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VERTEBRATE ROAD MORTALITY PREDOMINANTLY IMPACTS AMPHIBIANS

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Abstract.—**One potential contributor to global amphibian decline is mortality due to traffic ("road-kill"). Most studies of road-kill have focused on large mammals, but relatively little research has evaluated the impact of road-kill on other wild animals. We conducted multi-species road-kill surveys in Indiana, USA to develop a road-kill database and to identify habitat characteristics associated with road-kill. Four different routes were surveyed for vertebrate mortalities twice weekly from 8 March 2005 to 31 July 2006. We recorded 10,515 mortalities representing > 60 species (n = 496 surveys). The most common species we encountered were Bullfrogs (*Rana catesbeiana*, n = 1,671), Virginia Opossums (*Didelphis virginianus*, n = 79), and Chimney Swifts (*Chaetura pelagica*, n = 36). We recorded thousands of anuran, but most (> 7,500) could not be identified to species. Habitat variables that best predicted vertebrate mortality were water, forest, and urban/residential areas. Overall, our results suggested that road mortality impacts a wide variety of species and that habitat type strongly influences frequency of road-kill. Amphibians may be especially vulnerable because they often migrate *en masse* to or from breeding wetlands. Clearly, road-kill is a major source of amphibian mortality and may contribute to their global decline.**

Key Words.—anurans; habitat; Indiana; mammals, reptiles; road-kill

INTRODUCTION

Conflicts between wildlife and human interests have increased in recent decades because of human population growth and the resulting expansion of anthropogenic pressures into wildlife habitat. One area of particular concern is wildlife/vehicle collisions, which often result in human injury and monetary losses; as well as, high rates of mortality for many wildlife species. For example, Lalo (1987) estimated vertebrate mortality on United States roads at 1 million individuals per day. Although road mortality may be sustainable in abundant species with high reproductive rates, it can have a significant impact on populations of threatened or endangered species (e.g., Kushlan 1988; Foster and Humphrey 1995; Evink et al. 1996). For many such species, road mortality can serve as a limiting-factor, as their foraging and dispersal behaviors put them at risk of being struck on roadways (Gibbs and Shriver 2002; Aresco 2005).

There are many factors that can affect wildlife road mortality, including those that are taxon-specific (e.g., migrating female turtles; Steen et al. 2006), as well as those that are more taxon-general (e.g., traffic volume; Ray et al. 2006). Not surprisingly, species are more likely to be killed on roads adjacent to their preferred habitat (Cain et al. 2003; Forman et al. 2003). However, the situation is often more complex, particularly when human developments are considered. For example, Kanda et al. (2006) used a geographical information system (GIS) to determine landscape characteristics around Virginia opossum (*Didelphis virginiana*) road-kill sites in central Massachusetts and found that opossums were most often killed in low-elevation areas with minimal forest cover and more human development. In contrast, Bashore et al. (1985) found that deer-vehicle collisions decreased as the number of buildings and residences increased. The circumstances may be complicated further when animals use human structures (like bridges) as travel corridors (e.g., Hubbard et al. 2000).

Although most road mortality studies have centered on carnivores and ungulates, the effects of roads and road-kill also impact herpetofauna (Aresco 2005; Langen et al. 2006). Over the last two decades, amphibian populations have been declining worldwide (Blaustein and Wake 1990; Wake 1991; Fahrig et al. 1995; Becker et al. 2007) and these declines often are associated with some type of habitat fragmentation (Fahrig et al. 1995; Vos 1997). When considered jointly, habitat fragmentation and roads have the potential to impact strongly amphibian population dynamics. Indeed, a growing literature suggests that a significant amount of amphibian mortality is associated with road-kill (Fahrig et al. 1995; Ashley and Robinson 1996; Vos 1997). Indiana, whose state motto is "The Crossroads of America," is characterized by a highly fragmented, agriculturally dominated landscape that contains

> 150,000 km of roads. The biological effects of this road network are not well-understood, but the combination of habitat fragmentation and high road density may negatively impact many wildlife species.

Our research had three objectives: (1) identify, characterize, and evaluate road-kill sites in Indiana to develop a road-kill species index (with an emphasis on herpetofauna); (2) incorporate these empirical data into a Geographical Information System (GIS) to identify landscape characteristics of roads with high vertebrate mortality; and (3) investigate the effect of weather and season on the incidence of road-kill. These data are then interpreted in light of the global decline in amphibian populations.

Methods

Survey Routes.—We identified potential road-kill survey routes throughout Tippecanoe County, Indiana, USA using topographic maps (scale 1:156,000) and by consulting with regional biologists. Survey routes varied in length and were chosen to represent a mixture of geographic and anthropogenic conditions (e.g., upland vs. wetland, rural vs. suburban). Survey routes also were chosen based on safety and accessibility (e.g., visibility, available shoulder). We acquired available annual daily traffic volume data for survey routes (surveys from 2001-2004) from the Indiana Department of Transportation (Indiana Department of Transportation. 2003. Road Mileage and Control. Available from http://www.in.gov/dot/div/communications/2003annualreport/Road_Mileage_and_Control.pdf [Accessed 17 November 2006]) and the City Engineering Office of West Lafayette (Table 1).

Overall, we selected four survey routes (Lindberg Road, SR 26, US 231, and South River Road) covering a total of 12 linear km (Fig. 1). Lindberg Rd, located in West Lafayette, bisects the Celery Bog Nature Preserve. The bog is located entirely within the city limits and is surrounded by a variety of human developments: a golf course, shopping center, apartment complexes, and residential subdivisions. The SR 26 route is a two-lane highway located in rural western Tippecanoe County. There is a large wetland immediately south of the SR26 survey route with upland forest habitat directly north of it. The US 231 route is located northwest of Purdue University in an area dominated by agricultural fields. South River Rd parallels the Wabash River floodplain south of Purdue, Indiana and the landscape is comprised of mixed hardwood forest patches and many private residences.

Road-kill Sampling.—We performed road-kill detection surveys on all selected routes. Routes were driven at slow speeds (< 40 km/h) whenever possible to increase the probability of carcass detection while emphasizing surveyor safety. We surveyed each route twice per week from 8 March 2005 to 31 July 2006 for a total of 124 surveys per route. This intensive sampling was designed to enhance the detection of smaller carcasses (e.g., salamanders), which could rapidly disappear due to degradation and/or scavenging. Surveys accounted for all carcasses killed within the paved road shoulders, including those that were clearly

Table 1. Survey routes in Tippecanoe County, Indiana, USA with approximate distances and site descriptions.

Site	Survey Route	Length (km)	Site Description	Road Description	Urbanization Level	Avg. Daily Traffic[1]	# Land Cover Classes
Lindberg Road	Lindberg Road from US 231 to McCormick Road	1.8	wetland surrounded by golf course and bisected by 2-lane paved road	2-lane paved road with turning lane in center; no shoulder on south side, bike lane on north side; 30MPH	urban	6287	5
SR 26	SR 26 from 750W to CR925W	2.9	wetland surrounded by mixed hardwood woodlots and agricultural fields	2-lane paved road; very little shoulder, some roadside ditches; 50-55MPH	rural	1900	7
South River Road	S. River Rd. from US 231 bypass to CR300W	3.9	river bottom/flood plain, mixed hardwood woodlots near Purdue airport	2-lane paved road; little shoulder; 40-45MPH	suburban	3404	7
US 231	US 231 from US 52 to CR600N	3.4	primarily agricultural with ephemeral ditch system	2-lane paved road, large shoulder; 55MPH	rural	1930	6

[1]Data from Indiana Department of Transportation and the City of West Lafayette traffic surveys 2001-2004.

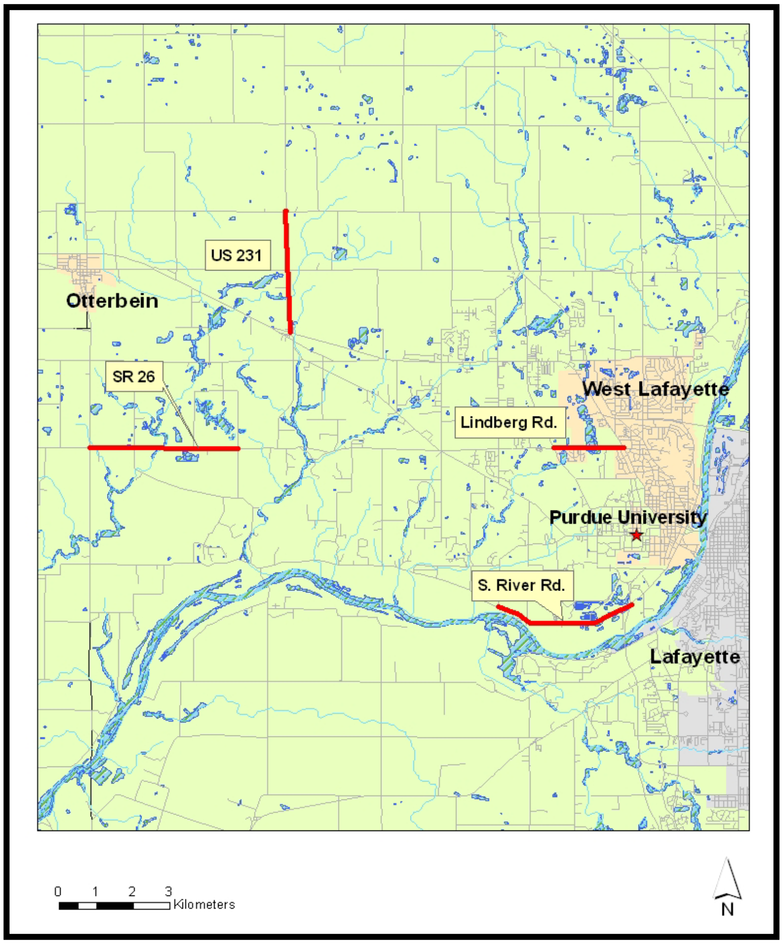

FIGURE 1. Locations of Tippecanoe County, Indiana road-kill survey routes (n = 4; routes highlighted in red).

killed by traffic but ejected from the road surface proper. We identified all road-kills to species whenever possible, marking them with spray paint or removing them to avoid recounting, and we assigned each a precise (sub-meter resolution) UTM coordinate using a Trimble GeoXT mobile GPS/GIS system. Species records were used to determine which species were most often encountered as road-kill along the survey routes.

Survey Route GIS.—We developed a GIS database for all survey routes using ArcGIS 9 (ESRI). We referred to aerial photographs obtained from the Indiana Spatial Data Service (Indiana Spatial Data Service. 2006. Available from http://www.indiana.edu/~gisdata/ [Accessed 5 June 2006]) to aid in interpretation of the spatial extent and location of habitat patches. The seamless information data file ("sid") for each raster download was added to an ArcMap project and served as a base map for digitizing survey route buffers and habitat types. We applied a 100 m buffer (from the center of the road) parallel to each survey route and overlaid it onto its corresponding aerial photo. The 100 m buffer included the habitat immediately adjacent to each survey route as habitat management and mitigation measures are typically implemented within 100 m of the roadways.

We downloaded road-kill location data using Terra-Sync and GPS Pathfinder Office software (Trimble 2003) and projected these data on their respective routes in the GIS database. We divided each route and its buffer into 100 m sections, essentially constructing a 100 m x 200 m analysis "window" from which the number of road-kills, corresponding habitat composition, and road characteristics within each section could be determined (see Fig. 2 for an example). The total numbers of 100 x 200 m sections were 21, 41, 30, and 35 for Lindberg Rd, S. River Rd, SR 26, and US 231, respectively.

We created seven land cover classes and then digitized features for each survey route and its associated buffer based on our interpretation of landscape features visible on the aerial imagery. Land cover classes included grass / shrub ditches (ditch), agriculture / pasture (ag), forest / woodlot (forest), urban / recreational grasses (urbrec), urban / residential (urbres), water / wetlands (water), and grassland / shrublands (shrub) (Glista 2006). Following the digitizing of habitat classes, we used the Calculate Area tool in ArcMap to determine the area (m^2) of each habitat polygon per 100 m x 200 m section of each survey route.

Road-kill/Habitat Association Analysis.—We divided road-kill data into their taxonomic categories for each route: mammals, birds, amphibians, reptiles, and overall. We examined the spatial distribution of road mortality events along all four routes to determine which habitat variable(s) most influenced road-kill numbers for each taxa. To evaluate whether mortality was uniformly distributed across routes, we used Kolmogorov-Smirnov tests ($\alpha = 0.05$). We then performed stepwise linear regressions and used r^2 values to determine which habitat variables best predicted total numbers of individuals killed within each category at each survey route ($\alpha = 0.05$). Each section (100 m x 200 m) on a route represented one sampling unit with the response variable being the number of road-kills per section and the predictor variables consisting of the proportion of each habitat feature class within each section. Log

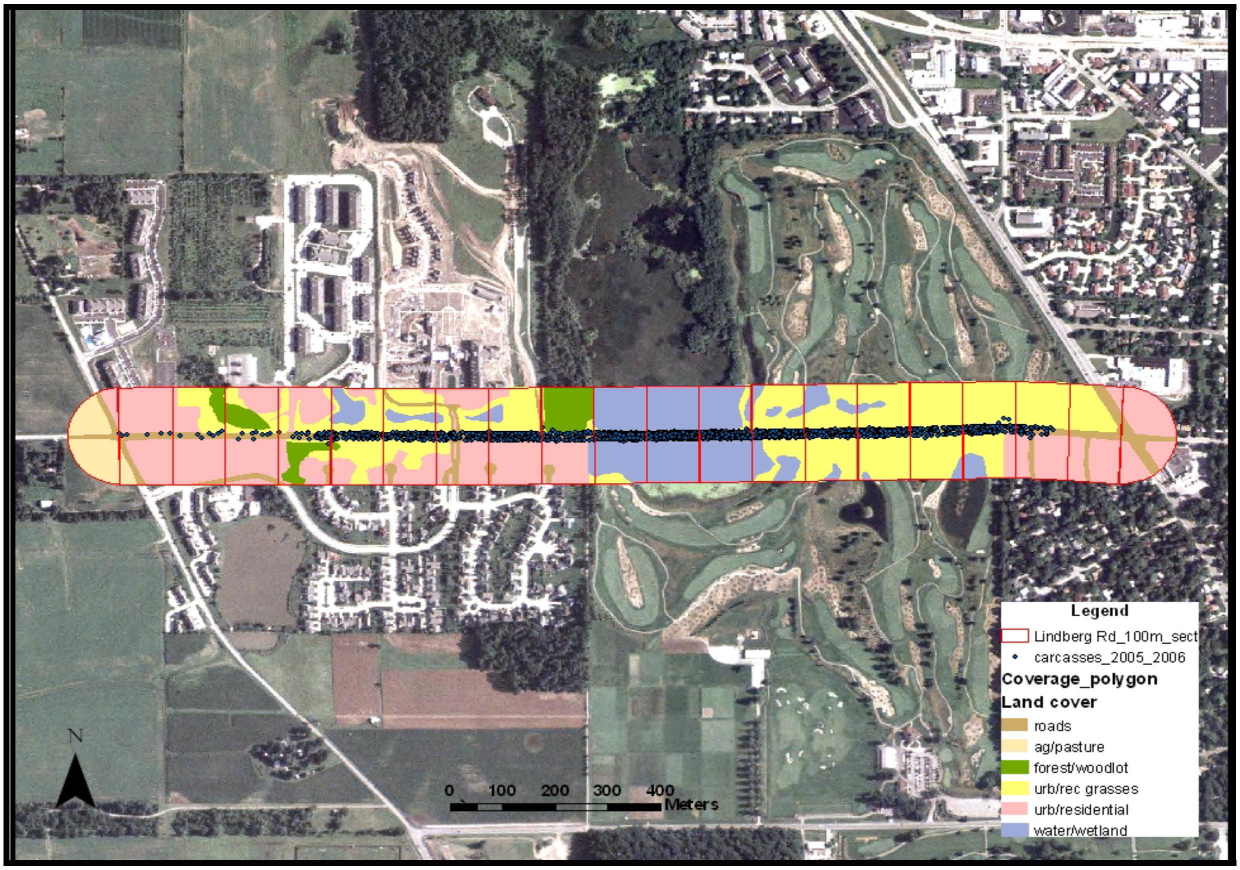

FIGURE 2. Map of Lindberg Road, (Tippecanoe County, Indiana, USA) survey route with associated road mortalities (n = 8,176), digitized habitat types, and 100 m buffer.

transformations were used in instances where the data were not normally distributed. We did not conduct analyses of habitat association and avian road-kill for all four routes because of a paucity of data, as was the case for amphibian and reptile data from the South River Rd route.

Weather Analysis.—We obtained weather data from the Indiana State Climate Office (Indiana State Climate Office. 2006. Available from http://shadow.agry.purdue.edu/sc.index.html [Accessed 2 September 2006]) and from these data we calculated monthly mean temperature and total precipitation levels. Mean temperatures and precipitation levels were plotted against the pooled number of road-kills per km surveyed during each month to evaluate general relationships between road-kill levels and weather factors. We used linear regression to determine which weather variables (temperature or precipitation) had the greatest influence on road-kill number across all four routes ($\alpha = 0.05$). All analyses were performed using SPSS 14.0 (SPSS 2006).

RESULTS

Road-kill Sampling.—From 8 March 2005 to 31 July 2006, we conducted 496 surveys, traveling a total of 1,488 km, and recorded 10,515 road mortality events for an average of 7.1 kills per km surveyed across all four survey routes. Of this total, 9,950 (95%) individuals were amphibians and reptiles, 360 (3%) mammals, and 205 (2%) birds (Table 2). We identified 69 species among the mortalities, including at least 25 mammals (Table 3a), 26 birds (Table 3b), and 9 amphibians (Table 3c) and 10 reptiles (Table 3d). The most frequently identified amphibian species was bullfrogs (*Rana catesbeiana*, n = 1,671). However, a substantial majority of the amphibian (n = 7,602) fell into the category of "unknown ranid" as they could only be identified to genus. For mammals and birds, the most frequently identified species were Opossums (n = 79) and Chimney Swifts (*Chaetura pelagica*, n = 36), respectively.

The routes with the highest occurrence of road-kill were Lindberg Rd (n = 8,231) with 8,069 amphibians

TABLE 2. Vertebrate mortalities by taxonomic group for all four Tippecanoe County, Indiana, USA survey routes, 8 March 2005 - 31 July 2006.

Route	Mammalia	Aves	Herpetofauna	Total Kills	Route Distance (km)	No. of Surveys	Total km surveyed	Kills/km Surveyed
Lindberg Rd	72	88	8,016	8,176	1.8	124	223.2	36.6
SR 26	80	33	1,648	1,761	2.9	124	359.6	4.9
US 231	76	33	237	346	3.4	124	421.6	0.8
S. River Rd.	132	51	49	232	3.9	124	483.6	0.5
TOTAL	360	205	9,950	10,515	12	496	1,488	7.1

and reptiles, 73 mammals, and 89 birds, and the SR 26 route (n = 1,736) with 1,624 amphibians and reptiles, 79 mammals, and 33 birds. The total for the US 231 route was 330, with 218 amphibians and reptiles, 79 mammals, and 33 birds. South River Rd totaled 218 road-kills, of which 39 were amphibians and reptiles, 129 mammals, and 50 birds (Table 2). The route with the highest mean road-kill per km was Lindberg Rd (36.6); whereas, the route with the lowest mean road-kill per km was S. River Rd (0.5; Table 2).

Road-kill/Habitat Association Analysis.—Mortalities were not uniformly distributed along the routes (Fig. 3; Kolmogorov-Smirnov tests, $P < 0.005$ for all routes), presumably indicating that surrounding habitats influenced frequency of road-kill. The best regression models for predicting total numbers of road-kill across all taxa were water/forest/urbres ($r^2 = 0.797$) for SR 26, urbres ($r^2 = 0.169$) for US 231, water/urbrec ($r^2 = 0.899$) for Lindberg Rd, and urbres ($r^2 = 0.097$) for S. River Rd (Table 4). The best models for predicting total numbers of amphibian and reptile road-kill were water/forest/urbres ($r^2 = 0.791$) for SR 26, urbres ($r^2 = 0.281$) for US 231, and water/urbrec ($r^2 = 0.897$) for Lindberg Rd. The best models for mammals were forest/water ($r^2 = 0.421$) for SR 26, water ($r^2 = 0.409$) for US 231, water/forest ($r^2 = 0.245$) for Lindberg Rd, and urbrec ($r^2 = 0.076$) for S. River Rd. We deemed the avian data too sparse for quantitative analyses.

Weather Analysis.—Although we detected road-kills in all months, there were weather-related and seasonal patterns in the data. Linear regression produced a model suggesting that monthly mean temperature had the greatest influence on road-kill numbers across all routes ($r^2 = 0.684$) and the majority of road-kills occurred from July through September, during the period of peak temperatures and precipitation levels (Fig. 4).

DISCUSSION

Road-kill Sampling.—During a 17-month period, we recorded > 10,000 road mortality events across four survey routes. Ashley and Robinson (1996) surveyed a 3.6 km section of road in Ontario, Canada over two 2-year periods and recorded > 32,000 road mortalities and of those 95% were reptiles and amphibians. Their proportion of amphibian and reptile road-kills was the same as our results for all four routes. In one year, Smith and Dodd (2003) counted > 1,800 mortalities along a 3.2 km section of highway in Florida, and of those, 91% were herpetofauna. Collectively, these studies indicate that roads that traverse wetlands can be major sources of amphibian and reptile mortality.

Two routes, Lindberg Rd. and SR 26, became focal points of our study because of large numbers of herpetofauna road mortalities. During our surveys, we recorded > 7,900 road-killed frogs (*Rana* sp.) on Lindberg Rd. There were fewer amphibian and reptile road-kills (n = 1,648) along the SR 26 route, but the species diversity (n = 16 amphibian and reptile species) was higher, probably because of the presence of all seven land cover classes within the survey route buffer. Collectively, nearly 10,000 amphibians and reptiles were killed along these two routes in 1.5 years. Furthermore, these are likely substantial underestimates of the true road-kill as carcasses degraded very rapidly and/or were scavenged during the summer months.

Degradation obfuscated not only the absolute number of carcasses, but in some cases their identity. For example, 46 Northern Leopard Frogs (*Rana pipiens*) were clearly documented on the Lindberg Rd and SR 26 routes over the course of the study. However, some of the 7,602 dead frogs identified to genus but not to species (Table 3) were probably also *Rana pipiens.* The impact of traffic on Northern Leopard Frogs is particularly noteworthy because they are officially listed as a species of special conservation concern in Indiana (Indiana Department of Natural Resources. 2006. Indiana's Species of Greatest Conservation Need. Available from http://www.in.gov/dnr/fishwild/endangered/endangered_list-Dec06.pdf [Accessed 23 March 2007]). We note that we heard *Rana pipiens* adults calling from wetlands near these roads during early spring surveys.

Bullfrogs were the most frequently killed species we recorded. They also were the species we heard most often and observed near the survey routes. Bullfrogs are prolific breeders, often laying several thousand eggs per female (Wright and Wright 1949; Trauth et al. 1990; Harding 1997). This may explain the large numbers of Bullfrogs that we recorded on both routes. Bullfrogs are

TABLE 3. Vertebrate species recorded along four Tippecanoe County, Indiana, USA survey routes, 8 March 2005-31 July 2006. Overall total = 10,515 road-kills. (* indicates species of special conservation concern in Indiana).

A. Mammalia.

Scientific Name	Common Name	Total
Blarina brevicauda	Northern Short-tailed Shrew	14
Canis familiaris	Domestic Dog	1
Canis latrans	Coyote	1
Didelphis virginiana	Opossum	79
Felis catus	Domestic Cat	5
*Lasiurus borealis**	Eastern Red Bat	1
Marmota monax	Woodchuck	1
Mephitis mephitis	Striped Skunk	16
Microtus ochrogaster	Prairie Vole	1
Microtus pennsylvanicus	Meadow Vole	15
Mus musculus	House Mouse	2
Mustela vison	Mink	6
Odocoileus virginianus	White-tailed Deer	4
Ondatra zibethicus	Muskrat	10
Peromyscus spp.	Deer/White-footed Mouse	39
Procyon lotor	Raccoon	43
Scalopus aquaticus	Eastern Mole	4
Sciurus carolinensis	Eastern Gray Squirrel	23
Sciurus niger	Eastern Fox Squirrel	27
Sorex cinereus	Masked Shrew	1
Spermophilus tridecemlineatus	13-lined Ground Squirrel	6
Sylvilagus floridanus	Eastern Cottontail	37
Tamiasciurus hudsonicus	Red Squirrel	6
Tamias striatus	Eastern Chipmunk	7
Vulpes vulpes	Red Fox	1
?	unknown bat	2
?	unknown mammal	8
Total		**360**

B. Aves.

Scientific Name	Common Name	Total
Agelaius phoeniceus	Red-winged Blackbird	8
Branta canadensis	Canada Goose	2
Butorides virescens	Green Heron	1
Cardeulis tristis	American Goldfinch	1
Cardinalis cardinalis	Northern Cardinal	9
Chaetura pelagica	Chimney Swift	36
Colaptes auratus	Northern Flicker	1
Dumetella carolinensis	Gray Catbird	1
Eremophila alpestris	Horned Lark	1
Hirundo rustica	Barn Swallow	5
Melanerpes erythrocephalus	Red-headed Woodpecker	2
Melospiza melodia	Song Sparrow	9
Molothrus ater	Brown-headed Cowbird	2
Otus asio	Eastern Screech Owl	6
Passer domesticus	House Sparrow	15
Passerina cyanea	Indigo Bunting	3
Phasianus colchicus	Ring-necked Pheasant	2
Porzana carolina	Sora	1
Quiscalus quiscula	Common Grackle	6
Spizella passerina	Chipping Sparrow	1
Sturnella magna	Eastern Meadowlark	2
Sturnus vulgaris	European starling	11
Tachycineta bicolor	Tree Swallow	1
Troglodytes aedon	House Wren	1
Turdus migratorius	American Robin	18
Zenaida macroura	Mourning Dove	4
?	unknown bird	56
Total		**205**

C. Amphibia

Scientific Name	Common Name	Total
Ambystoma tigrinum	Eastern Tiger Salamander	142
Bufo americanus	American Toad	111
Hyla spp.	Tree Frog	1
Pseudacris crucifer	Spring Peeper	8
Rana catesbeiana	Bullfrog	1,671
Rana clamitans	Green Frog	172
Rana palustris	Pickerel Frog	18
*Rana pipiens**	Northern Leopard Frog	74
Rana spp.	unknown ranid	7,602
?	unknown frog	10
Total		**9,809**

D. Reptilia.

Scientific Name	Common Name	Total
Chelydra serpentina	Snapping Turtle	23
Chrysemys picta	Midland Painted Turtle	28
Elaphe obsoleta	Black Rat Snake	5
Elaphe vulpina	Fox Snake	9
Graptemys geographica	Northern Map Turtle	1
Nerodia sipedon	Northern Water Snake	1
Storeria dekayi wrightorum	Midland Brown Snake	19
Terrapene carolina	Eastern Box Turtle	1
Thamnophis sirtalis	Common Garter Snake	35
Trachemys scripta	Red-eared Slider	13
?	unknown snake	4
?	unknown turtle	2
Total		**141**

voracious predators that will not only out-compete other species but also prey on them, which may explain the disproportionate number of Bullfrogs relative to other frog species. Many of the frogs we identified as *Rana* spp. were presumably Bullfrogs, but the exact proportion of each species could not be determined.

Although anurans made up the majority of road-kill on Lindberg Rd and SR26, there were some other notable mortality events. For example, between 17 February 2006 and 7 April 2006, we recorded 30 road-killed Tiger Salamanders (*Ambystoma tigrinum*) on Lindberg Rd and 70 on SR26, presumably killed during their spring migration to breeding areas. During a 46-day period between April and June 2006, we found 34 dead Chimney Swifts on the Lindberg Rd route. Most swift carcasses were located on the sections of road bisecting the bog and were probably a result of low-flying birds striking vehicles while pursuing insects. The modest numbers of salamanders and swifts were documented over a temporally contracted period, which suggests that migrating animals are ephemerally exposed to vehicular hazards while using the bog as a stopover or breeding area.

Road-kill/Habitat Association.—The Lindberg Rd habitat analysis model (Table 4) included water as a key predictor of both herpetofauna and overall road-kill numbers, which is intuitive considering the high numbers of amphibians and reptiles recorded along those routes and the fact that Lindberg Rd bisects the Celery Bog Nature Preserve. Both the Ashley and Robinson (1996) as well as Smith and Dodd (2003) studies were conducted on stretches of road that bisected wetland complexes and both documented high numbers of herpetofauna road-kill. Celery Bog notwithstanding, there are several other sources of water such as apartment complex retention ponds and golf course water "hazards" that could be used by various amphibian and reptile species as breeding, cover, and feeding areas. The presence of these artificial water sources could explain why we found amphibian and reptile carcasses in such high numbers along the entire route.

As with the Lindberg Rd route, the presence of water along the SR 26 route was important in predicting frequency of road-kill. The mixture of upland and water habitat likely contributed to the relatively high frequency of Tiger Salamander road-kill along that stretch of road, as it provides both breeding and over-wintering areas for this species. Furthermore, both SR 26 and Lindberg Rd had multiple farm ponds and creeks along the routes. We found Green Frogs (*Rana clamitans*) in sections near creeks, whereas Bullfrogs were prevalent in areas closer to farm ponds. Green Frogs prefer relatively cool, clear, permanent bodies of water, whereas Bullfrogs need permanent bodies of warm water (up to ~ 21°C; Minton 2001). The distribution of both Green Frogs and Bullfrogs along SR 26 seemed to be consistent with each species habitat requirements, although we did not consider specific species in our analyses.

Weather.—Weather and season influenced road-kill numbers. Monthly mean temperature had the greatest influence on the amount of road mortality. Road-kills

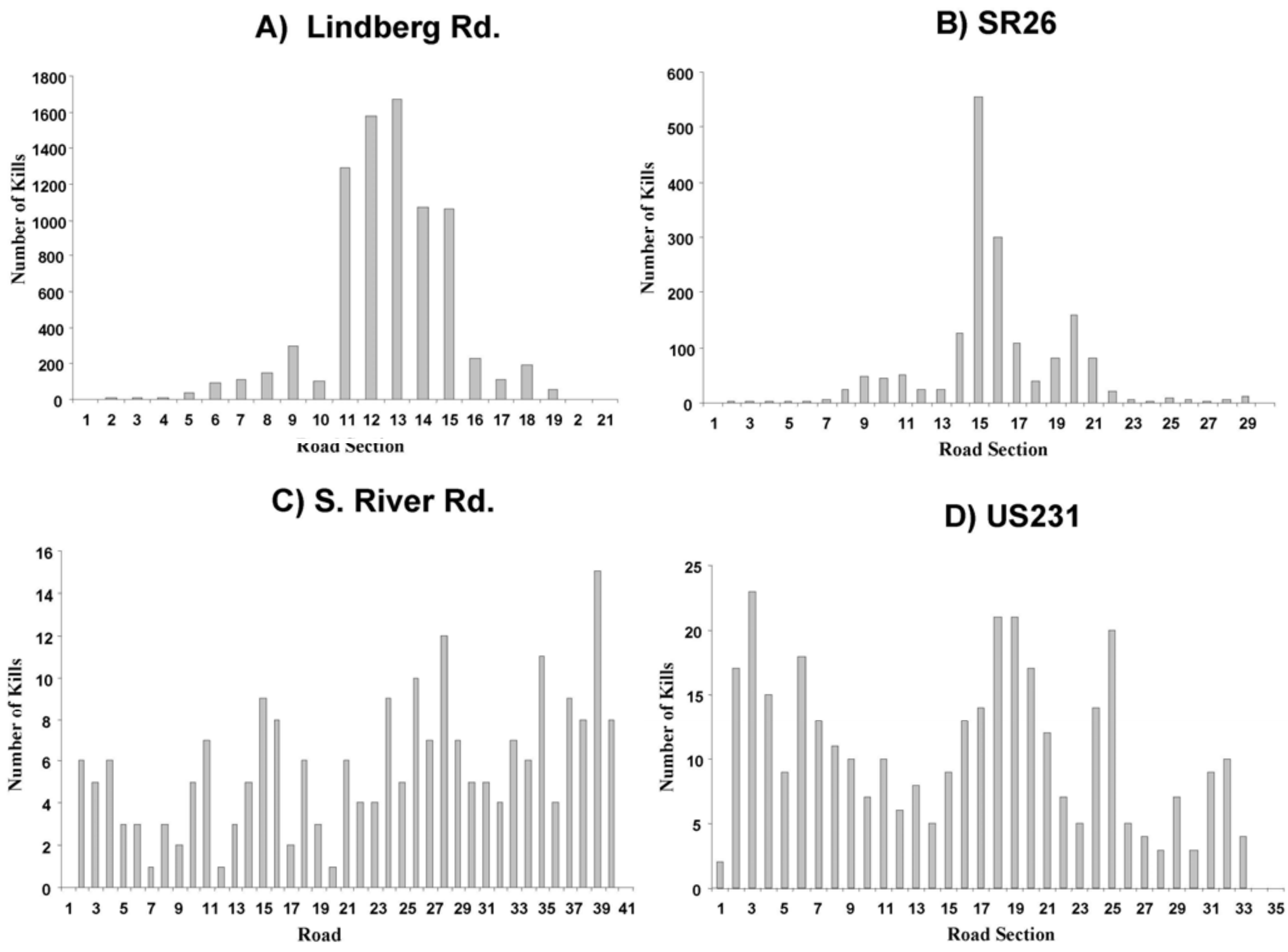

FIGURE 3. Distribution of road-kills (n = 10,515) per 100 x 200m section on all four Tippecanoe County, Indiana, USA survey routes, 8 March 2006 - 31 July 2006. Road section orientation is left = west, and right = east, except for US231 in which left = north and right = south. A) Lindberg Rd., B) SR 26, C) South River Road, D) US 231

TABLE 4. Linear regression models of road-kill numbers and surrounding habitat types using seven predictor variables (ditch, agriculture, forest, urbrec, urbres, water, and shrub). See *Survey Route GIS* section for further description of habitat variables. Birds were not included in analyses due to a paucity of data.

Route	Model R^2	Model P	Variable	Coefficient B	SE	*P*
			(Amphibians and Reptiles)			
Lindberg Rd.	0.897	0.000	constant	-38.680	72.840	0.602
			water	1,842.915	147.368	0.000
			urbrec	289.631	148.864	0.067
US 231	0.281	0.003	constant	7.532	0.921	0.000
			urbres	-19.004	5.848	0.003
SR 26	0.791	0.000	constant	-13.344	16.737	0.433
			water	912.316	98.084	0.000
			forest	163.241	81.226	0.055
			urbres	43.746	60.21	0.474
			(Mammals)			
Lindberg Rd.	0.245	0.080	constant	1.746	0.708	0.024
			forest	11.807	5.957	0.063
			water	3.480	1.955	0.092
US 231	0.409	0.000	constant	2.000	0.283	0.000
			water	163.265	34.195	0.000
South River Rd.	0.076	0.081	constant	2.810	0.367	0.000
			urbrec	5.086	2.844	0.081
SR 26	0.421	0.001	constant	1.477	0.385	0.001
			forest	8.014	2.483	0.003
			water	7.493	2.962	0.018
			(All Taxa)			
Lindberg Rd.	0.899	0.000	constant	-35.648	73.168	0.632
			water	1,865.336	148.032	0.000
			urbrec	288.077	149.535	0.070
US 231	0.169	0.014	constant	11.705	1.161	0.000
			urbres	-13.094	5.053	0.014
South River Rd.	0.097	0.047	constant	4.384	0.732	0.000
			urbres	4.489	2.189	0.047
SR 26	0.797	0.000	constant	-11.309	16.727	0.505
			water	924.073	98.024	0.000
			forest	172.147	81.176	0.044
			urbres	45.238	60.174	0.459

across all routes were highest during the summer months (highest monthly mean temperatures) and peaked in September. Conversely, road mortality was lowest in winter.

The mortality patterns of amphibians in response to seasonal changes can be explained by life histories of the various species. Key factors include breeding seasonality, dispersal of juveniles, and movements to over-wintering areas. The majority of amphibians and reptiles we encountered (e.g., Bullfrogs and Green Frogs) breed from mid-May through July (Minton 2001). Ashley and Robinson (1996) recorded monthly road mortalities for four species of anurans (Northern Leopard Frogs, Bullfrogs, Green Frogs, and American Toads) and discovered distinct patterns for each species. Leopard Frog mortalities were unimodal with the peak in late summer. Bullfrog, Green Frog, and American Toad mortalities were bimodal with peaks both in mid-spring and late summer. Smith and Dodd (2003) also documented weather and season–related patterns in their road-kill data, as they recorded high kill frequencies for frogs in July and August and an overall higher number of road-kills throughout the summer months.

Detection Biases.—We think our counts of mortality are conservative. Amphibian and reptile movements often are associated with breeding migrations and/or weather-related events (Langton 1989). Sampling during the first year did not begin until March; therefore, many of the early salamander and anuran migrations may have been missed. However, during the second year of sampling, we were able to document several early migrations, such as Tiger Salamanders and Northern Leopard Frogs.

Detection and positive identification of carcasses often was taxing. Small species such as Spring Peepers were very difficult to locate and were undoubtedly missed on occasion. Carcass degradation made identification difficult and was a constant problem, especially for amphibians and reptiles during the summer months. Additionally, some carcasses may have been eaten by scavengers prior to marking and some animals may have

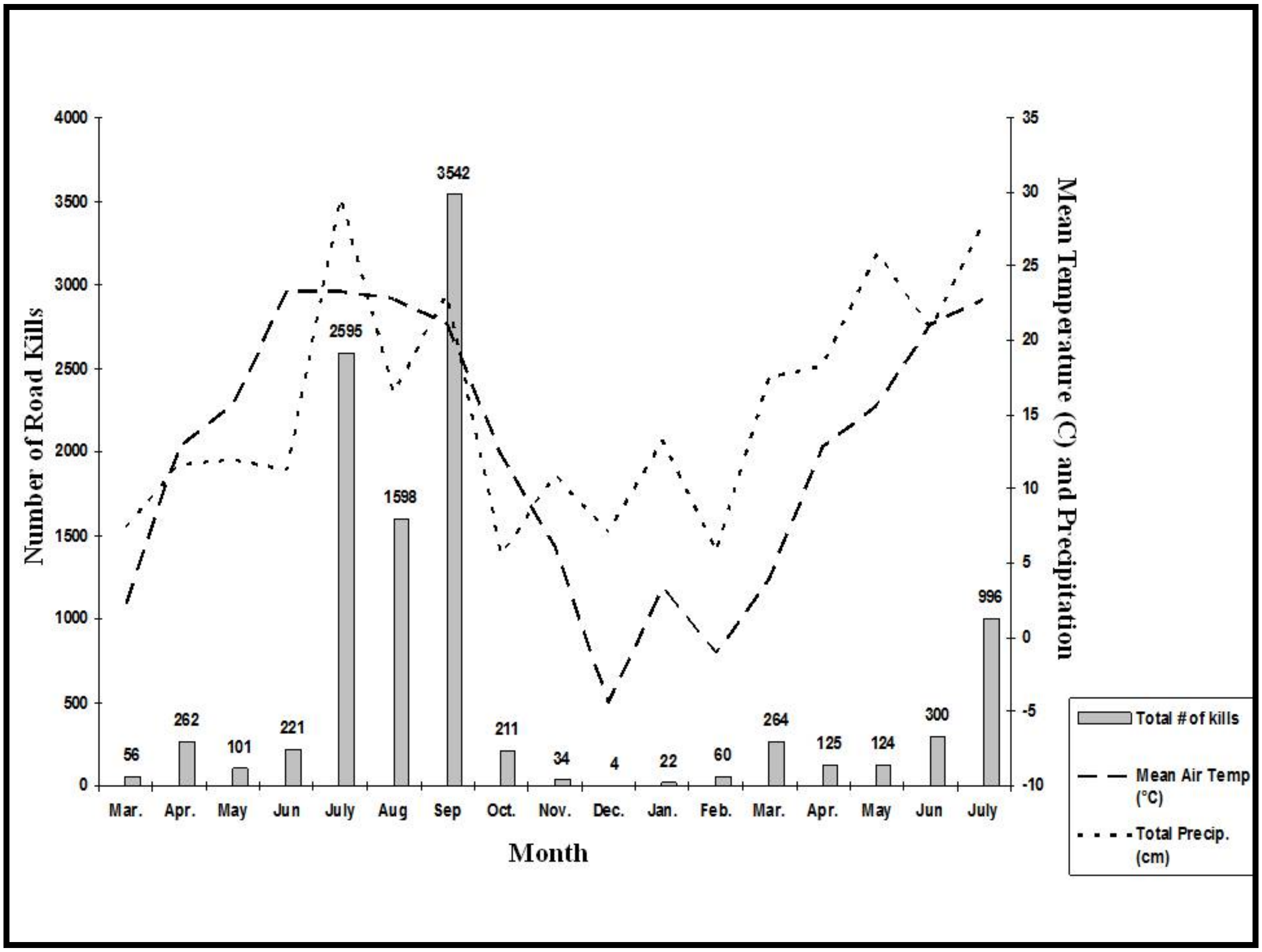

FIGURE 4. Monthly road-kill levels vs. monthly mean temperature and monthly total precipitation across four Tippecanoe County, Indiana, USA survey routes, March 2005 - July 2006.

left the roadside after being hit (DeVault et al. 2003; Smith and Dodd 2003). Carcass removal by other means such as road crews and snow removal equipment also may have affected our final numbers. Finally, visibility was limited on some days due to fog, rain, or snow. Given all these caveats, it is notable that 10,088 of 10,515 road-killed individuals were small (< 1 kg), and thus, might be disproportionately underrepresented. Note that these individuals represent a substantial fraction (96%) of the overall species diversity (Table 3).

Conclusions.—Road-kill can pose serious threats to a variety of species. Vehicle traffic on roads can be a direct source of wildlife mortality and, in some instances, can be catastrophic (Langton 1989). For many species, road mortality can serve as a population-limiting factor because their foraging and dispersal behaviors put them at risk of being struck on roadways. Although road mortality may not affect abundant populations, it can have a significant impact on populations of threatened or endangered species.

We have documented significant wildlife road mortality that may deserve consideration for mitigation, most notably involving areas where roads bisect or are in proximity to wetlands. Connectivity of habitat and passibility of road systems are important factors to consider when developing road-kill mitigation systems (Yanes et al. 1995). Unfortunately, there is no panacea for mitigating road-kill; what works for one species or suite of species may not be the best option for others. There are, however, various measures that may be more effective for the areas of highest road mortality (Lindberg Rd and SR 26 in our study), such as underpasses or culvert and barrier wall systems (Clevenger and Waltho 2000; Jackson and Griffin 2000; Dodd et al. 2004; Glista 2006).

Our results emphasize that road-kill may be a significant factor in the overall decline of amphibian and reptile populations, particularly frogs and other amphibians. Consider our results and those of two other studies: Ashley and Robinson (1996) plus Smith and Dodd (2003). Collectively, these three studies document

42,502 dead amphibians and reptiles across four routes which span a total of only 11.5 km of road. The total number of survey days was 488, which translates into a mean of roughly 7.6 dead amphibians and reptiles/km/day. We do not mean to imply this number is universally applicable, but use it to illustrate the potential magnitude of road mortality on declining populations of reptiles and amphibians. Habitat destruction, climate change, infectious diseases, and UV radiation may be the major factors involved in the decline of many populations, but the effects of road-kill should not be underestimated.

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