranid_S0224	gii 156788556 gb EF525888.1	Rana pipiens voucher MAS006i-05 CO1	99.46	0.00	1007	
unknown_ranid_S0228	gii 156788492 gb EF525856.1	Rana catesbeiana voucher MAS062-05 CO1	99.63	0.00	977	
	gii 156788538 gb EF525879.1	Rana clamitans voucher MAS237-05 CO1	95.11	0.00	841	
unknown_ranid_S0234	gii 156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	99.83	0.00	1099	
	gii 156788476 gb EF525848.1	Rana catesbeiana voucher GANA737-06 CO1	94.65	0.00	928	
unknown_ranid_S0235	gii 156788476 gb EF525848.1	Rana catesbeiana voucher GANA737-06 CO1	99.24	0.00	1182	
	gii 156788538 gb EF525879.1	Rana clamitans voucher MAS237-05 CO1	94.5	0.00	1013	
unknown_ranid_S0240	gii 156788556 gb EF525888.1	Rana pipiens voucher MAS006i-05 CO1	99.79	0.00	887	
unknown_ranid_S0241	gii 156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	100	0.00	1035	
	gii 156788492 gb EF525856.1	Rana catesbeiana voucher MAS062-05 CO1	94.82	0.00	874	
unknown_ranid_S0245	gii 156788246 gb EF525733.1 	Bufo americanus voucher MAS026-05 CO1	99.51	0.00	1105	
	gii 156788382 gb EF525801.1 	Bufo fowleri voucher MAS1233-02 CO1	99.18	0.00	1098	
unknown_ranid_S0248	gii 156788556 gb EF525888.1	Rana pipiens voucher MAS006i-05 CO1	99.83	0.00	1055	
unknown_ranid_S0249	gii 156078921 gb EU096752.1 	Lasiurus blossevillei voucher ROM 117457 CO1	85.77	0.00	566	
unknown_ranid_S0254	gii 156788382 gb EF525801.1 	Bufo fowleri voucher MAS1233-02 CO1	99.83	0.00	1064	
	gii 156788230 gb EF525725.1 	Bufo americanus voucher MAS-902-01 CO1	99.83	0.00	1064	
unknown_ranid_S0257	gii 156788318 gb EF525769.1 	Bufo fowleri voucher MAS-1210-02 CO1	99.85	0.00	1199	
	gii 156788230 gb EF525725.1 	Bufo americanus voucher MAS-902-01 CO1	99.85	0.00	1199	
unknown_ranid_S0277	gii 31158395 gb AY129166.1 	Phytophthora capsici CO1	88.73	0.00	86.1	plant fungus
unknown_ranid_S0305	gii 156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	99.84	0.00	1116	
	gii 156788492 gb EF525856.1	Rana catesbeiana voucher MAS062-05 CO1	94.89	0.00	950	
unknown_ranid_S0345	gii 156788492 gb EF525856.1	Rana catesbeiana voucher MAS062-05 CO1	99.48	0.00	1044	
	gii 156788538 gb EF525879.1	Rana clamitans voucher MAS237-05 CO1	94.77	0.00	896	
unknown_ranid_S0353	gii 156788246 gb EF525733.1	Bufo americanus voucher MAS026-05 CO1	99.66	0.00	1072	
	gii 156788382 gb EF525801.1	Bufo fowleri voucher MAS1233-02 CO1	99.32	0.00	1061	
unknown_ranid_S0360	gii 4530187 gb AF069423.1 	Chrysemys picta mitochondrion, complete genome	99.85	0.00	1208	
	gii 211906383 gb FJ392294.1 	Trachemys scripta mitochondrion, complete genome	92.53	0.00	941	
unknown_ranid_S0367	gii 189343261 gb EU770566.1 	Tropocyclops parvus voucher ZPLMX789 CO1	96.54	0.00	1005	copepod
unknown_ranid_S0407	gii 156788492 gb EF525856.1	Rana catesbeiana voucher MAS062-05 CO1	99.24	0.00	944	
	gii 156788538 gb EF525879.1	Rana clamitans voucher MAS237-05 CO1	95.41	0.00	835	
unknown_ranid_S0457	gii 49066264 gb AY659992.1 	Ambystoma tigrinum tigrinum mitochondrion, complete genome	99.09	0.00	994	
	gii 49066250 gb AY659991.1 	Ambystoma mexicanum mitochondrion, complete genome	94.76	0.00	861	
unknown_ranid_S0458	gii 156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	99.83	0.00	1066	
	gii 156788492 gb EF525856.1	Rana catesbeiana voucher MAS062-05 CO1	95	0.00	911	
unknown_ranid_S0459	gii 49066264 gb AY659992.1 	Ambystoma tigrinum tigrinum mitochondrion, complete genome	99.18	0.00	1101	
	gii 49066278 gb AY659993.1 	Ambystoma andersoni mitochondrion, complete genome	94.77	0.00	952	

	gi 49066250 gb AY659991.1	Ambystoma mexicanum mitochondrion, complete genome	94.77	0.00	952
	gi 44843533 emb AJ584639.1	Ambystoma dumerilii mitochondrion, complete genome	94.77	0.00	952
unknown_ranid_S0490	gi 156788424 gb EF525822.1	Hyla versicolor voucher MAS216-05 CO1	100	0.00	1138
unknown_ranid_S0528	gi 156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	99.83	0.00	1081
	gi 156788492 gb EF525856.1	Rana catesbeiana voucher MAS062-05 CO1	95.07	0.00	926
unknown_ranid_S0529	gi 156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	99.84	0.00	1173
	gi 156788492 gb EF525856.1	Rana catesbeiana voucher MAS062-05 CO1	94.81	0.00	992
unknown_ranid_S0533	gi 156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	100	0.00	1099
	gi 156788492 gb EF525856.1	Rana catesbeiana voucher MAS062-05 CO1	94.94	0.00	929
unknown_ranid_S0544	gi 156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	100	0.00	1042
	gi 156788492 gb EF525856.1	Rana catesbeiana voucher MAS062-05 CO1	94.89	0.00	887
unknown_ranid_S0548	gi 156788424 gb EF525822.1	Hyla versicolor voucher MAS216-05 CO1	99.82	0.00	1026
unknown_ranid_S0549	gi 156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	99.39	0.00	1192
	gi 156788476 gb EF525848.1	Rana catesbeiana voucher GANA737-06 CO1	94.37	0.00	1009
unknown_ranid_S0550	gi 156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	100	0.00	1033
	gi 156788492 gb EF525856.1	Rana catesbeiana voucher MAS062-05 CO1	94.81	0.00	872
unknown_ranid_S0555	gi 156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	99.39	0.00	1192
	gi 156788476 gb EF525848.1	Rana catesbeiana voucher GANA737-06 CO1	94.37	0.00	1009
unknown_ranid_S0557	gi 156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	99.15	0.00	1055
	gi 156788492 gb EF525856.1	Rana catesbeiana voucher MAS062-05 CO1	94.71	0.00	911
unknown_ranid_S0597	gi 156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	100	0.00	1002
	gi 156788492 gb EF525856.1	Rana catesbeiana voucher MAS062-05 CO1	94.81	0.00	841
unknown_ranid_S0612	gi 156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	99.38	0.00	1171
	gi 156788492 gb EF525856.1	Rana catesbeiana voucher MAS062-05 CO1	94.58	0.00	1000
unknown_ranid_S0658	gi 156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	100	0.00	1214
	gi 156788476 gb EF525848.1	Rana catesbeiana voucher GANA737-06 CO1	94.37	0.00	1009
unknown_ranid_S0659	gi 156788556 gb EF525888.1	Rana pipiens voucher MAS006i-05 CO1	99.36	0.00	1146
unknown_ranid_S0660	gi 156788224 gb EF525722.1	Bufo americanus voucher MAS-003-02 CO1	99.07	0.00	1160
	gi 156788394 gb EF525807.1	Bufo fowleri voucher MAS-138-01 CO1	98.91	0.00	1155
unknown_ranid_S0664	gi 156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	99.85	0.00	1194
	gi 156788476 gb EF525848.1	Rana catesbeiana voucher GANA737-06 CO1	94.75	0.00	1009
unknown_ranid_S0672	gi 156788492 gb EF525856.1	Rana catesbeiana voucher MAS062-05 CO1	98.85	0.00	1090
	gi 156788538 gb EF525879.1	Rana clamitans voucher MAS237-05 CO1	94.55	0.00	944
unknown_ranid_S0673	gi 156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	99.85	0.00	1208
	gi 156788476 gb EF525848.1	Rana catesbeiana voucher GANA737-06 CO1	94.52	0.00	1014
unknown_ranid_S0701	gi 156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	99.84	0.00	1164
	gi 156788492 gb EF525856.1	Rana catesbeiana voucher MAS062-05 CO1	94.79	0.00	987
unknown_ranid_S0719	gi 156788536 gb EF525878.1	Rana clamitans voucher MAS246-05 CO1	100	0.00	1173
	gi 156788476 gb EF525848.1	Rana catesbeiana voucher GANA737-06 CO1	94.76	0.00	981
unknown_salamander_S0383	gi 156788202 gb EF525711.1	Ambystoma laterale x Ambystoma jeffersonianum voucher MAS094-06 CO1	91.79	0.00	881
	gi 49066264 gb AY659992.1	Ambystoma tigrinum mitochondrion, complete genome	87.65	0.00	765
	gi 51979827 gb AY728218.1	Ambystoma laterale mitochondrion, complete genome	86.88	0.00	737
	gi 156788204 gb EF525712.1	Ambystoma laterale x Ambystoma jeffersonianum voucher MAS084-06 CO1	87.99	0.00	734
	gi 49066278 gb AY659993.1	Ambystoma andersoni mitochondrion, complete genome	86.75	0.00	732
	gi 49066250 gb AY659991.1	Ambystoma mexicanum mitochondrion, complete genome	86.62	0.00	726
	gi 44843533 emb AJ584639.1	Ambystoma mexicanum complete mitochondrial genome	86.62	0.00	726
	gi 156788200 gb EF525710.1	Ambystoma laterale x Ambystoma jeffersonianum voucher MAS061-06 CO1	86.79	0.00	721

Table 11. Top BLAST hits for all reptile samples that were successfully sequenced at the CO1 gene. Samples that are in bold text resulted in an unexpected BLAST result (e.g. incorrect genus).

Sample	Accession Number	Description	Max Identity	E-value	Score	Comments
Chelydra_serpentina_S0359	gi 118412500 gb EF122793.1	Chelydra serpentina mitochondrion, complete genome	99.81	0.0	948	Night snake Batjan Frog-eating Snake
Storeria_dekayi_wrightorum_S0378	gi 126041411 gb EF417389.1	Storeria dekayi voucher CAS196039 CO1	98.77	0.0	721	
unknown_snake_S0308	gi 189164442 gb EU728584.1 	Hypsiglena slevini voucher MVZ 234613 mitochondrion, genome	84.49	9.0E-171	608	
unknown_snake_S0308	gi 237846041 gb FJ952363.1 	Stegonotus batjanensis isolate BJE01110 CO1	84.92	9.0E-171	608	
unknown_snake_S0348	gi 189164484 gb EU728587.1	Hypsiglena chlorophaea deserticola voucher MVZ 241611 mitochondrion	84.62	1.0E-173	617	
unknown_snake_S0396	gi 126041411 gb EF417389.1	Storeria dekayi voucher CAS196039 CO1	99.16	0.0	647	
unknown_snake_S0417	gi 189164484 gb EU728587.1	Hypsiglena chlorophaea deserticola voucher MVZ 241611 mitochondrion	84.59	5.0E-173	616	
unknown_snake_S0417	gi 237846041 gb FJ952363.1	Stegonotus batjanensis isolate BJE01110 CO1	84.99	2.0E-172	614	
unknown_snake_S0590	gi 237846041 gb FJ952363.1 	Stegonotus batjanensis isolate BJE01110 CO1	84.84	3.0E-150	540	
unknown_snake_S0590	gi 133900906 gb EF460849.1 	Thamnophis chrysocephalus CO1	94.91	3.0E-145	523	
unknown_turtle_S0601	gi 211906383 gb FJ392294.1	Trachemys scripta mitochondrion, complete genome	99.7	0.0	1205	Garter Snake

Table 12. Top BLAST hits for all birds samples that were successfully sequenced at the COI gene. Samples that are in bold text resulted in an unexpected BLAST result (e.g. incorrect genus).

Sample	Accession Number	Description	Max Identity	E-value	Score	Comments
Archilochus_colubris_S0253	gi 143024812 gb EF532935.1	Archilochus colubris mitochondrion, complete genome	100	0.0	1098	Chimney Swift
Dendroica_coronato_	gi 7406869 gb AF140366.1 AF140366	Dendroica coronata COI	100	0.0	1133	
Dumetalla_carolinensis_S0489	gi 117372199 gb DQ434588.1	Dumetella carolinensis voucher LPBO1681-39234 COI	99.66	0.0	1090	
Dumetella_carolinensis_S0489b	gi 117372201 gb DQ434589.1	Dumetella carolinensis voucher HBO163189227 COI	99.54	0.0	798	
Stelgidopteryx_serripennis_S0032	gi 116832047 gb DQ432837.1 	Chaetura pelagica voucher USNM 607660 COI	100	0.0	1072	
Toxostoma_rufum_	gi 151336352 gb EF484233.1	Toxostoma rufum isolate Truf0490 COI	100	0.0	1120	
Turdus_migratorius_	gi 117372605 gb DQ434791.1	Turdus migratorius voucher HBO1192-19029 COI	99	0.0	1249	
Turdus_migratorius_S0362	gi 117372605 gb DQ434791.1	Turdus migratorius voucher HBO1192-19029 COI	99.82	0.0	1007	
unknown_bird_S0030	gi 116832047 gb DQ432837.1	Chaetura pelagica voucher USNM 607660 COI	100	0.0	1018	
unknown_bird_S0031	gi 116832047 gb DQ432837.1	Chaetura pelagica voucher USNM 607660 COI	100	0.0	1186	
unknown_bird_S0039	gi 116832047 gb DQ432837.1	Chaetura pelagica voucher USNM 607660 COI	99.84	0.0	1181	
unknown_bird_S0041	gi 116832047 gb DQ432837.1	Chaetura pelagica voucher USNM 607660 COI	100	0.0	1181	
unknown_bird_S0091	gi 117371519 gb DQ434248.1	Agelaius phoeniceus voucher STA8011-35700 COI	100	0.0	1112	
unknown_bird_S0098	gi 117371519 gb DQ434248.1	Agelaius phoeniceus voucher STA8011-35700 COI	99.85	0.0	1218	
unknown_bird_S0131	gi 117372605 gb DQ434791.1	Turdus migratorius voucher HBO1192-19029 COI	100	0.0	1133	
unknown_bird_S0153	gi 117372687 gb DQ434832.1	Zenaida macroura voucher SPP1293-87989 COI	100	0.0	1070	
unknown_bird_S0192	gi 116832751 gb DQ433189.1	Spiza americana voucher MVZ:Bird:178371 COI	100	0.0	1064	
unknown_bird_S0199	gi 169882758 gb EU525509.1	Spizella passerina voucher VS 91 COI	98.85	0.0	1234	
unknown_bird_S0214	gi 117372443 gb DQ434710.1	Passerina cyanea voucher SPP2171-22282 COI	99.52	0.0	1147	
unknown_bird_S0225	gi 151336354 gb EF484234.1	Bombycilla cedrorum isolate BcedrBT0745 COI	100	0.0	1068	
unknown_bird_S0260	gi 117372609 gb DQ434793.1	Turdus migratorius voucher SPP1192-20843 COI	100	0.0	944	
unknown_bird_S0265	gi 116878133 gb DQ434236.1	Zenaida macroura voucher ARBNC015 COI	99.85	0.0	1227	mislabeled
unknown_bird_S0336	gi 116878133 gb DQ434236.1	Zenaida macroura voucher ARBNC015 COI	100	0.0	1179	
unknown_bird_S0346	gi 169882758 gb EU525509.1	Spizella passerina voucher VS 91 COI	100	0.0	1188	
unknown_bird_S0400	gi 116832411 gb DQ433019.1	Melospiza melodia voucher USNM 601592 COI	99.51	0.0	1109	
unknown_bird_S0450	gi 51101824 gb AY666364.1	Spizella arborea voucher MKP 1530 COI	99.84	0.0	1186	
unknown_bird_S0488	gi 117372687 gb DQ434832.1	Zenaida macroura voucher SPP1293-87989 COI	99.84	0.0	1182	
unknown_bird_S0491	gi 117372687 gb DQ434832.1	Zenaida macroura voucher SPP1293-87989 COI	99.55	0.0	1223	
unknown_bird_S0534	gi 262073353 gb GU013600.1	Gryllidae sp. DS-Test-027 COI	94.17	0.0	942	
unknown_bird_S0553	gi 116876305 gb DQ433322.1	Archilochus colubris mitochondrion, complete genome	99.41	0.0	1234	
unknown_bird_S0554	gi 6469728 gb AF197836.1 AF197836	Turdus migratorius COI	98.44	0.0	1240	
unknown_bird_S0560	gi 169882756 gb EU525508.1	Spizella passerina voucher VS 90 COI	98.73	0.0	1253	
unknown_bird_S0562	gi 156788382 gb EF525801.1 	Bufo fowleri voucher MAS1233-02 COI	100	0.0	994	
unknown_bird_S0651	gi 116878077 gb DQ434208.1	Tyrannus forficatus voucher MCZ.335886 COI	95.02	0.0	1042	

Table 13. Top BLAST hits for all mammal samples that were successfully sequenced at the CO1 gene. Samples that are in bold text resulted in an unexpected BLAST result (e.g. incorrect genus).

Sample	Accession Number	Description	Max Identity	E-value	Score	Comments
Lasiurus_borealis_S0063	gi 156078921 gb EU096752.1	Lasiurus blossevillei voucher ROM 117457 CO1	85.37	0.0	603	
Lasiurus_borealis_S0630	gi 117936820 gb EF080371.1	Lasiurus atratus voucher ROM 107228 CO1	85.02	0.0	665	
Mus_musculus_S0430	gi 3150274 gb L07095.1 MUSMTHYP	Mus domesticus strain NZB/B1NJ mitochondrion genome	99.84	0.0	1181	
Mustela_vison_S0657	gi 37575659 gb AY377152.1	Mustela vison CO1	99.84	0.0	1134	
Ondatra_zibethicus_S0408	gi 223470488 gb FJ527882.1	Mus musculus domesticus isolate 3 CO1	84.96	1.0E-169	604	
unknown_bat_S0242	gi 156078921 gb EU096752.1	Lasiurus blossevillei voucher ROM 117457 CO1	85.25	5.0E-169	603	
unknown_bat_S0243	gi 117936820 gb EF080371.1	Lasiurus atratus voucher ROM 107228 CO1	84.92	1.0E-170	608	
unknown_mammal_S0049	gi 156078497 gb EU095480.1	Proechimys gularis voucher ROM 106108 CO1	84.55	0.0	641	Ecuadoran spiny rat
unknown_mammal_S0049	gi 206583715 gb DQ989636.3	Cervus unicolor swinhoei mitochondrion, complete genome	83.33	3.0E-171	610	Formosan sambar
unknown_mammal_S0056	gi 12802821 gb AF330007.1	Spermophilus tridecemlineatus	98.44	0.0	1242	
unknown_mammal_S0217	gi 12802821 gb AF330007.1	Spermophilus tridecemlineatus	98.72	0.0	1247	
unknown_mammal_S0315	gi 156078497 gb EU095480.1 	Proechimys gularis voucher ROM 106108	84.4	1.0E-150	542	Ecuadoran spiny rat
unknown_mammal_S0320	gi 156079063 gb EU096823.1 	Hylaeamys megacephalus voucher ROM 117346	85.23	6.0E-178	632	Large-headed rice rat
unknown_mammal_S0320	gi 156078443 gb EU095453.1 	Oecomys rutilus voucher ROM 104473	85.41	6.0E-178	632	Red arboreal rice rat
unknown_mammal_S0333	gi 8347068 emb AJ238588.1	Sciurus vulgaris complete mitochondrial genome CO1	86.99	4.0E-175	623	
unknown_mammal_S0333	gi 156078501 gb EU095482.1	Proechimys hoplomys voucher ROM 115908 CO1	85.02	5.0E-159	569	
unknown_mammal_S0384	gi 223470488 gb FJ527882.1	Mus musculus domesticus isolate 3 CO1	85.07	3.0E-171	610	
unknown_mammal_S0384	gi 215882397 gb FJ483847.1	Eothenomys chinensis mitochondrion, complete genome	84.58	3.0E-166	593	Sichuan red-backed vole
unknown_mammal_S0438	gi 215882397 gb FJ483847.1 	Eothenomys chinensis mitochondrion, complete genome	84.25	2.0E-177	630	Pratt's vole
unknown_mammal_S0438	gi 14599791 gb AF348082.1 	Microtus kikuchii mitochondrion, complete genome	84.53	2.0E-177	630	Taiwan vole
unknown_mammal_S0604	gi 12802821 gb AF330007.1	Spermophilus tridecemlineatus	99.17	0.0	1088	
unknown_mammal_S0674	gi 213516889 gb FJ463038.1 	Proedromys sp. BBH-2008 mitochondrion, complete genome	86.63	0.0	734	
unknown_mammal_S0674	gi 156078517 gb EU095490.1 	Rhipidomys nitela voucher ROM 103290 CO1	83.7	5.0E-169	603	
unknown_mammal_S0717	gi 213516889 gb FJ463038.1	Proedromys sp. BBH-2008 mitochondrion, complete genome	86.96	0.0	725	
unknown_mammal_S0717	gi 156079065 gb EU096824.1 	Hylaeamys megacephalus voucher ROM 117384 CO1	83.78	6.0E-168	599	Large-headed rice rat
unknown_sciuridae_S0338	gi 8347068 gb AJ238588.1	Sciurus vulgaris complete mitochondrial genome	86	1.0E-174	621	
Zapus_hudsonius_S0418	gi 189343263 gb EU770567.1	Tropocyclops parvus voucher ZPLMX793 CO1	95	0.0	1009	
unknown_rodent_S0302	gi 156078465 gb EU095464.1 	Euryoryzomys macconnelli voucher ROM 106056 CO1	85	0.0	675	MacConnell's rice rat
unknown_rodent_S0302	gi 156078443 gb EU095453.1	Oecomys rutilus voucher ROM 104473 CO1	85	0.0	660	
unknown_rodent_S0429	gi 3150274 gb L07095.1 MUSMTHYP	Mus domesticus strain NZB/B1NJ mitochondrion genome	100	0.0	1061	

Table 14. Akaike Information Criterion (AIC) model selection for predicting roadkill using a negative binomial model (NBL). Models with a smaller AIC value are considered to be a better fitting model and should be used for data analysis. The total model includes ground cover, average temperature, average precipitation, and traffic volume.

Model	Factors	AIC	Chi-square	DF
Total	Total Model	5716.26	1324.33	1042
	Ground Cover, Temperature, Precipitation	5752.54	1342.87	1043
	Ground Cover and Temperature	5816.19	1377.43	1044
	Ground Cover	6652.9	1840.31	1045
Amphibians	Total Model	4940.75	1291.17	1042
	Ground Cover, Temperature, Precipitation	5036.97	1230.24	1043
	Ground Cover and Temperature	5111.97	1256.84	1044
	Ground Cover	5910.57	1696.91	1045
Reptiles	Total Model	1298.41	1030.93	1042
	Ground Cover, Temperature, Precipitation	1306.25	1204.68	1043
	Ground Cover and Temperature	1376.11	1338.02	1044
	Ground Cover	1437.13	1288.42	1045
Mammals	Total Model	2397.54	1183.67	1042
	Ground Cover, Temperature, Precipitation	2451.31	1127.97	1043
	Ground Cover and Temperature	2462.45	1205.99	1044
	Ground Cover	2545.78	1200.04	1045
Birds	Total Model	1748.27	1058.46	1042
	Ground Cover, Temperature, Precipitation	1777.33	1097.77	1043
	Ground Cover and Temperature	1776.76	1065.61	1044
	Ground Cover	1939.2	1097.77	1045

Table 15. Akaike Information Criterion (AIC) model selection for predicting roadkill using a binary logistic regression model (BLR). Models with a smaller AIC value are considered to be a better fitting model and should be used for data analysis. The total model includes ground cover, average temperature, average precipitation, and traffic volume.

Model	Factors	AIC	Chi-square	DF
Total	Total Model	722.71	1014.85	1042
	Ground Cover, Temperature, Precipitation	739.52	977.61	1043
	Ground Cover and Temperature	761.21	983.44	1044
	Ground Cover	1193.01	983.44	1045
Amphibians	Total Model	669.41	1037.28	1042
	Ground Cover, Temperature, Precipitation	670.17	1058.64	1043
	Ground Cover and Temperature	694.56	1254.84	1044
	Ground Cover	1418.18	1063	1045
Reptiles	Total Model	739.5	926.48	1042
	Ground Cover, Temperature, Precipitation	745.2	998.98	1043
	Ground Cover and Temperature	805.3	975.7	1044
	Ground Cover	889.76	1063	1045
Mammals	Total Model	1249.91	1046.39	1042
	Ground Cover, Temperature, Precipitation	1313.9	1044.92	1043
	Ground Cover and Temperature	1312.03	1046.77	1044
	Ground Cover	1381.36	1056	1045
Birds	Total Model	1104.68	1050.19	1042
	Ground Cover, Temperature, Precipitation	1116.48	1043.09	1043
	Ground Cover and Temperature	1114.77	1043.91	1044
	Ground Cover	1260.1	1055	1045

Table 16. Pre- and post-harvest road mortalities at 5 study sites.

Pre-Harvest											
	231 Ag		52 Ag		26 Ag		26 Mix		52 Mix		Total
	Corn	Soy	Corn	Soy	Corn	Soy	Corn	Soy	Corn	Soy	
Mammals	15	0	9	N/A	4	7	1	4	0	7	47
Birds	13	1	6	N/A	0	0	0	1	3	4	28
Herps	34	2	117	N/A	208	78	7	13	34	31	524
Post-Harvest											
	231 Ag		52 Ag		26 Ag		26 Mix		52 Mix		Total
	Corn	Soy	Corn	Soy	Corn	Soy	Corn	Soy	Corn	Soy	
Mammals	6	2	8	N/A	3	2	2	1	0	3	27
Birds	0	0	0	N/A	0	0	0	0	0	0	0
Herps	0	0	0	N/A	0	0	0	1	0	1	2

Figure 1. Map of all 12 roadkill survey routes in Indiana.

Figure 2. Monthly roadkill totals throughout a 20-month field season. a) monthly amphibian mortality across all 12 sites; b) monthly mortality across all 12 sites including birds, mammals, and reptiles but not amphibians (note the difference in scale compared to panel a); c) total monthly mortality across all 12 sites and all 4 taxa plotted against monthly mean temperature and monthly mean precipitation.

Figure 3. Neighbor-joining tree that illustrates the benefits and consequences of DNA barcoding for roadkill identification. Clade A illustrates how the reference sample for *Anaxyrus americanus* collected during this study matches a sequence for the same species from GenBank. Also, this clade contains some samples incorrectly identified in the field because an “unknown frog” was more closely related to toads than to frogs. Clade B represents that some of the unknown frogs paired with a *Lithobates catesbeianus* reference sample, indicated the unknown specimens were probably bullfrogs. Clade C serves as an outgroup for this tree and simply shows both bird samples had the highest level of sequence divergence versus all the other reptile and amphibian samples in this tree.

Figure 4. Map of US 231 Wetland survey route, 100-meter sections, and manual digitization of land cover classes.

Figure 5. Map of US 231 Dynamic Wetland survey route, 100-meter sections, and manual digitization of land cover classes.

Figure 6. Map of SR 26 Ag/Forest Mix survey route, 100-meter sections, and manual digitization of land cover classes.

Figure 7. Map of SR 26 Ag survey route, 100-meter sections, and manual digitization of land cover classes.

Figure 8. Map of Lindberg survey route, 100-meter sections, and manual digitization of land cover classes.

Figure 9. Map of SR26 Wet survey route, 100-meter sections, and manual digitization of land cover classes.

Figure 10. Map of Prophetstown survey route, 100-meter sections, and manual digitization of land cover classes

Figure 11. Map of SR38 Ag/Forest Mix survey route, 100-meter sections, and manual digitization of land cover classes.

Figure 12. Map of US 231 South Ag survey route, 100-meter sections, and manual digitization of land cover classes.

Figure 13. Map of US 52 Wet survey route, 100-meter sections, and manual digitization of land cover classes.

Figure 14. Map of US 52 Ag survey route, 100-meter sections, and manual digitization of land cover classes.

Figure 15. Map of 52 Ag/Forest Mix survey route, 100-meter sections, and manual digitization of land cover classes.

Figure 16. Distribution of total road mortalities across the SR 26 Ag survey route by 100 meter section (Blue – amphibian; Green – reptile; Red – bird; Gold – mammal).

Figure 17. Distribution of total road mortalities across the Lindberg survey route by 100 meter section (Blue – amphibian; Green – reptile; Red – bird; Gold – mammal).

Figure 18. Distribution of total road mortalities across the SR 26 Wet survey route by 100 meter section (Blue – amphibian; Green – reptile; Red – bird; Gold – mammal).

Figure 19. Live box turtle on side of roadway at SR 26 Mix survey route.

Figure 20. Coyote on road shoulder at US 52 Mix survey route used to illustrate marking technique which was used to avoid double-counting individuals.

Figure 21. Photo of SR 26 Wet survey route.

Panel A. Total monthly amphibian mortality (across all 12 sites) during this 20-month survey.

Panel B. Total monthly roadkill mortality (across all 12 sites) and divided by taxonomic category during this 20-month survey.

Panel C. Total monthly mortality plotted with average temperature and precipitation to allow clarification of the relationship between road mortality, temperature, and precipitation.

0 125 250 500 Meters

Legend

Landcover_231Wet_Intersect

LandCoverT

- Ag/Pasture
- Grass/Shrub Ditch
- Prairie/Grassland
- Road
- Urban/Rec Grass
- Urban/Res
- Water/Wetland

0 125 250 500 Meters

Legend

Landcover_26Mix_Intersect

LandCoverT

- Ag/Pasture
- Forest/Woodlot
- Road
- Urban/Rec Grass
- Urban/Res

0 125 250 500 Meters

Legend

Landcover_26Ag_Inters ect

LandcoverT

- Ag/Pasture
- Forest/Woodlot
- Grass/Shrub Ditch
- Prairie/Grassland
- Road
- Urban/Rec Grass
- Urban/Res

0 125 250 500 Meters

Legend

Landcover_Lindberg_Intersect

LandCoverT

- Forest/Woodlot
- Road
- Urban/Rec Grass
- Urban/Res
- Water/Wetland

0 125 250 500 Meters

0 125 250 500 Meters

Legend

Landcover_Ptown_Intersect

LandCoverT

- Forest/Woodlot
- Grass/Shrub Ditch
- Prairie/Grassland
- Road
- Urban/Rec Grass
- Urban/Res
- Water/Wetland

0 125 250 500 Meters

Legend

Landcover_38Mix_Intersect

LandCoverT

- Ag/Pasture
- Forest/Woodlot
- Road
- Urban/Rec Grass
- Urban/Res

0 125 250 500 Meters

Legend

Landcover_231SAg_Intersect

LandCoverT

- Ag/Pasture
- Road
- Urban/Rec Grass
- Urban/Res

0 125 250 500 Meters

Legend

Landcover_52Wet_Intersect

LandCoverT

- Ag/Pasture
- Forest/Woodlot
- Grass/Shrub/Ditch
- Prairie/Grassland
- Road
- Urban/Recreational Grass
- Urban/Residential
- Water/Wetland

0 125 250 500 Meters

Legend

Landcover_52Ag_Intersect

LandCover

- Ag/Pasture
- Grass/Shrub Ditch
- Road

Legend**Landcover_52Mix_Intersect****LandCoverT**

- Ag/Pasture
- Forest/Woodlot
- Grass/Shrub Ditch
- Road
- Urban/Rec Grass
- Urban/Res

APPENDIX

Table A1. US 231 Dynamic land cover classification based on 100-meter section and side of the roadway. The CalculateArea tool was used to determine the dominant land cover class per section via the total area in square meters was used. Side “0” represents the road/transect and was excluded to determine the habitat type on each side of the road. Side “1” represents the north or west side of the roadway and Side “2” represents the south or east side of the road.

Section	Side of Road	Land Cover Type	Area (m ²)
0	0	Road	1018
0	1	Forest/Woodlot	5806
0	1	Urban/Res	182
0	2	Ag/Pasture	4755
1	0	Road	961
1	1	Forest/Woodlot	7039
1	1	Urban/Rec Grass	286
1	1	Urban/Res	251
1	2	Ag/Pasture	6775
2	0	Road	1029
2	1	Forest/Woodlot	5925
2	1	Urban/Rec Grass	1526
2	2	Ag/Pasture	6716
3	0	Road	1122
3	1	Forest/Woodlot	6706
3	1	Urban/Rec Grass	717
3	2	Ag/Pasture	2608
3	2	Forest/Woodlot	4040
4	0	Road	1194
4	1	Forest/Woodlot	7301
4	2	Forest/Woodlot	6705
5	0	Road	1235
5	1	Forest/Woodlot	4386
5	1	Urban/Rec Grass	2576
5	1	Urban/Res	210
5	2	Ag/Pasture	5090
5	2	Forest/Woodlot	1618
6	0	Road	1784
6	1	Urban/Rec Grass	6732
6	2	Ag/Pasture	6864
7	0	Road	1366
7	1	Urban/Rec Grass	7026
7	2	Ag/Pasture	6901
8	0	Road	1316
8	1	Ag/Pasture	3647
8	1	Urban/Rec Grass	3168
8	2	Ag/Pasture	6807
9	0	Road	1389

9	1	Ag/Pasture	6817
9	2	Ag/Pasture	6997
10	0	Road	1296
10	1	Ag/Pasture	6873
10	2	Ag/Pasture	7122
11	0	Road	2471
11	1	Ag/Pasture	2552
11	1	Urban/Rec Grass	1887
11	1	Urban/Res	151
11	2	Ag/Pasture	4937

Table A2. US 231 S Ag land cover classification based on 100-meter section and side of the roadway. The CalculateArea tool was used to determine the dominant land cover class per section via the total area in. Side “0” represents the road/transect and was excluded to determine the habitat type on each side of the road. Side “1” represents the north or west side of the roadway and Side “2” represents the south or east side of the road.

Section	Side of Road	Land Cover Type	Area (m²)
0	0	Road	924
0	1	Ag/Pasture	5467
0	2	Ag/Pasture	5382
1	0	Road	978
1	1	Ag/Pasture	7089
1	2	Ag/Pasture	7001
2	0	Road	1046
2	1	Ag/Pasture	7848
2	2	Ag/Pasture	7829
3	0	Road	1025
3	1	Ag/Pasture	7038
3	2	Ag/Pasture	6630
3	2	Urban/Res	304
4	0	Road	1076
4	1	Ag/Pasture	7118
4	2	Ag/Pasture	6961
5	0	Road	1094
5	1	Ag/Pasture	7283
5	2	Ag/Pasture	7061
6	0	Road	1065
6	1	Ag/Pasture	7041
6	2	Ag/Pasture	4086
6	2	Urban/Rec Grass	2438
6	2	Urban/Res	291
7	0	Road	1083
7	1	Ag/Pasture	7182
7	2	Ag/Pasture	6899
7	2	Urban/Rec Grass	56
8	0	Road	1050
8	1	Ag/Pasture	7463
8	2	Urban/Rec Grass	6712
8	2	Urban/Res	508
9	0	Road	836
9	1	Ag/Pasture	7196
9	2	Urban/Res	352
9	2	Urban/Rec Grass	6610
10	0	Road	921
10	1	Ag/Pasture	7329

10	2	Ag/Pasture	1826
10	2	Urban/Rec Grass	4705
10	2	Urban/Res	468
11	0	Road	1334
11	1	Ag/Pasture	7122
11	2	Ag/Pasture	5594
11	2	Urban/Rec Grass	1096
12	0	Road	1665
12	1	Ag/Pasture	5430
12	2	Ag/Pasture	4138
12	2	Urban/Rec Grass	698

Table A3. US 231 Wet land cover classification based on 100-meter section and side of the roadway. The CalculateArea tool was used to determine the dominant land cover class per section via the total area in square meters was used. Side “0” represents the road/transect and was excluded to determine the habitat type on each side of the road. Side “1” represents the north or west side of the roadway and Side “2” represents the south or east side of the road.

Section	Side of Road	Land Cover Type	Area (m ²)
0	0	Road	1269
0	1	Ag/Pasture	5568
0	1	Grass/Shrub Ditch	497
0	2	Ag/Pasture	4571
1	0	Road	1151
1	1	Ag/Pasture	6967
1	1	Grass/Shrub Ditch	530
1	2	Ag/Pasture	6467
2	0	Road	1208
2	1	Ag/Pasture	6671
2	1	Grass/Shrub Ditch	567
2	2	Ag/Pasture	6696
3	0	Road	1448
3	1	Ag/Pasture	6245
3	1	Grass/Shrub Ditch	597
3	2	Ag/Pasture	6852
4	0	Road	2698
4	1	Ag/Pasture	2789
4	1	Grass/Shrub Ditch	592
4	1	Prairie/Grassland	2810
4	2	Ag/Pasture	3180
4	2	Urban/Rec Grass	3197
5	0	Road	1216
5	1	Prairie/Grassland	6531
5	2	Ag/Pasture	1594
5	2	Urban/Rec Grass	5652
6	0	Road	1318
6	1	Prairie/Grassland	6311
6	1	Water/Wetland	516
6	2	Ag/Pasture	7231
7	0	Road	1420
7	1	Prairie/Grassland	3752
7	1	Water/Wetland	3179
7	2	Ag/Pasture	7041
8	0	Road	1377
8	1	Prairie/Grassland	3839
8	1	Urban/Rec Grass	1803
8	1	Urban/Res	454

8	1	Water/Wetland	85
8	2	Ag/Pasture	6601
8	2	Urban/Rec Grass	594
9	0	Road	1482
9	1	Ag/Pasture	7039
9	1	Urban/Rec Grass	117
9	2	Ag/Pasture	6765
10	0	Road	1364
10	1	Ag/Pasture	7136
10	2	Ag/Pasture	6377
11	0	Road	1190
11	1	Ag/Pasture	7548
11	2	Ag/Pasture	6666
12	0	Road	1129
12	1	Ag/Pasture	6159
12	2	Ag/Pasture	5520

Table A4. SR 26 Ag land cover classification based on 100-meter section and side of the roadway. The CalculateArea tool was used to determine the dominant land cover class per section via the total area in square meters was used. Side “0” represents the road/transect and was excluded to determine the habitat type on each side of the road. Side “1” represents the north or west side of the roadway and Side “2” represents the south or east side of the road.

Section	Side of Road	Land Cover Type	Area (m²)
0	0	Road	1320
0	1	Ag/Pasture	5568
0	1	Grass/Shrub Ditch	252
0	2	Ag/Pasture	4709
0	2	Grass/Shrub Ditch	646
1	0	Road	908
1	1	Ag/Pasture	9296
1	1	Grass/Shrub Ditch	360
1	2	Ag/Pasture	9474
1	2	Grass/Shrub Ditch	386
2	0	Road	890
2	1	Ag/Pasture	9406
2	1	Grass/Shrub Ditch	278
2	2	Ag/Pasture	9311
2	2	Grass/Shrub Ditch	329
3	0	Road	830
3	1	Ag/Pasture	9504
3	1	Grass/Shrub Ditch	277
3	2	Ag/Pasture	9232
3	2	Grass/Shrub Ditch	385
4	0	Road	1434
4	1	Ag/Pasture	8009
4	1	Grass/Shrub Ditch	1107
4	2	Ag/Pasture	4351
4	2	Grass/Shrub Ditch	134
4	2	Prairie/Grassland	4825
5	0	Road	782
5	1	Ag/Pasture	9528
5	1	Grass/Shrub Ditch	236
5	2	Ag/Pasture	6782
5	2	Grass/Shrub Ditch	309
5	2	Prairie/Grassland	2351
6	0	Road	810
6	1	Ag/Pasture	9433
6	1	Grass/Shrub Ditch	282
6	2	Ag/Pasture	9150
6	2	Grass/Shrub Ditch	361
7	0	Road	773

7	1	Ag/Pasture	9521
7	1	Grass/Shrub Ditch	260
7	2	Ag/Pasture	9059
7	2	Grass/Shrub Ditch	364
8	0	Road	808
8	1	Ag/Pasture	9551
8	1	Grass/Shrub Ditch	253
8	2	Ag/Pasture	9038
8	2	Grass/Shrub Ditch	327
9	0	Road	839
9	1	Ag/Pasture	9525
9	1	Grass/Shrub Ditch	232
9	2	Ag/Pasture	9080
9	2	Grass/Shrub Ditch	183
10	0	Road	824
10	1	Ag/Pasture	9394
10	1	Forest/Woodlot	258
10	1	Grass/Shrub Ditch	198
10	2	Ag/Pasture	4307
10	2	Grass/Shrub Ditch	116
10	2	Urban/Rec Grass	4270
10	2	Urban/Res	604
11	0	Road	842
11	1	Ag/Pasture	4783
11	1	Forest/Woodlot	4619
11	1	Grass/Shrub Ditch	23
11	1	Urban/Res	207
11	1	Urban/Rec Grass	318
11	2	Ag/Pasture	8220
11	2	Grass/Shrub Ditch	432
11	2	Urban/Rec Grass	590
12	0	Road	855
12	1	Ag/Pasture	3762
12	1	Forest/Woodlot	2859
12	1	Grass/Shrub Ditch	642
12	1	Urban/Rec Grass	2318
12	1	Urban/Res	454
12	2	Ag/Pasture	8322
12	2	Grass/Shrub Ditch	762
13	0	Road	1876
13	1	Ag/Pasture	5085
13	1	Grass/Shrub Ditch	297
13	2	Ag/Pasture	4034
13	2	Grass/Shrub Ditch	708

Table A5. SR 26 Mix land cover classification based on 100-meter section and side of the roadway. The CalculateArea tool was used to determine the dominant land cover class per section via the total area in square meters was used. Side “0” represents the road/transect and was excluded to determine the habitat type on each side of the road. Side “1” represents the north or west side of the roadway and Side “2” represents the south or east side of the road.

Section	Side of Road	Land Cover Type	Area (m ²)
0	0	Road	579
0	1	Ag/Pasture	5635
0	2	Ag/Pasture	5341
1	0	Road	937
1	1	Ag/Pasture	11385
1	2	Ag/Pasture	10885
2	0	Road	829
2	1	Ag/Pasture	9771
2	2	Ag/Pasture	9550
2	2	Urban/Rec Grass	14
3	0	Road	741
3	1	Ag/Pasture	6206
3	1	Urban/Rec Grass	3034
3	1	Urban/Res	119
3	2	Ag/Pasture	8519
3	2	Urban/Rec Grass	842
4	0	Road	1475
4	1	Ag/Pasture	8084
4	1	Urban/Rec Grass	1528
4	1	Urban/Res	62
4	2	Ag/Pasture	1172
4	2	Urban/Rec Grass	7293
4	2	Urban/Res	577
5	0	Road	1537
5	1	Ag/Pasture	9567
5	2	Urban/Rec Grass	7837
5	2	Urban/Res	1216
6	0	Road	874
6	1	Ag/Pasture	8662
6	1	Forest/Woodlot	857
6	2	Ag/Pasture	9070
6	2	Urban/Rec Grass	643
7	0	Road	753
7	1	Forest/Woodlot	9355
7	2	Ag/Pasture	9555
8	0	Road	1151
8	1	Ag/Pasture	5990
8	1	Forest/Woodlot	3543

8	2	Ag/Pasture	1741
8	2	Urban/Rec Grass	7218
8	2	Urban/Res	338
9	0	Road	741
9	1	Ag/Pasture	9584
9	2	Urban/Rec Grass	9509
9	2	Urban/Res	203
10	0	Road	624
10	1	Ag/Pasture	9579
10	2	Ag/Pasture	9215
10	2	Urban/Rec Grass	418
11	0	Road	736
11	1	Ag/Pasture	1602
11	1	Forest/Woodlot	1960
11	1	Urban/Rec Grass	7636
11	1	Urban/Res	523
11	2	Ag/Pasture	96
11	2	Forest/Woodlot	4789
11	2	Urban/Rec Grass	6007
11	2	Urban/Res	707
12	0	Road	1121
12	1	Forest/Woodlot	1355
12	1	Urban/Rec Grass	4835
12	1	Urban/Res	319
12	2	Forest/Woodlot	3656
12	2	Urban/Rec Grass	2102

Table A6. SR 26 Wet land cover classification based on 100-meter section and side of the roadway. The CalculateArea tool was used to determine the dominant land cover class per section via the total area in square meters was used. Side “0” represents the road/transect and was excluded to determine the habitat type on each side of the road. Side “1” represents the north or west side of the roadway and Side “2” represents the south or east side of the road.

Section	Side of Road	Land Cover Type	Area (m ²)
0	0	Road	544
0	1	Forest/Woodlot	5490
0	2	Ag/Pasture	3810
0	2	Forest/Woodlot	113
0	2	Urban/Rec Grass	1955
1	0	Road	691
1	1	Forest/Woodlot	5036
1	1	Grass/Shrub Ditch	63
1	1	Prairie/Grassland	1410
1	1	Urban/Rec Grass	1612
1	1	Water/Wetland	1092
1	2	Forest/Woodlot	6721
1	2	Urban/Rec Grass	2396
1	2	Urban/Res	269
2	0	Road	868
2	1	Ag/Pasture	341
2	1	Grass/Shrub Ditch	167
2	1	Prairie/Grassland	1650
2	1	Water/Wetland	577
2	1	Urban/Rec Grass	6408
2	1	Urban/Res	681
2	2	Forest/Woodlot	3893
2	2	Urban/Rec Grass	5511
2	2	Urban/Res	81
2	2	Water/Wetland	66
3	0	Road	773
3	1	Ag/Pasture	9580
3	1	Grass/Shrub Ditch	230
3	2	Forest/Woodlot	5029
3	2	Urban/Rec Grass	3690
3	2	Water/Wetland	855
4	0	Road	782
4	1	Ag/Pasture	8615
4	1	Grass/Shrub Ditch	520
4	1	Road	581
4	2	Forest/Woodlot	7158
4	2	Urban/Rec Grass	1636
4	2	Urban/Res	598

4	2	Water/Wetland	97
5	0	Road	765
5	1	Road	19
5	1	Ag/Pasture	9152
5	1	Grass/Shrub Ditch	722
5	2	Water/Wetland	3131
5	2	Urban/Res	139
5	2	Urban/Rec Grass	4585
5	2	Forest/Woodlot	1543
5	2	Urban/Rec Grass	70
6	0	Road	745
6	1	Ag/Pasture	7076
6	1	Forest/Woodlot	2072
6	1	Grass/Shrub Ditch	293
6	1	Water/Wetland	203
6	2	Urban/Rec Grass	1033
6	2	Water/Wetland	8353
7	0	Road	779
7	1	Ag/Pasture	4682
7	1	Forest/Woodlot	4423
7	1	Grass/Shrub Ditch	225
7	1	Water/Wetland	450
7	2	Grass/Shrub Ditch	88
7	2	Urban/Rec Grass	4487
7	2	Water/Wetland	5373
8	0	Road	707
8	1	Ag/Pasture	3562
8	1	Forest/Woodlot	5364
8	1	Grass/Shrub Ditch	103
8	1	Urban/Rec Grass	39
8	2	Forest/Woodlot	436
8	2	Urban/Rec Grass	8855
9	0	Road	840
9	1	Ag/Pasture	4322
9	1	Forest/Woodlot	3608
9	1	Grass/Shrub Ditch	78
9	1	Urban/Rec Grass	1686
9	2	Forest/Woodlot	8186
9	2	Urban/Rec Grass	1370
9	2	Urban/Res	417
10	0	Road	699
10	1	Ag/Pasture	9167
10	1	Grass/Shrub Ditch	319
10	2	Forest/Woodlot	6844
10	2	Urban/Rec Grass	2578
10	2	Urban/Res	219
11	0	Road	739

11	1	Ag/Pasture	7626
11	1	Forest/Woodlot	1805
11	1	Grass/Shrub Ditch	220
11	2	Forest/Woodlot	285
11	2	Urban/Rec Grass	8647
11	2	Urban/Res	717
12	0	Road	620
12	1	Forest/Woodlot	5831
12	1	Grass/Shrub Ditch	122
12	2	Urban/Rec Grass	5569
12	2	Urban/Res	284

Table A7. SR 38 Mix land cover classification based on 100-meter section and side of the roadway. The CalculateArea tool was used to determine the dominant land cover class per section via the total area in square meters was used. Side “0” represents the road/transect and was excluded to determine the habitat type on each side of the road. Side “1” represents the north or west side of the roadway and Side “2” represents the south or east side of the road.

Section	Side of Road	Land Cover Type	Area (m ²)
0	0	Road	691
0	1	Ag/Pasture	6872
0	2	Urban/Rec Grass	4197
0	2	Urban/Res	239
1	0	Road	1323
1	1	Ag/Pasture	6819
1	1	Urban/Rec Grass	535
1	2	Ag/Pasture	7443
1	2	Forest/Woodlot	957
2	0	Road	917
2	1	Ag/Pasture	9385
2	1	Urban/Rec Grass	730
2	2	Urban/Res	407
2	2	Forest/Woodlot	8336
3	0	Road	861
3	1	Ag/Pasture	9869
3	1	Urban/Rec Grass	795
3	2	Forest/Woodlot	9040
4	0	Road	864
4	1	Ag/Pasture	9672
4	1	Urban/Rec Grass	690
4	2	Forest/Woodlot	8627
4	2	Urban/Rec Grass	34
4	2	Urban/Res	94
5	0	Road	840
5	1	Ag/Pasture	9403
5	1	Urban/Rec Grass	665
5	2	Ag/Pasture	151
5	2	Forest/Woodlot	6509
5	2	Urban/Rec Grass	1452
5	2	Urban/Res	434
6	0	Road	849
6	1	Ag/Pasture	9828
6	1	Urban/Rec Grass	687
6	2	Ag/Pasture	8442
6	2	Urban/Rec Grass	446
7	0	Road	756
7	1	Ag/Pasture	7053

7	1	Urban/Rec Grass	3292
7	2	Ag/Pasture	8411
7	2	Urban/Rec Grass	475
8	0	Road	1204
8	1	Ag/Pasture	6651
8	1	Urban/Rec Grass	3074
8	2	Ag/Pasture	8391
8	2	Urban/Rec Grass	397
9	0	Road	858
9	1	Ag/Pasture	10222
9	2	Ag/Pasture	8595
9	2	Urban/Rec Grass	543
10	0	Road	840
10	1	Ag/Pasture	10234
10	2	Ag/Pasture	8698
10	2	Urban/Rec Grass	676
11	0	Road	2158
11	1	Ag/Pasture	11946
11	2	Ag/Pasture	3233
11	2	Urban/Rec Grass	6964
11	2	Urban/Res	593
12	0	Road	1797
12	1	Ag/Pasture	4985
12	2	Urban/Rec Grass	4479
12	2	Urban/Res	526

Table A8. US 52 Ag land cover classification based on 100-meter section and side of the roadway. The CalculateArea tool was used to determine the dominant land cover class per section via the total area in square meters was used. Side “0” represents the road/transect and was excluded to determine the habitat type on each side of the road. Side “1” represents the north or west side of the roadway and Side “2” represents the south or east side of the road.

Section	Side of Road	Land Cover Type	Area (m ²)
0	0	Grass/Shrub Ditch	1673
0	0	Road	2499
0	1	Ag/Pasture	3073
0	1	Grass/Shrub Ditch	690
0	2	Ag/Pasture	4242
0	2	Grass/Shrub Ditch	947
1	0	Grass/Shrub Ditch	737
1	0	Road	2383
1	1	Ag/Pasture	6202
1	1	Grass/Shrub Ditch	657
1	2	Ag/Pasture	6114
1	2	Grass/Shrub Ditch	795
2	0	Grass/Shrub Ditch	821
2	0	Road	2000
2	1	Ag/Pasture	6448
2	1	Grass/Shrub Ditch	738
2	2	Ag/Pasture	6752
2	2	Grass/Shrub Ditch	736
3	0	Grass/Shrub Ditch	753
3	0	Road	2038
3	1	Ag/Pasture	6353
3	1	Grass/Shrub Ditch	772
3	2	Ag/Pasture	6647
3	2	Grass/Shrub Ditch	821
4	0	Grass/Shrub Ditch	734
4	0	Road	1970
4	1	Ag/Pasture	6571
4	1	Grass/Shrub Ditch	770
4	2	Ag/Pasture	6756
4	2	Grass/Shrub Ditch	797
5	0	Grass/Shrub Ditch	688
5	0	Road	1884
5	1	Ag/Pasture	6590
5	1	Grass/Shrub Ditch	659
5	2	Ag/Pasture	6688
5	2	Grass/Shrub Ditch	745
6	0	Grass/Shrub Ditch	635
6	0	Road	1889

6	1	Ag/Pasture	6663
6	1	Grass/Shrub Ditch	736
6	2	Ag/Pasture	6687
6	2	Grass/Shrub Ditch	756
7	0	Grass/Shrub Ditch	634
7	0	Road	1860
7	1	Ag/Pasture	6634
7	1	Grass/Shrub Ditch	851
7	2	Ag/Pasture	6855
7	2	Grass/Shrub Ditch	624
8	0	Grass/Shrub Ditch	653
8	0	Road	2042
8	1	Ag/Pasture	5855
8	1	Grass/Shrub Ditch	1195
8	2	Ag/Pasture	6603
8	2	Grass/Shrub Ditch	687
9	0	Grass/Shrub Ditch	466
9	0	Road	3656
9	1	Ag/Pasture	4625
9	1	Grass/Shrub Ditch	1977
9	2	Ag/Pasture	5548
9	2	Grass/Shrub Ditch	1036
10	0	Grass/Shrub Ditch	680
10	0	Road	2277
10	1	Ag/Pasture	6436
10	1	Grass/Shrub Ditch	1027
10	2	Ag/Pasture	6024
10	2	Grass/Shrub Ditch	969
11	0	Grass/Shrub Ditch	586
11	0	Road	2237
11	1	Ag/Pasture	7307
11	1	Grass/Shrub Ditch	1119
11	2	Ag/Pasture	7485
11	2	Grass/Shrub Ditch	646
12	0	Grass/Shrub Ditch	591
12	0	Road	2008
12	1	Ag/Pasture	5504
12	1	Grass/Shrub Ditch	1041
12	2	Ag/Pasture	3436
12	2	Grass/Shrub Ditch	911

Table A9. US 52 Mix land cover classification based on 100-meter section and side of the roadway. The CalculateArea tool was used to determine the dominant land cover class per section via the total area in square meters was used. Side “0” represents the road/transect and was excluded to determine the habitat type on each side of the road. Side “1” represents the north or west side of the roadway and Side “2” represents the south or east side of the road.

Section	Side of Road	Land Cover Type	Area (m²)
0	0	Grass/Shrub Ditch	494
0	0	Road	1782
0	1	Forest/Woodlot	705
0	1	Grass/Shrub Ditch	43
0	1	Urban/Rec Grass	1653
0	1	Urban/Res	1335
0	2	Ag/Pasture	4517
0	2	Grass/Shrub Ditch	1179
1	0	Grass/Shrub Ditch	587
1	0	Road	1938
1	1	Ag/Pasture	1036
1	1	Forest/Woodlot	4597
1	1	Grass/Shrub Ditch	227
1	1	Urban/Rec Grass	1480
1	1	Urban/Res	173
1	2	Ag/Pasture	6152
1	2	Grass/Shrub Ditch	1229
2	0	Grass/Shrub Ditch	642
2	0	Road	1888
2	1	Urban/Rec Grass	3007
2	1	Forest/Woodlot	4238
2	1	Grass/Shrub Ditch	303
2	2	Ag/Pasture	6096
2	2	Grass/Shrub Ditch	1255
3	0	Grass/Shrub Ditch	622
3	0	Road	1949
3	1	Ag/Pasture	1174
3	1	Forest/Woodlot	5694
3	1	Grass/Shrub Ditch	394
3	1	Urban/Rec Grass	268
3	2	Ag/Pasture	5849
3	2	Grass/Shrub Ditch	1262
4	0	Grass/Shrub Ditch	570
4	0	Road	2141
4	1	Ag/Pasture	6608
4	1	Forest/Woodlot	325
4	1	Grass/Shrub Ditch	425
4	1	Urban/Rec Grass	252

4	2	Ag/Pasture	5760
4	2	Grass/Shrub Ditch	1217
5	0	Grass/Shrub Ditch	571
5	0	Road	2358
5	1	Ag/Pasture	2265
5	1	Forest/Woodlot	2208
5	1	Grass/Shrub Ditch	305
5	1	Urban/Rec Grass	2991
5	1	Urban/Res	177
5	2	Ag/Pasture	2855
5	2	Forest/Woodlot	221
5	2	Grass/Shrub Ditch	1390
5	2	Urban/Rec Grass	2080
5	2	Urban/Res	549
6	0	Grass/Shrub Ditch	645
6	0	Road	2049
6	1	Ag/Pasture	6540
6	1	Urban/Rec Grass	860
6	2	Forest/Woodlot	4853
6	2	Urban/Rec Grass	1632
6	2	Urban/Res	211
7	0	Grass/Shrub Ditch	625
7	0	Road	2108
7	1	Ag/Pasture	4404
7	1	Forest/Woodlot	1448
7	1	Road	322
7	1	Urban/Rec Grass	1508
7	1	Urban/Res	72
7	2	Ag/Pasture	3210
7	2	Forest/Woodlot	608
7	2	Grass/Shrub Ditch	88
7	2	Urban/Rec Grass	2689
7	2	Urban/Res	179
8	0	Grass/Shrub Ditch	489
8	0	Road	2444
8	1	Forest/Woodlot	3930
8	1	Urban/Rec Grass	1244
8	1	Urban/Res	2038
8	2	Ag/Pasture	7628
8	2	Forest/Woodlot	1736
8	2	Grass/Shrub Ditch	275
8	2	Urban/Rec Grass	448
9	0	Grass/Shrub Ditch	522
9	0	Road	4310
9	1	Forest/Woodlot	371
9	1	Urban/Rec Grass	6115
9	2	Forest/Woodlot	1145
9	2	Grass/Shrub Ditch	332

9	2	Urban/Rec Grass	1720
10	0	Grass/Shrub Ditch	487
10	0	Road	4186
10	1	Urban/Rec Grass	6525
10	2	Ag/Pasture	6074
10	2	Grass/Shrub Ditch	710
11	0	Grass/Shrub Ditch	604
11	0	Road	2455
11	1	Ag/Pasture	97
11	1	Urban/Rec Grass	6182
11	1	Urban/Res	1723
11	2	Ag/Pasture	7298
11	2	Grass/Shrub Ditch	1048
12	0	Grass/Shrub Ditch	465
12	0	Road	2048
12	1	Ag/Pasture	4068
12	1	Urban/Rec Grass	1712
12	2	Ag/Pasture	3609
12	2	Grass/Shrub Ditch	754

Table A10. US 52 Wet land cover classification based on 100-meter section and side of the roadway. The CalculateArea tool was used to determine the dominant land cover class per section via the total area in square meters was used. Side “0” represents the road/transect and was excluded to determine the habitat type on each side of the road. Side “1” represents the north or west side of the roadway and Side “2” represents the south or east side of the road.

Section	Side of Road	Land Cover Type	Area (m ²)
0	0	Grass/Shrub Ditch	464
0	0	Road	1259
0	1	Ag/Pasture	1427
0	1	Grass/Shrub Ditch	818
0	2	Grass/Shrub Ditch	812
0	2	Forest/Woodlot	4037
1	0	Grass/Shrub Ditch	571
1	0	Road	1951
1	0	Water/Wetland	67
1	1	Ag/Pasture	3416
1	1	Forest/Woodlot	1850
1	1	Grass/Shrub Ditch	1310
1	1	Water/Wetland	31
1	2	Forest/Woodlot	7347
1	2	Water/Wetland	741
2	0	Grass/Shrub Ditch	446
2	0	Road	2027
2	0	Water/Wetland	133
2	1	Ag/Pasture	809
2	1	Forest/Woodlot	3839
2	1	Urban/Rec Grass	265
2	1	Water/Wetland	1639
2	2	Forest/Woodlot	7186
2	2	Water/Wetland	797
3	0	Grass/Shrub Ditch	776
3	0	Road	1811
3	1	Ag/Pasture	4538
3	1	Forest/Woodlot	2017
3	2	Ag/Pasture	566
3	2	Forest/Woodlot	7577
4	0	Grass/Shrub Ditch	735
4	0	Road	1951
4	1	Ag/Pasture	5306
4	1	Forest/Woodlot	354
4	1	Grass/Shrub Ditch	922
4	2	Ag/Pasture	1128
4	2	Forest/Woodlot	6983
5	0	Grass/Shrub Ditch	727

5	0	Road	1903
5	1	Ag/Pasture	5450
5	1	Grass/Shrub Ditch	1065
5	2	Ag/Pasture	2751
5	2	Forest/Woodlot	5300
6	0	Grass/Shrub Ditch	733
6	0	Road	1870
6	1	Ag/Pasture	5904
6	1	Grass/Shrub Ditch	986
6	2	Ag/Pasture	4982
6	2	Forest/Woodlot	3604
7	0	Grass/Shrub Ditch	644
7	0	Road	1662
7	1	Ag/Pasture	5501
7	1	Grass/Shrub Ditch	1025
7	2	Ag/Pasture	6285
7	2	Forest/Woodlot	938
7	2	Grass/Shrub Ditch	599
8	0	Grass/Shrub Ditch	645
8	0	Road	1696
8	1	Ag/Pasture	3015
8	1	Grass/Shrub Ditch	845
8	1	Prairie/Grassland	2842
8	2	Ag/Pasture	2227
8	2	Grass/Shrub Ditch	463
8	2	Prairie/Grassland	5183
9	0	Grass/Shrub Ditch	699
9	0	Road	1717
9	1	Grass/Shrub Ditch	544
9	1	Prairie/Grassland	3051
9	1	Water/Wetland	3406
9	2	Grass/Shrub Ditch	392
9	2	Prairie/Grassland	2687
9	2	Water/Wetland	4673
10	0	Grass/Shrub Ditch	744
10	0	Road	1876
10	1	Grass/Shrub Ditch	1011
10	1	Prairie/Grassland	1965
10	1	Urban/Res	137
10	1	Urban/Rec Grass	1118
10	1	Water/Wetland	3092
10	2	Grass/Shrub Ditch	656
10	2	Prairie/Grassland	6528
10	2	Water/Wetland	351
11	0	Grass/Shrub Ditch	633
11	0	Road	2938
11	1	Ag/Pasture	3460
11	1	Grass/Shrub Ditch	862

11	1	Prairie/Grassland	31
11	1	Urban/Res	9
11	1	Urban/Rec Grass	2335
11	2	Grass/Shrub Ditch	297
11	2	Prairie/Grassland	1691
11	2	Urban/Rec Grass	4303
11	2	Urban/Res	831
12	0	Grass/Shrub Ditch	666
12	0	Road	2216
12	1	Ag/Pasture	6287
12	2	Ag/Pasture	1489
12	2	Urban/Rec Grass	1410
12	2	Urban/Res	14

Table A11. Lindberg land cover classification based on 100-meter section and side of the roadway. The CalculateArea tool was used to determine the dominant land cover class per section via the total area in square meters was used. Side “0” represents the road/transect and was excluded to determine the habitat type on each side of the road. Side “1” represents the north or west side of the roadway and Side “2” represents the south or east side of the road.

Section	Side of Road	Land Cover Type	Area (m ²)
0	0	Road	1133
0	1	Road	370
0	1	Urban/Rec Grass	4158
0	1	Water/Wetland	1172
0	2	Road	423
0	2	Urban/Rec Grass	4377
0	2	Urban/Res	250
1	0	Road	1498
1	1	Road	2246
1	1	Urban/Rec Grass	6327
1	1	Water/Wetland	904
1	2	Road	1786
1	2	Urban/Rec Grass	6139
1	2	Urban/Res	682
2	0	Road	1458
2	1	Road	169
2	1	Urban/Rec Grass	8687
2	1	Water/Wetland	1288
2	2	Road	874
2	2	Urban/Rec Grass	6715
2	2	Urban/Res	1310
3	0	Road	1473
3	1	Forest/Woodlot	6294
3	1	Road	812
3	1	Urban/Rec Grass	2968
3	2	Road	1083
3	2	Urban/Rec Grass	5891
3	2	Urban/Res	1596
4	0	Road	1577
4	1	Forest/Woodlot	1613
4	1	Urban/Rec Grass	1382
4	1	Water/Wetland	7028
4	2	Urban/Rec Grass	3190
4	2	Water/Wetland	5167
5	0	Road	1520
5	1	Urban/Rec Grass	464
5	1	Water/Wetland	9575
5	2	Urban/Rec Grass	37

5	2	Water/Wetland	8035
6	0	Road	1487
6	1	Urban/Rec Grass	598
6	1	Water/Wetland	9647
6	2	Water/Wetland	8385
7	0	Road	1510
7	1	Urban/Rec Grass	4495
7	1	Water/Wetland	5733
7	2	Urban/Rec Grass	808
7	2	Water/Wetland	7702
8	0	Road	1298
8	1	Urban/Rec Grass	10007
8	2	Urban/Rec Grass	7269
8	2	Water/Wetland	1359
9	0	Road	1181
9	1	Urban/Rec Grass	9926
9	2	Urban/Rec Grass	8581
9	2	Water/Wetland	280
10	0	Road	1216
10	1	Urban/Rec Grass	9791
10	2	Urban/Rec Grass	8965
11	0	Road	1529
11	1	Urban/Rec Grass	10187
11	2	Urban/Rec Grass	6054
11	2	Water/Wetland	2929
11	2	Forest/Woodlot	887
12	0	Road	980
12	1	Urban/Rec Grass	5506
12	2	Forest/Woodlot	5664

Table A12. Prophetstown land cover classification based on 100-meter section and side of the roadway. The CalculateArea tool was used to determine the dominant land cover class per section via the total area in square meters was used. Side “0” represents the road/transect and was excluded to determine the habitat type on each side of the road. Side “1” represents the north or west side of the roadway and Side “2” represents the south or east side of the road.

Section	Side of Road	Land Cover Type	Area (m ²)
0	0	Road	1525
0	1	Forest/Woodlot	2964
0	1	Grass/Shrub Ditch	421
0	1	Urban/Rec Grass	746
0	1	Urban/Res	363
0	1	Water/Wetland	7115
0	2	Forest/Woodlot	4050
0	2	Urban/Rec Grass	1459
0	2	Urban/Res	499
1	0	Road	805
1	1	Forest/Woodlot	1748
1	1	Urban/Rec Grass	181
1	1	Water/Wetland	6037
1	2	Forest/Woodlot	3598
1	2	Water/Wetland	4680
2	0	Road	783
2	1	Water/Wetland	8260
2	2	Water/Wetland	8359
3	0	Road	845
3	1	Urban/Rec Grass	1082
3	2	Urban/Rec Grass	1087
3	2	Water/Wetland	7262
4	0	Road	1825
4	1	Prairie/Grassland	6498
4	1	Urban/Rec Grass	1596
4	2	Forest/Woodlot	66
4	2	Urban/Rec Grass	7450
5	0	Road	1073
5	1	Prairie/Grassland	8017
5	2	Forest/Woodlot	6811
5	2	Urban/Rec Grass	1128
6	0	Road	1174
6	1	Prairie/Grassland	8258
6	2	Urban/Rec Grass	101
6	2	Forest/Woodlot	7703
6	2	Prairie/Grassland	342
7	0	Road	1244
7	1	Prairie/Grassland	8020

7	2	Forest/Woodlot	3139
7	2	Prairie/Grassland	4600
8	0	Road	1125
8	1	Prairie/Grassland	8401
8	1	Road	71
8	2	Prairie/Grassland	8040
9	0	Road	1022
9	1	Prairie/Grassland	7398
9	1	Road	946
9	2	Road	950
9	2	Prairie/Grassland	6892
10	0	Road	1150
10	1	Prairie/Grassland	8275
10	2	Prairie/Grassland	7957
11	0	Road	1166
11	1	Prairie/Grassland	8033
11	2	Prairie/Grassland	7909
12	0	Road	1673
12	1	Prairie/Grassland	7791
12	2	Road	1539
12	2	Prairie/Grassland	4542
12	2	Urban/Rec Grass	835
12	2	Urban/Res	1608
13	0	Road	1231
13	1	Prairie/Grassland	6656
13	2	Urban/Rec Grass	1833
13	2	Urban/Rec Grass	788
13	2	Urban/Res	1543