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SPATIO-TEMPORAL PATTERNS OF MAMMAL ROAD MORTALITY IN MIDDLE MAGDALENA VALLEY, COLOMBIA

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Abstract: Wildlife-vehicle collisions are acknowledged as the leading source of vertebrate mortality worldwide. Here, we examined spatio-temporal patterns of mammal road mortality in three routes on a fragmented landscape in the Middle Magdalena Valley, Santander department, Colombia. We identified the species affected by road mortality, estimated the roadkill rates for each road segment, and identified roadkill hotspots related to multi-scale structural connectivity. During an eigth-month period, we recorded a total of 152 mammal roadkills of 12 native species. The roads with the highest roadkill rates were Departmental Route 01 (DR01) and National Route 66 (NR66), but roadkill aggregations corresponding to potential roadkill hotspots were only identified on the route DR01. These hotspots intersect with sections of the landscape with lower connectivity, suggesting that mammal species are forced to leave suitable habitats in response to habitat fragmentation, increasing the risk of wildlife-vehicle collisions. Our results contribute to understanding spatio-temporal factors potentially promoting mammal roadkill events and offer a unique opportunity to designing effective mitigation measures that will work properly.

Keywords: landscape connectivity; mitigation measures; mortality rates; roadkills.

INTRODUCTION

Transport infrastructure, including roads and railways, have a crucial role in modern economy and society (Forman *et al.* 2003). However, the physical presence of roads and railroads in the landscape affects nature creating new habitat edges, altering hydrological dynamics, and disrupting natural processes and habitats (Seiler 2001). Wildlife responds differentially to roads and traffic, showing negative, positive or neutral effects on the individual, population, and community levels (Fahrig & Rytwinski 2009, Rosa & Bager 2013, Rytwinski & Fahrig 2013). Despite this fact, most of population-level responses to roads are negative (Fahrig & Rytwinski 2009, Rytwinski & Fahrig 2013). Roads and traffic impact wildlife directly in several negative ways, decreasing habitat quality, facilitating the introduction and spread of exotic species, acting as barriers limiting movement, reducing genetic diversity, subdividing animal populations, promoting metapopulation extinction, and increasing wildlife mortality due to wildlife-vehicle collisions (Seiler 2001, Forman *et al.* 2003, Jaeger *et al.* 2005, Marsh & Jaeger 2015).

Wildlife roadkills are perhaps the greatest anthropogenic source of direct mortality for vertebrates on Earth, representing a growing phenomenon of significant dimensions (Forman & Alexander 1998, Fabrizio *et al.* 2019). Wildlife-vehicle collisions are of special concern for vertebrate species lacking avoidance behaviour, having greater mobility, larger body size, and home range, and lower reproductive rates and densities (Jaeger *et al.* 2005, Fahrig & Rytwinski 2009, Rytwinski & Fahrig 2013). Recent efforts to mitigate the negative effects of roads and traffic on wildlife includes the construction of crossings structures, fences, warning signs, among others (Clevenger *et al.* 2003, van der Grift *et al.* 2013, Rytwinski *et al.* 2016). Since the implementation of such measures are expensive, they must be located on the most critical road sections (*e.g.*, areas with high mortality rates and/or places where increasing landscape connectivity is needed) to enhance cost-effectiveness (Clevenger *et al.* 2003, van der Grift *et al.* 2013, Bueno *et al.* 2015, Mimet *et al.* 2016).

In Colombia, most studies investigating road impacts on wildlife populations have been focused on counts of animals killed by vehicles (*e.g.*, de la Ossa-Nadjar & de la Ossa-V 2013, 2015, Castillo *et al.* 2015, de la Ossa-V & Galván-Guevara 2015, Meza-Joya *et al.* 2015, Monroy 2015), without testing spatio-temporal patterns of wildlife-vehicle collisions (but see Payan *et al.* 2013, Ramos & Meza-Joya 2018) or landscape connectivity models to evaluate roadkill patterns. Beyond the scarce knowledge of how road mortality might affect wildlife populations, most roads in the country lack mitigation measures or they are installed intuitively without studies supporting their location and effectiveness (Payan *et al.* 2013). Therefore, wildlife-vehicle collisions remain a latent threat for national biodiversity that requires urgent evaluation, effective mitigation and management. Identify spatial and temporal patterns of wildlife-vehicle collisions and understand how native species moves through landscape is critical to avoid high-risk areas and install or improve mitigation measures on existing, new or projected roads (Bueno *et al.* 2015, Gunson & Teixeira 2015, Kazemi *et al.* 2016).

Wildlife roadkills are assumed to be spatially and temporally aggregated in function of species' traits, road attributes, landscape features, and climate (Jaeger *et al.* 2005, Gunson *et al.* 2011, Marsh & Jaeger 2015). Road segments with significant roadkill aggregations (hotspots) are frequently used as priority locations for mitigation measures (Coelho *et al.* 2008, van der Grift *et al.* 2013, Ascensão *et al.* 2017). However, the identification of hotspots location is insufficient to understand how different species occupy and move through the landscape surrounding road networks (Clevenger *et al.* 2003, Beckmann *et al.* 2010). Thus, the challenge is to identify wildlife crossing locations capable of improving multispecies connectivity at regional scales over the long term (Mimet *et al.* 2016). In this sense, roadkill research should integrate connectivity analyses with hotspot identification as criteria for ranking where to install mitigation measures (see Ascensão *et al.* 2017).

In this context, our goal was to describe and to analyse mammal road mortality in two-lane paved roads on a fragmented landscape in the Middle Magdalena Valley, Colombia. For this, we aimed to (1) describe the species richness of mammals roadkilled and estimate mortality rates for the surveyed road segments, (2) assess whether roadkills are spatially clustered forming hotspots of mortality, (3) evaluate if observed roadkill events differs among climatic seasons, (4) quantify multi-scale structural connectivity (*i.e.*, landscape and local-scale) around surveyed roads, and (5) assess whether numbers of roadkills differs among areas of low and high landscape connectivity. Our results will help identify the routes that require most attention from governmental agencies and provide information to the decision makers on specific wildlife mitigation measures in order to reduce the trade-off between their cost and effectiveness.

MATERIAL AND METHODS

Study area

We conducted this study in the Middle Magdalena Valley, municipality of Barrancabermeja, Santander department, Colombia (06°59'41.6"N, 73°44'51.5"W; *datum* WGS84; 90 m elevation). This region comprises extensive lowland alluvial plains with swampy ecosystems interspersed with non-flooded areas (Garzón & Gutiérrez 2013). The climate is tropical humid, with a bimodal rainfall pattern, mean annual precipitation of 2,917 mm, mean annual temperature of 27.9°C, and a relative humidity of 80% (IDEAM 2016). We monitored a total of 75 km of two-lane paved roads on three different routes: 26 km of the Departmental Route 01 (DR01), 18 km of the National Route 45 (NR45),

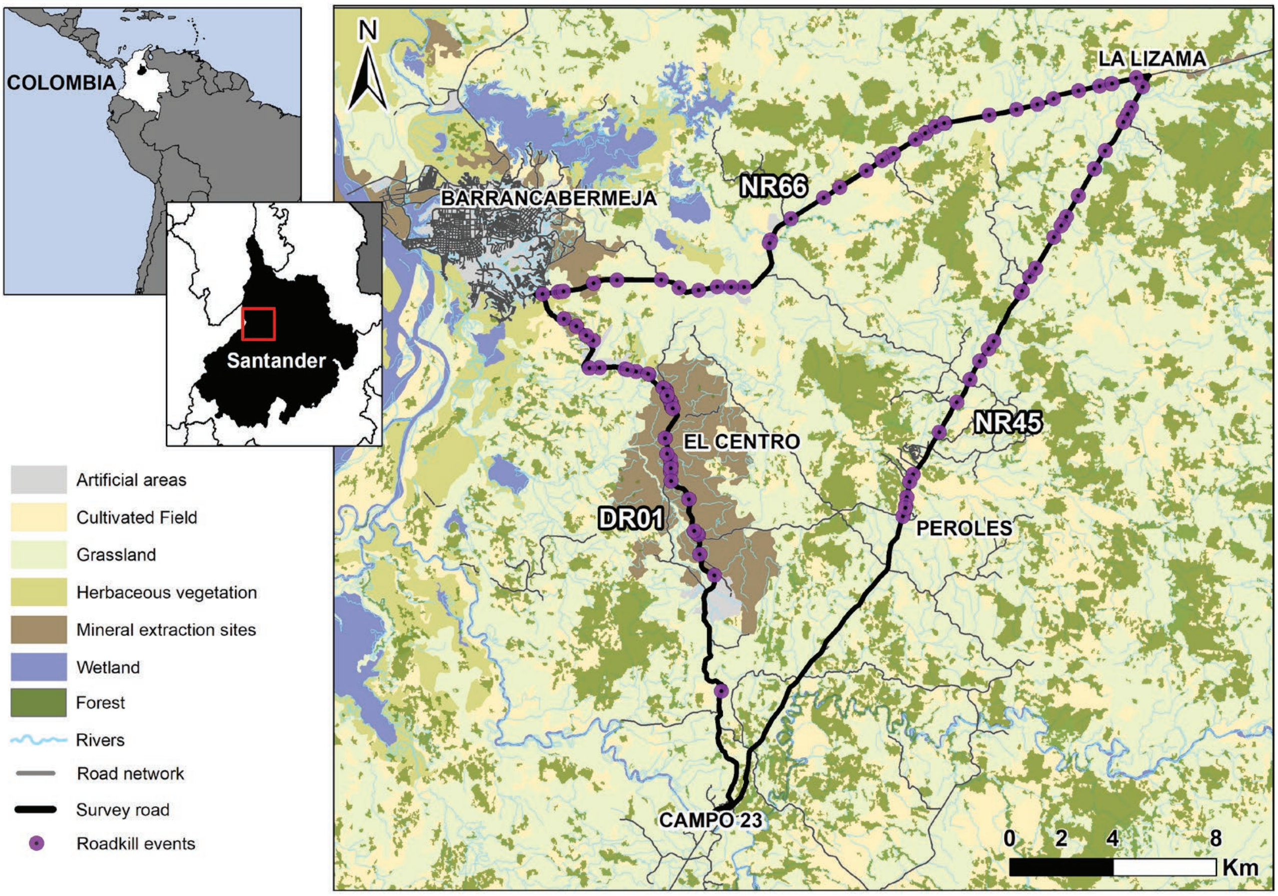

Figure 1. Map showing the three surveyed road segments in the middle Magdalena Valley, Santander department, Colombia. Purple dots indicate the locations of roadkilled specimens. Inset: location of the study area in Santander department, Colombia. Abbreviations: (DR01) Departmental Route 01, (NR45) National Route 45, and (NR66) National Route 66.

and 31 km of the National Route 66 (NR66) (Figure 1; Supplementary Material – Table S1).

Data collection

We conducted roadkill surveys by car (driver and passenger) at slow speed (≤ 40 km/h), twice per month from June 2013 to January 2014, during rainy (August to November 2013) and dry seasons (June to July 2013 and December 2013 to January 2014). Surveys always started at 06:00 h and were concluded between 11:00 and 14:00 h. The observers drove along the roadside searching on both sides of the roads looking for mammal mortalities. Roadkills detected out of the regular sampling surveys were considered as incidental encounters and were excluded from the analyses but included in the species account. We excluded domestic animals and unidentified carcasses from our counts. Roadkilled animals were identified to the lowest possible taxonomic level based on morphological features and then were removed from the road to avoid subsequent recounts. For each observation, we recorded specimen location (geographic coordinates), age (as immature or adult), and approximate number of days since death. We also recorded four life history variables that may be important in explaining species' vulnerability to roadkill (*e.g.*, Cáceres 2011, Cook & Blumstein 2013, Mimet *et al.* 2016): time of activity, locomotor habit, diet, and mean body mass. These variables were obtained mainly from Animal Diversity Web database (Myers *et al.* 2017), with additions from Robinson & Redford (1986) and Cáceres (2011).

Roadkill estimates

We estimated mammal roadkill richness as the number of species (S) found dead on the surveyed road segment. A relative road mortality index (RMI) was also estimated by dividing the number of roadkills for each species on the total number of roadkills for all species. Overall mortality rates (*i.e.*, per day and km) were estimated using Siriema software v2.0 (Coelho *et al.* 2014). To avoid overestimation in mortality rates, we used the upper value for searchers' efficiency (detectability) for mammals (*i.e.*, 0.60) and the upper value for the removal time characteristic for mammals (*i.e.*, 5.32 days) estimated by Teixeira *et al.* (2013a). Our estimates of daily mortality rates were multiplied by 365 to generate an annual estimate. For practical purposes, we compared our results with those from other studies estimating a relative road mortality rate by dividing the number of roadkill occasions into the total sampling effort (*i.e.*, the overall number of km surveyed). We used a Mann-Whitney U test to determine if differences in observed counts of roadkills among climatic seasons were statistically significant, whereas a Chi-square test was used to investigate if there were significant variations in roadkill numbers among months ($p < 0.05$).

Roadkill hotspots

We evaluated the dispersion of roadkill events using the modified Ripley's K (Coelho *et al.* 2008) with an initial radius of 100 m, a radius increase of 400 m, confidence level of 95%, and 1,000 Monte Carlo simulations (Teixeira *et al.* 2013b). To detect the road sections with high mortality rates (hotspots), we implemented the 2D HotSpot Identification analysis. For this, we divided each road into 100 m segments and a circle of 200 m radius (*i.e.*, the smallest radius at which the roadkill aggregations were significant in 2D Ripley's K test) was centered at the midpoint of the first segment, summing the values for all roadkill events inside the circle area. This sum was multiplied by a correction factor that considers the length of the road analyzed inside the circle in this position. This procedure was repeated for all segments, resulting in a roadkill aggregation intensity value for each segment of road (for details see Coelho *et al.* 2014). We evaluated the significance of potential aggregations at a confidence level of 95% and 1,000 simulations. Values for aggregation intensity above the upper confidence level of 95% indicate significant roadkill hotspots. For analyses in this section we used Siriema v2.0 (Coelho *et al.* 2014).

Structural connectivity

We quantify multi-scale structural connectivity around surveyed roads for roadkills by building a resistance cost surface to represent the hypothesized relationships between environmental features and multi-scale structural connectivity (see Rayfield *et al.* 2016), using Landsat 8 satellite image (OLI/TIRS) at 30 m spatial resolution (USGS 2017). Resistance was quantified by combining several landscape features (Supplementary Material – Table S2 and Figure S1): (i) the normalized difference vegetation index (NDVI), (ii) the surface temperature, (iii) the Colombian Corine Land Cover classes, (iv) the fragmentation index (entropy metric) calculated with the GuidosToolbox software (Vogt & Riitters 2017), (v) a river proximity map (Euclidean distance), and (vi) a road proximity map (Euclidean distance). Resistance values were then reclassified in a numerical range as a function of cost, from 1 (permeable) to 100 (impermeable). Finally, we estimated the cumulative resistance surface by overlapping the landscape feature maps (Supplementary Material – Figure S2).

We used circuit theory to evaluate landscape-scale connectivity by defining a core area of 1,269 km^2 (equivalent to our study area) and a buffer of 656 km^2 around the core. To obtain a current density map we randomly selected 178 nodes around the perimeter of the buffer and connected all node pairs considering all possible pathways across landscape at once (omnidirectionality), using the advanced mode option in Circuitscape software (McRae *et al.* 2008). We removed the buffer area from the current density map to avoid current associated saturation (Koen *et al.* 2014). In addition, we used graph-theory for modelling local-scale connectivity using Graphab software v.2 (Foltête *et al.* 2012). The complete graph among all node pairs was created based on the resistance surface (Supplementary Material – Figure S1) with links defined by the cumulative cost distance (cost unit), without link thresholding of habitat patches. The contribution of each habitat patch within the entire landscape was estimated using the delta Probability of Connectivity Index (d-PC) (Foltête *et al.* 2012).

We assess whether counts of roadkills differs among areas of low and high landscape-scale connectivity by adding a circular buffer of 100-m radius to each roadkill location. Then, we calculated the current density statistics (minimum, maximum, range, mean, standard error [SE]) for each buffer. We used a t-test to assess whether counts of roadkills differs among areas of low (< 0.50 amperes) and high landscape connectivity (> 0.50 amperes).

RESULTS

Roadkill estimates

During an eight-month period and 16 monitoring trips totalling 1,200 km of road surveys, we recorded a total of 152 roadkilled wild mammals belonging to 12 species: seven carnivores, two didelphids, one cingulate, one pilosa, and one rodent (Table 1; Figure 1). Of this total, we found 112 roadkilled mammals during the surveys and 40 by incidental encounters. Based on their body size, all roadkilled specimens were catalogued as adults. The most frequently roadkilled mammal during monitoring surveys were the common opossum *Didelphis marsupialis* (Didelphimorphia, Didelphidae; N = 35; RMI = 0.31), the crab-eating fox *Cerdocyon thous* (Carnivora, Canidae; N = 25; RMI = 0.22), the northern tamandua *Tamandua mexicana* (Pilosa, Myrmecophagidae; N = 22; RMI = 0.20), and the crab-eating raccoon *Procyon cancrivorus* (Carnivora, Procyonidae; N = 20; RMI = 0.18). Together, these species represent about 91% of the mammal road mortalities in the study area. The least frequently encountered species were the tayra *Eira barbara* (Carnivora, Mustelidae), the greater grison *Galictis vittata* (Carnivora, Mustelidae), the northern naked-tailed armadillo *Cabassous centralis* (Cingulata, Chlamyphoridae), the Neotropical otter *Lontra longicaudis* (Carnivora, Mustelidae), and the jaguarondi *Herpailurus yagouaroundi* (Carnivora, Felidae), which were detected once (Table 1).

Most roadkills were individuals of small and medium species (≤ 7 kg), summing 82% of the mortalities (N = 124; 10 species). Almost three-quarters of the roadkilled specimens (72%) were nocturnal (N = 109; 6 species). Terrestrial (41%; N = 63; 5 species) and scansorial (32%; N = 49; 4 species) specimens were most frequently found roadkilled. Omnivorous species had the highest rates of mortality (69%; N = 107; 5 species). Most roadkill events occurred during the dry season (Z = 1.98; df = 1; p = 0.041; Figure 2). Roadkills also showed non-uniform monthly distribution ($\chi^2 = 52.7$; df = 3; $p < 0.05$), with two roadkill peaks, the first between June and July (N = 32) and a second but more intense peak between December and January (N = 41). The road segments with the highest number of roadkill events were DR01 (N = 40) and NR66 (N = 40); whereas the lowest number of roadkills

Table 1. Mammal species recorded as roadkilled during monitoring surveys in the middle Magdalena Valley, Santander department, Colombia, with the species-specific life history traits included in this study, number of roadkill specimens (incidental encounters in parenthesis), and the road mortality index (RMI). For estimates of RMI, we excluded the data from incidental encounters.

Taxa	Mean body mass (Kg)	Time of activity	Locomotor habit	Diet	Roadkills	RMI
CARNIVORA						
Canidae						
Cerdocyon thous (Linnaeus, 1766)	6.5	Nocturnal	Terrestrial	Omnivorous	25 (9)	0.22
Felidae						
Leopardus pardalis (Linnaeus, 1758)	10.5	Nocturnal	Scansorial	Carnivorous	1 (1)	0.01
Herpailurus yagouaroundi (Geoffroy Saint-Hilaire, 1803)	5.0	Cathemeral	Terrestrial	Carnivorous	0 (1)	---
Mustelidae						
Eira barbara (Linnaeus, 1758)	4.8	Diurnal	Scansorial	Omnivorous	0 (1)	---
Galictis vittata (Schreb, 1776)	2.9	Cathemeral	Terrestrial	Carnivorous	1 (0)	0.01

Table 1. Continued on next page…

Table 1. ...Continued

Taxa	Mean body mass (Kg)	Time of activity	Locomotor habit	Diet	Roadkills	RMI
Lontra longicaudis (Olfers, 1818)	5.8	Diurnal	Aquatic	Piscivorous	0 (1)	---
Procyonidae						
Procyon cancrivorus (Cuvier, 1798)	10.1	Nocturnal	Terrestrial	Omnivorous	20 (6)	0.18
CINGULATA						
Chlamyphoridae						
Cabassous centralis (Miller, 1899)	2.5	Nocturnal	Terrestrial	Insectivorous	0 (1)	---
DIDELPHIMORPHIA						
Didelphidae						
Caluromys lanatus (Olfers, 1818)	0.3	Nocturnal	Scansorial	Omnivorous	2 (1)	0.02
Didelphis marsupialis Linnaeus, 1758	1.0	Nocturnal	Scansorial	Omnivorous	35 (8)	0.31
PILOSA						
Myrmecophagidae						
Tamandua mexicana (Saussure, 1860)	4.2	Cathemeral	Arboreal	Insectivorous	22 (11)	0.20
RODENTIA						
Sciuridae						
Sciurus granatensis Humboldt, 1811	0.3	Diurnal	Arboreal	Herbivorous	6	0.05
Total road mortalities					**112 (40)**	---

was recorded in NR45 (N = 32; Table 2). Similarly, the road segments DR01 and NR66 showed the highest roadkill rates relative to the road segment NR45 (Table 2). The overall estimated relative road mortality rate (roadkills per km) was higher when compared to other studies (Table 3).

Roadkill hotspots

Ripley's K analyses showed significant roadkill aggregations at the 0.1–16.9 km scale for the route DR01, without significant overdispersion. For national routes (NR45 and NR66) there was no significant spatial clustering but significant spatial overdispersion was detected. 2D HotSpot analyses identified four roadkill hotspots in route DR01 at: km 4.2 to 4.8, 7.3 to 8.4, 10.3 to 11.1, and 13.8 to 14.1 (Figure 3). There was no spatial aggregation of roadkills in the other studied roads (*i.e.*, NR45 and NR66), indicating the absence of roadkill hotspots.

Structural connectivity

The omnidirectional connectivity mosaic (mean = 0.62 ± 0.42) shows a variety of current flow patterns across the full extent of the study area (Figure 3a). Contiguous patches of high connectivity values reflect large patches of relatively unaltered lands with low resistance values. Current flows through low and medium resistance areas with a relatively high probability of use as movement corridors (high current density), such as those associated with native forests and oil palm plantations. Eastern regions tended to have lower resistance values leading to higher corridor values, but many western regions also host relatively well-connected networks that are critical corridors for overall landscape connectivity maintenance.

The graph-connectivity model contains 1,506 habitat patches ranging from 0.45 to 6,886.8 ha (mean 20.1 ha) and 5,144 links (Figure 3c).

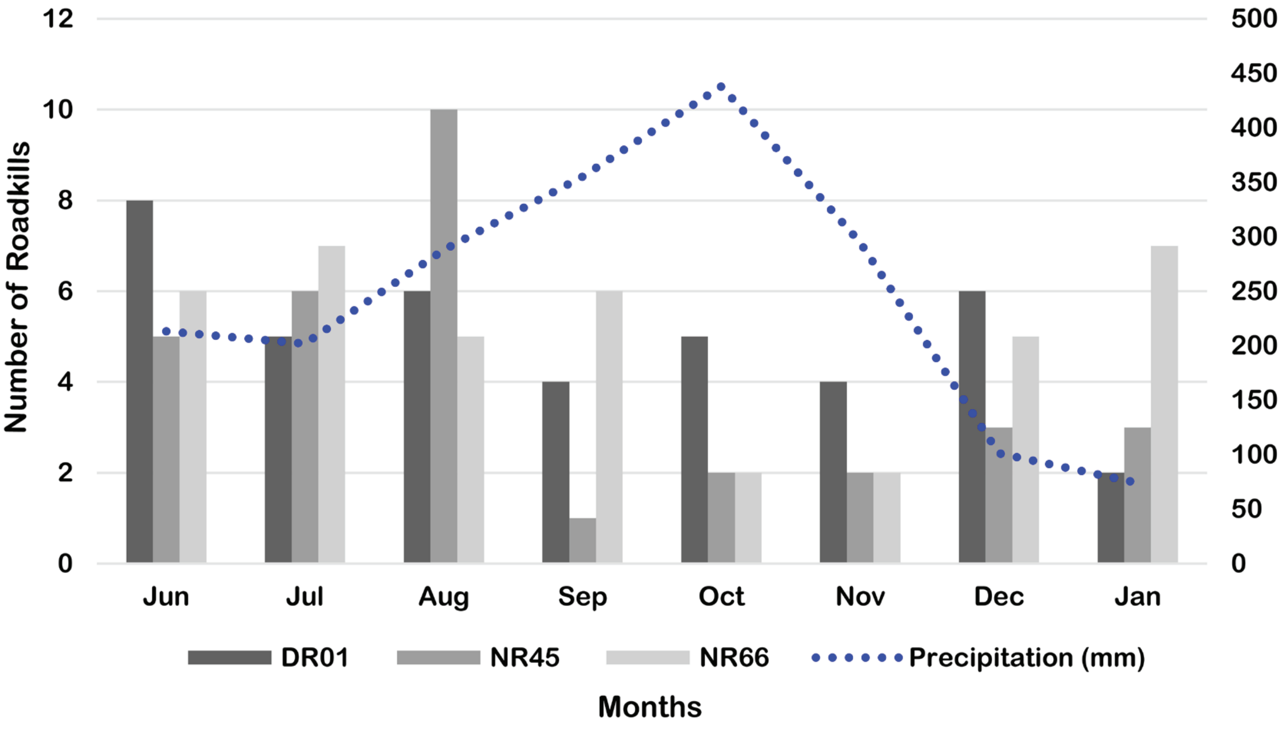

Figure 2. Roadkilled mammals against total precipitation per month in each surveyed road segment during monitoring trips in the middle Magdalena Valley, Santander department, Colombia. For estimates, we excluded the data from incidental encounters. Abbreviations: (DR01) Departmental Route 01, (NR45) National Route 45, and (NR66) National Route 66.

Cumulative cost path values among habitat patches were variable (mean = 2,928.2 ± 1,943.7), with shortest least-cost paths traced through native forest and oil palm plantations. High-cost paths occur in open areas with little or no associated vegetation, particularly along road networks. Node importance based on the probability of connectivity (d-PC) shows that landscape adjacent to studied roads is composed of small, isolated habitat patches with lower connectivity values (< 0.004). Higher d-PC values (> 0.04) are related to habitat patches in the eastward of the study area, indicating that comparatively, they are most important to maintaining overall connectivity (Figure 3c).

Roads on the study area do not constitute an impenetrable barrier to the movement of wild mammals (Figure 3a, c). Some roadkill locations (N = 31) intersect with sections of the landscape with high connectivity (current density > 0.51 amperes) that are critical for animal movement, especially on the northern (NR66) and eastern

Table 2. Mammal road mortality and roadkill rate estimates for each surveyed road segment during monitoring trips in the middle Magdalena Valley, Santander department, Colombia. For estimates, we excluded the data from incidental encounters.

Road segment	Roadkill animals	Roadkill species (S)	Length (km)	Total surveys	Total km surveyed	Roadkill rate per km	Roadkill rate per day	Roadkill rate per year
DR01	40	6	26	16	416	0.042	1.10	402
NR45	32	7	18	16	288	0.049	0.88	321
NR66	40	4	31	16	496	0.036	1.10	402
Total	**112**	**8**	**75**	**16**	**1,200**	**0.041**	**3.08**	**1,125**

Table 3. Survey methods and relative road mortality rate (RMR, roadkills per km surveyed) for roadkill studies including wild mammals in Colombia. RMR estimates include only roadkill events of wild mammal recorded during monitoring trips.

Study	Survey methods	Location	Elevation (m)	Roadkill animals	Total km surveyed	RMR
This study	Car (≤ 40 km/h), twice per month, 8 months	Middle Magdalena Valley, Santander department	74-100	112	1,200	0.093
Castillo *et al.* (2015)	Motorcycle (25 km/h), four times per week, 5 months	Andean region, Cordillera Occidental, Cauca department	639-1757	259	4,600	0.056
Monroy *et al.* (2015)	Motorcycle (15 km/h), twice per week, 6 months	Caribbean region, Sucre department	14-32	104	2,352	0.044
de la Ossa-Nadjar & de la Ossa-V (2015)	Motorcycle (15 km/h), six times per week, 8 months	Caribbean region, Sucre department	45-307	104	19,066	0.005
de La Ossa-V & Galván-Guevara (2015)	Motorcycle (14 km/h), twice per week, 6 months	Caribbean region, Sucre department	6-64	121	1,306	0.092
de la Ossa-Nadjar & de la Ossa-V (2013)	Motorcycle, four times per week, 6 months	Caribbean region, Sucre department	45-307	114	9,536	0.012
Payan *et al.* (2013)	Motorcycle (velocity no reported), 17 day between April and June	Magdalena Valley, para-Caribbean dry belt, Cesar department	No reported	190	2,753	0.069

(NR45) study area; whereas most of roadkill locations (N = 81) intersect with areas of low landscape connectivity (current density < 0.50 amperes; Figure 3a, c; Supplementary Material – Table S3). Higher mortality rates were detected in areas of low landscape connectivity ($t = -11.604$, $p < 0.05$). Roadkill hotspots identified in route DR01 (km 4.2 to 4.8, 7.3 to 8.4, 10.3 to 11.1, and 13.8 to 14.1; Figure 3b, d) coincides with areas of low landscape connectivity dominated by matrix habitats (*e.g.*, grasslands, cultivated fields, and mineral extraction sites).

DISCUSSION

Previous studies have shown that species-specific life history traits are an influential factor in the occurrence of wildlife roadkills, with species with omnivorous diets, nocturnal habits, small body size, and high mobility being more prone to be roadkilled (*e.g.*, Ford & Fahrig 2007, Cáceres 2011, Cook & Blumstein 2013, Mimet *et al.* 2016). In this study, road mortality was concentrated on four usually abundant, nocturnal, highly mobile, habitat generalist species: *D. marsupialis*, *C. thous*, *T. mexicana*, and *P. cancrivorus*. Our observations are consistent with findings from previous studies in Colombia, which have shown that these species of wild mammals are more prone to roadkill events (*e.g.*, de la Ossa-Nadjar & de la Ossa-V 2013, 2015, Payan *et al.* 2013, Castillo *et al.* 2015, Monroy *et al.* 2015). We hypothesize that the continued loss of reproductive-age adults of these species due to roadkill in the study area can have severe populational and ecological consequences with cumulative effects that might take multiple

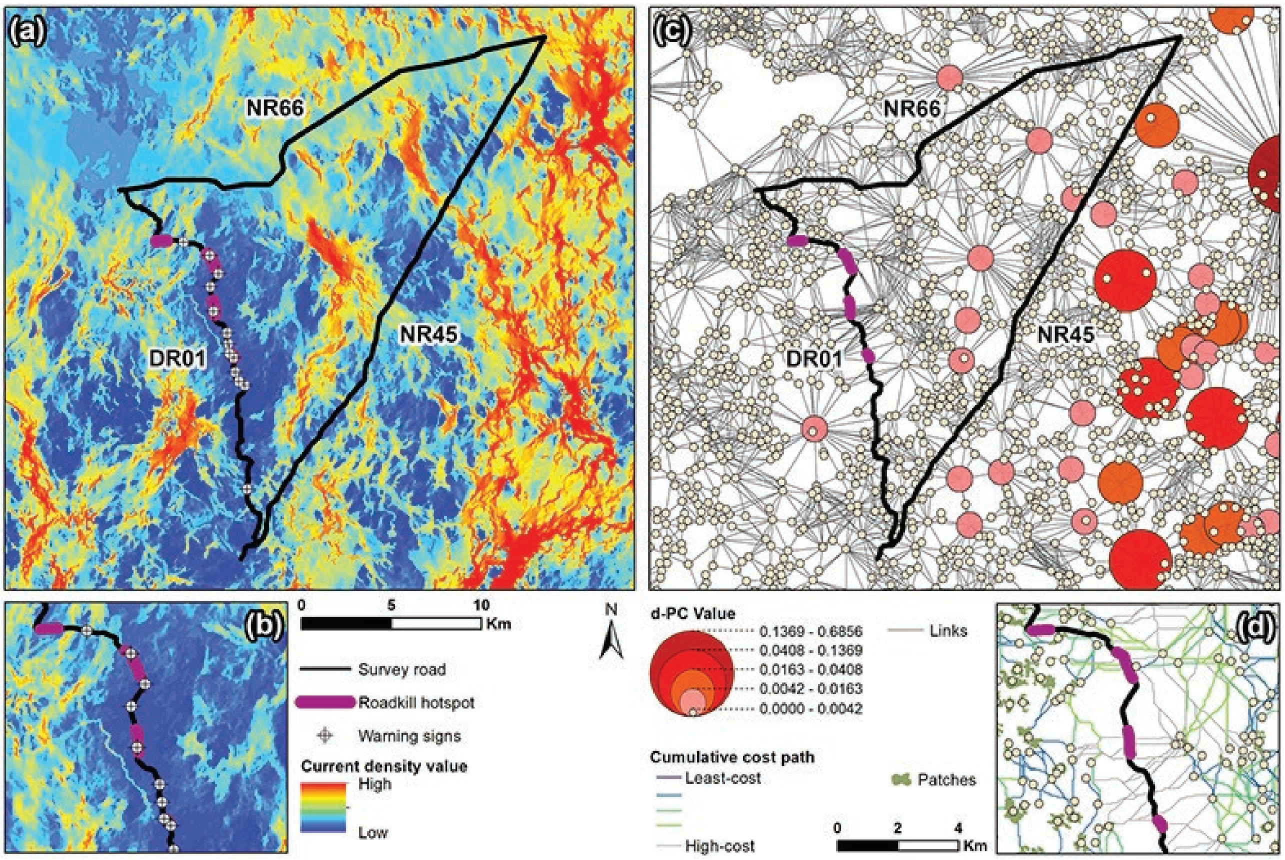

Figure 3. Multi-scale structural connectivity maps and roadkill hotspots in the Middle Magdalena Valley, Santander department, Colombia. (a) Landscape-scale omnidirectional connectivity map (Circuitscape analysis). (b) Detailed circuitscape analysis map indicating the location of roadkill hotspots on DR01. (c) Local-scale connectivity (graph theory) map without link thresholding. (d) Detailed spatial graph map showing the location of roadkill hotspots on DR01. Abbreviations: (DR01) Departmental Route 01, (NR45) National Route 45, and (NR66) National Route 66.

generations to recover, or they might never recover at all. Alternatively, high mortality rates for these species may be due to a combination of factors, including high population densities and higher ecological resiliency to roadkills. Further studies (*e.g.*, genetic diversity and population demography) at local and regional level are needed to test these hypotheses.

On the other hand, road and traffic impacts on animal populations may depend on species-specific behavioural responses (Jaeger *et al.* 2005, Fahrig & Rytwinski 2009). For example, the generalist behaviour of *D. marsupialis* and *P. cancrivorus*, the freeze antipredator response of *C. thous*, and the clumsy and slow terrestrial locomotion of *T. mexicana* have been invoked to explain the high road mortality rates of these species (Artavia *et al.* 2015). Likewise, many species do not show car avoidance behaviour, increasing their roadkill probability, especially when population's densities are high (Jaeger *et al.* 2005, Ascensão *et al.* 2017). Moreover, wildlife communities with high species richness will be more susceptible to road mortality effects because a higher number of species are prone to be roadkilled (Ascensão *et al.* 2017). Road size, road quality, and vehicular speed represent additional factors that could influence the number of local wildlife roadkills and deserve further investigation.

Seasonal and monthly peaks of road mortalities coincide with holiday times in Colombia (especially June to July and December to January) when high rates of recreational trips intensify the traffic volume in the study area. Increase in roadkills during the dry season is probably linked to species mating seasons, when individuals tend to move more frequently or larger distances to find a mate (as suggested by Smith-Patten & Patten 2008); and low resource availability, that force animals to foraging in larger areas to find food (Bueno & Almeida 2010).

However, seasonal differences in roadkills may be also related to the persistence time of carcasses during rainy seasons, since the action of rainfall and runoff promotes faster degradation of carcass and washes away carcass debris (see Santos *et al.* 2011, 2016).

We found that routes DR01 and NR66 presented the highest number of roadkill events (N = 40, each), involving six and four species, respectively. Our estimates of roadkill rate per year suggest that at least 1,125 native mammals are killed by vehicle collisions in the study area. However, our numbers are likely conservative because for the analyses we used the upper limits for searcher detectability and carcass removal characteristic time parameters estimated by Teixeira *et al.* (2013a). Roadside mowing and delayed mortality of hurt animals may also lead to underestimates of roadkill rates. Similarly, surveys at larger time intervals (*e.g.*, fortnightly) may also lead to underestimations due to small animals are usually not registered because they are rapidly obliterated by the daily traffic flow. Small carcasses are expected to remain for shorter periods, particularly in roads with high traffic flow given the faster degradation caused by vehicles passing by (Slater 2002, Santos *et al.* 2011, 2016). Moreover, activity by diurnal scavenger birds (mainly *Coragyps atratus*, Accipitriformes, Cathartidae; *Caracara cheriway*, Falconiformes, Falconidae; and *Milvago chimachima*, Falconiformes, Falconidae) in road vicinities may positively enhancing the carcass removal process (see Ratton *et al.* 2014, Hill *et al.* 2018).

We identified several hotspot sections along route DR01 coinciding with sections adjacent to human settlements and oil extraction infrastructure in La Cira Infantas oil field, an area with high daily vehicular traffic mainly associated with petroleum industry operations. The location of these roadkill hotspots seems associated with areas with low regional structural connectivity, where the current flow is influenced by the presence of highly human-modified habitats. This may be due to the fact that animals living in forested areas are probably forced to travel through lower quality habitats when searching for potential habitat patches (see Spear et al. 2010, Trainor et al. 2013), whereas habitat generalist species probably used the land-cover matrix as habitat and/or as movement paths (Bueno et al. 2015, Freitas et al. 2015), which in both cases increases roadkill risk because of higher road crossing rates. Up to now, the only measure taken to address wildlife roadkills has been the installation of 12 wildlife warning signs (Figure 3b) along route DR01. Unfortunately, this strategy appears to be ineffective because their location largely coincides with roadkill hotspots. This finding is consistent with previous studies suggesting that warning signs, as an individual mitigation strategy, has low effectiveness (Rytwinski et al. 2016).

The tremendous expansion of national infrastructure included in the governmental program “Generation (4G) of Road Concessions in Colombia” increases the need to understand and assess adequately the impacts of roads and traffic on ecosystems and wildlife. Based on our results, we propose the implementation of multi-scale management measures to diminish the impact caused by roads on wildlife (see Ascensão *et al.* 2017). From a local-scale perspective, actions should be focused on installing traffic speed reducers and signage in the locations of roadkill hotspots, as well as environmental education to raise public awareness and improve driver attitudes to benefit wildlife conservation. From the metapopulation- and landscape-scale perspectives, strategies should include the installation of crossing structures such as underpasses and overpasses in areas of high structural connectivity, accompanied by drift fencing to channel wildlife to passages. These crossings should not be intended to eliminate roadkills but to provide wildlife movement corridors, linking larger functional landscape between core areas to maintain connectivity among populations (Beckmann *et al.* 2010, Bueno *et al.* 2015). Other mitigation measures should also be considered, including the protection of priority habitat patches (*i.e.*, nodes with high dPC values) and the restoration of degraded areas and critical corridors (*i.e.*, pinch points) to reduce habitat fragmentation and increase habitat connectivity among local and regional populations (see Mcrae *et al.* 2012, Langen *et al.* 2015). We believe that functional connectivity analysis combining landscape attributes, road-related factors, and species-specific traits may be a promising approach to understand roadkill spatiotemporal patterns. Finally, we emphasize the urgent need of integrate road ecology research with road planning and environmental licensing for road construction, paving, and widening, through a multidisciplinary approach involving infrastructure planners, engineers, road ecologist, and environmental authorities to effectively mitigate wildlife mortality on national roads (see Gunson & Teixeira 2015, Teixeira *et al.* 2016).

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REFERENCES

Artavia, A., Jiménez, M., Martínez-Salinas, A., Pomadera, E., Araya-Gamboa, D., & Arévalo-Huezo, E. 2015. Registro de mamíferos silvestres en la sección de la ampliación de la Ruta 32, Limón, Costa Rica. Brenesia, 83-84, 37–46.

Ascensão, F., Desbiez, A. L. J., Medici, E. P., & Bager, A. 2017. Spatial patterns of road mortality of medium–large mammals in Mato Grosso do Sul, Brazil. Wildlife Research, 44(2), 135–146. DOI: 10.1071/WR16108

Beckmann, J. P., Clevenger, A. P., Huijser, M. P. & Hilty, J. A., 2010. Safe passages: highways, wildlife, and habitat connectivity. Washington, D.C.: Island Press: p. 396.

Bueno, C., & Almeida, P. J. A. L. 2010. Sazonalidade de atropelamentos e os padrões de movimentos em mamíferos na BR-040 (Rio de Janeiro-Juiz de Fora). Revista Brasileira de Zoociências, 12(3), 219–226.

Bueno, C., Sousa, C. O. M., & de Freitas, S. R. 2015. Habitat or matrix: which is more relevant to predict road-kill of vertebrates? Brazilian Journal of Biology, 75(4), S228–S238. DOI: 10.1590/1519-6984.12614

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

Castillo, J. C., Urmendez-M, D., & Zambrano-G, G. 2015. Mortalidad de fauna por atropello vehicular en un sector de la Vía Panamericana entre Popayán y Patía. Boletín Científico Museo de Historia Natural de la Universidad de Caldas, 19(2), 207–219.

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

Coelho, I. P., Kindel, A., & Coelho, A. V. P. 2008. Roadkills of vertebrate species on two highways through the Atlantic Forest Biosphere Reserve, southern Brazil. European Journal of Wildlife Research, 54, 689–699. DOI: 10.1007/s10344-008-0197-4

Coelho A. V. P., Coelho I. P., Teixeira F. Z., & Kindell, A. 2014. SIRIEMA, spatial evaluation of road mortality software. (Retrieved on January 12th, 2018, from http://www.ufrgs.br/siriema/).

Cook T. C., & Blumstein D. T. 2013. The omnivore’s dilemma: diet explains variation in vulnerability to vehicle collision mortality. Biological Conservation, 167, 310–315. DOI: 10.1016/j.biocon.2013.08.016

de la Ossa-Nadjar, O., & de la Ossa-V, J. 2013. Fauna silvestre atropellada en dos vías principales que rodean los Montes de María, Sucre, Colombia. Revista Colombiana de Ciencia Animal, 5(1), 158–164.

de la Ossa-Nadjar, O., & de la Ossa-V, J. 2015. Vehicle collisions with wild fauna on the two roads that pass through the Montes de María, Sucre, Colombia. Revista U.D.C.A. Actualidad & Divulgación Científica, 18(2), 503–511.

de la Ossa-V, J., & Galván-Guevara, S. 2015. Registro de mortalidad de fauna silvestre por colisión vehicular en la carretera Toluviejo–ciénaga La Caimanera, Sucre, Colombia. Biota Colombiana, 16(1), 67–77. DOI: 10.21068/c0001

Fabrizio, M., Di Febbraro, M., D’Amico, M., Frate, L., Roscioni, F., & Loy, A. 2019. Habitat suitability vs. landscape connectivity determining road-kill risk at a regional scale: a case study on European badger (*Meles meles*). European Journal of Wildlife Research, 65, 7. DOI: 10.1007/s10344-018-1241-7

Fahrig, L., & Rytwinski, T. 2009. Effects of roads on animal abundance: an empirical review and synthesis. Ecology and Society, 14(1), 21.

Foltête, J.-C., Clauzel, C., & Vuidel, G. 2012. A software tool dedicated to the modelling of landscape networks. Environmental Modelling and Software, 38, 316–327. DOI: 10.1016/j.envsoft.2012.07.002

Ford, A. T., & Fahrig, L. 2007. Diet and body size of North American mammal road mortalities. Transportation Research Part D 12, 498–505. DOI: 10.1016/j.trd.2007.07.002

Forman, R. T., & Alexander, L. E. 1998. Roads and their major ecological effects. Annual Review of Ecology and Systematics, 29, 207–231. DOI: 10.1146/annurev.ecolsys.29.1.207

Forman, R. T., Sperling, D., Bissonette, J. A., Clevenger, A. P., Cutshall, C. D., Dale, V. H., Fahrig, L., France, R., Goldman, C. R., Heanue, K., Jones, J. A., Swanson, F. J., Turrentine, T., & Winter T. C. 2003. Road ecology: science and solutions. Washington, D. C.: Island Press: p. 481.

Freitas, S. R., Nepomuceno de Oliveira, A., Ciocheti, G., Vieira, M. V., Maria, D., & Matos, S. 2015. How landscape features influence road-kill of three species of mammals in the Brazilian savanna? Oecologia Australis, 18, 35–45. DOI: 10.4257/oeco.2014.1801.02

Garzón, N. V., & Gutiérrez, J. C. 2013. Deterioro de humedales en el Magdalena Medio: un llamado para su conservación. Bogotá D.C.: Fundación Alma – Instituto de Investigación de Recursos Biológicos Alexander von Humboldt: p. 145.

Gunson, K. E., Mountrakis, G., & Quackenbush, L. J. 2011. Spatial wildlife-vehicle collision models: a review of current work and its application to transportation mitigation projects. Journal of Environmental Management, 92, 1074–1082. DOI: 10.1016/j.jenvman.2010.11.027

Gunson, K. E., & Teixeira, F. Z. 2015. Road–wildlife mitigation planning can be improved by identifying the patterns and processes associated with wildlife-vehicle collisions. In: R. van der Ree, D. J. Smith & C. Grilo (Eds.), Handbook of road ecology. pp. 101–109. West Sussex: John Wiley & Sons Ltd.

Hill, J. E., DeVault, T.L., Beasley, J.C., Rhodes Jr., O. E. & Belant, J. L. 2018. Effects of vulture exclusion on carrion consumption by facultative scavengers. Ecology and Evolution, 8, 2518–2526. DOI: 10.1002/ece3.3840

IDEAM - Instituto de Hidrología, Meteorología y Estudios Ambientales. 2016. Promedios climatológicos. (Retrieved on May 23rd, 2018, from http://www.ideam.gov.co/).

Jaeger, J. A. G., Bowman, J., & Brennan, J. 2005. Predicting when animal populations are at risk from roads: an interactive model of road avoidance behavior. Ecological Modelling, 185, 329–348. DOI: 10.1016/j.ecolmodel.2004.12.015

Kazemi, V. D., Jafari, H., & Yavari, A. 2016. Spatio-temporal patterns of wildlife road mortality in Golestan National Park-North East of Iran. Open Journal of Ecology, 6, 312–324. DOI: 10.4236/oje.2016.66031

Koen, E. L., Bowman, J., Sadowski, C., & Walpole, A. A. 2014. Landscape connectivity for wildlife: development and validation of multispecies linkage maps. Methods in Ecology and Evolution, 5, 626–633. DOI: 10.1111/2041-210X.12197

Langen, T. A., Gunson, K. E., Jackson, S. D., Smith, D. J., & Ruediger, W. 2015. Planning and designing mitigation of road effects on small animals. In: K. M. Andrews, P. Nanjappa & S. P. D. Riley (Eds.), Roads and ecological infrastructure, concepts and applications for small animals. pp. 146–176. Baltimore: Johns Hopkins University Press.

Marsh, D. M., & Jaeger, J. A. C. 2015. Direct effects of roads on small animal populations. In: K. M. Andrews, P. Nanjappa & S. P. D. Riley (Eds.), Roads and ecological infrastructure, concepts and applications for small animals. pp. 42–56. Baltimore: Johns Hopkins University Press.

McRae, B. H., Dickson, B. G., Keitt, T. H., & Shah, V. B. 2008. Using circuit theory to model connectivity in ecology, evolution, and conservation. Ecology, 89(10), 2712–2724. DOI: 10.1890/07-1861.1

Mcrae, B. H., Hall, S. A., Beier, P., & Theobald, D. M. 2012. Where to restore ecological connectivity? Detecting barriers and quantifying restoration benefits. PLoS One, 7(12), e52604. DOI: 10.1371/journal.pone.0052604

Meza-Joya, F. L., Ramos-Pallares, E., Cardona, D., & Torres, M. 2015. Atropellamiento vehicular de mamíferos silvestre en el Magdalena Medio Santandereano. Mammalogy Notes, 2, 190.

Mimet, A., Clauzel, C., & Foltête, J. C. 2016. Locating wildlife crossings for multispecies connectivity across linear infrastructures. Landscape Ecology, 31(9), 1955–1973. DOI: 10.1007/s10980-016-0373-y

Monroy, M. C., 2015. Tasa de atropellamiento de fauna silvestre en la vía San Onofre-María la baja, Caribe Colombiano. Revista de la Asociación Colombiana de Ciencias Biológicas, 27, 88–95.

Myers, P., Espinosa, R., Parr, C. S., Jones, T., Hammond, G. S., & Dewey, T. A. 2017. The Animal Diversity Web. (Retrieved on June 11th, 2018, from https://animaldiversity.org/

Payan, E., Soto, C., Díaz-Pulido, A., Benítez, A., & Hernández, A. 2013. Wildlife road crossing and mortality: lessons for wildlife friendly road design in Colombia. Proceedings of the 2013 international conference on ecology and transportation, Scottsdale, USA.

Ramos, E., & Meza-Joya, F. L. 2018. Reptile road mortality in a fragmented landscape of the Middle Magdalena Valley, Colombia. Herpetology Notes, 11, 81–9.

Ratton, P., Secco, H., & Rosa, C.A. 2014. Carcass permanency time and its implications to the roadkill data. European Journal of Wildlife Research, 60(3), 543–543. DOI: 10.1007/s10344-014-0798-z

Rayfield, B., Pelletier, D., Dumitru, M., Cardille, J. A., & Gonzalez, A. 2016. Multipurpose habitat networks for short-range and long-range connectivity: A new method combining graph and circuit connectivity. Methods in Ecology and Evolution, 7, 222–231. DOI: 10.1111/2041-210X.12470

Robinson, J. G., & Redford, K. H. 1986. Body size, diet, and population density of Neotropical forest mammals. American Naturalist, 128, 65–680. DOI:10.1086/284596

Rosa, C. A., & Bager, A. 2013. Review of the factors underlying the mechanisms and effects of roads on vertebrates. Oecologia Australis, 17(1), 6–19. DOI: 10.4257/oeco.2013.1701.02

Rytwinski, T., & Fahrig, L. 2013. Why are some animal populations unaffected or positively affected by roads? Oecologia, 173(3), 1143–56. DOI: 10.1007/s00442-013-2684-x

Rytwinski, T., Soanes, K., Jaeger, J. A. G., Fahrig, L., Findlay, C. S., Houlahan, J., van der Ree, R., & van der Grift, E. A. 2016. How effective is road mitigation at reducing road-kill? A meta-analysis. PLoS ONE, 11(11): e0166941. DOI: 10.1371/journal.pone.0166941

Santos, S. M., Carvalho, F., & Mira, A. 2011. How long do the dead survive on the road? Carcass persistence probability and implications for road-kill monitoring surveys. PLoS ONE, 6(9), e25383. DOI: 10.1371/journal.pone.0025383

Santos, R. A. L., Santos, S. M., Santos-Reis, M., Picanco de Figueiredo, A., Bager, A., Aguiar, L. M. S. & Ascensão, F. 2016. Carcass persistence and detectability: reducing the uncertainty surrounding wildlife–vehicle collision surveys. PLoS ONE, 11(11), e0165608. DOI: 10.1371/journal.pone.0165608

Seiler, A. 2001. Ecological effects of roads: A review. Introductory Research Essay, 9. Riddarhyttan, Sweden: Department of Conservation Biology, University of Agricultural Sciences.

Slater, F. M. 2002. An assessment of wildlife road casualties – the potential discrepancy between numbers counted and numbers killed. Web Ecology, 3, 33–42. DOI: 10.5194/we-3-33-2002

Smith-Patten, B. D., & Patten, M. A. 2008. Diversity, seasonality, and context of mammalian roadkills in the Southern Great Plains. Environmental Management, 41: 844–852. DOI: 10.1007/s00267-008-9089-3

Spear, S. F., Balkenhol, N., Fortín, M. J., McRae, B. H., & Scribner, K. 2010. Use of resistance surfaces for landscape genetic studies: considerations for parameterization and analysis. Molecular Ecology, 19, 3576–3591. DOI: 10.1111/j.1365-294X.2010.04657.x

Teixeira, F. Z., Coelho, A. V. P., Esperandio, I. B., & Kindel, A. 2013a. Vertebrate road mortality estimates: effects of sampling methods and carcass removal. Biological Conservation, 157, 317–323. DOI: 10.1016/j.biocon.2012.09.006

Teixeira, F. Z., Coelho, I. P., Esperandio, I. B., Oliveira, N. R, Porto-Peter, F., Dornelles, S. S., Delazeri, N. R., Tavares, M., Borges-Martins, M., & Kindel, A. 2013b. Are road-kill hotspots coincident among different vertebrate groups? Oecologia Australis, 17, 36–47. DOI: 10.4257/oeco.2013.1701.04.

Teixeira, F. Z., Coelho, I. P., Lauxen, M., Esperandio, I. B., Hartz, S. M., & Kindel, A. 2016. The need to improve and integrate science and environmental licensing to mitigate wildlife mortality on roads in Brazil. Tropical Conservation Science, 9(1), 34–42. DOI: 10.1177/194008291600900104

Trainor, A. M., Walters, J. R., Morris, W. F., Sexton, J., & Moody, A. 2013. Empirical estimation of dispersal resistance surfaces: a case study with red-cockaded woodpeckers. Landscape Ecology, 28(4), 755–767. DOI: 10.1007/s10980-013-9861-5

USGS - United States Geological Survey. 2017. Geological Survey, Landsat 8 OLI (Operational Land Imager) and TIRS (Thermal Infrared Sensor). Retrieved on February 5th, 2018, from https://lta.cr.usgs.gov/

van der Grift, E. A., van der Ree, R., Fahrig, L., Findlay, S., Houlahan, J., Jaeger, J. A. G., Klar, N., Madriñan, L. F., & Olson, L. 2013. Evaluating the effectiveness of road mitigation measures. Biodiversity and Conservation, 22(2), 425–448. DOI: 10.1007/s10531-012-0421-0

Vogt, P., & Riitters, K., 2017. GuidosToolbox : universal digital image object analysis. European Journal of Remote Sensing, 50(1), 352–361. DOI: 10.1080/22797254.2017.1330650

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Supplementary Material - Table S1. General description of the surveyed route segments, landscape, management, anthropogenic features (urbanization level), and wildlife roadkill management measures.

Supplementary Material - Table S2. Resistance values assigned to different landscape features in the study area.

Supplementary Material - Table S3. Current density statistics from 100-m circular buffers around each roadkill location. Low landscape connectivity (< 0.50 amperes), high landscape connectivity (> 0.50, gray highlighted). *Mortality events associated to roadkill hotspots.

Supplementary Material - Figure S1. Landscape features used for createating a resistance surface of the study area. Spatial resolution of 30 m.

Supplementary Material - Figure S2. Resistance surfaces of the study area representing the hypothesized relationships between environmental features and multi–scale structural connectivity. Spatial resolution of 30 m.