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Wildlife roadkills in the Colombian Andes: Hotspots, causes, and most affected species

Atropellamientos de vida silvestre en los Andes colombianos: Puntos críticos, causas y especies más afectadas

Atropelamentos de vida selvagem nos Andes colombianos: Pontos críticos, causas e espécies mais afetadas

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Abstract

Background: Despite the improvements in connectivity and mobility of the national highways, road infrastructure promotes significant environmental disturbances, including wildlife-vehicles collisions. **Objective:** To estimate the direct road mortality of wildlife species during the two-lane Autopista al Mar 1 highway construction, Antioquia (Colombia), 2018-2021. **Methods:** A retrospective observational study was conducted, using primary sources of information. Descriptive statistics were used to estimate the direct road mortality for each taxonomic group, and two roadkill rates were calculated (Roadkills/day and Roadkills/km/day). Events and month/year relations were explored using ANOVA, critical points were identified using a conglomerates analysis and Ripley's K statistic, and a logistic regression was performed to determine which environmental parameters best-predicted wildlife roadkill along the road. **Results:** A total of 295 events were recorded, being *Didelphis marsupialis* (18.3%) and *Iguana iguana* (13.6%) the most frequent roadkilled species in the study area. The number of roadkills/day was 0.7553 and the number of roadkills/km/day was 0.0218; at least 275.68 individuals are road killed every year in this highway. A greater value of spatial aggregation was found in eight critical points of the highway but were not associated to any of the environmental parameters explored. **Conclusion:** Opossums and iguanas represented more than a third of the animal mortality recorded on the road of interest. Environmental and temporal factors did not explain this mortality, but explanatory factors associated with the COVID-19 pandemic are hypothesized. The road mortality data presented can serve the management of road accidents, mainly the implementation of mitigation measures that can reduce the mortality of wildlife species on the road.

Keywords: *Boa imperator; Coragyps atratus; Didelphis marsupialis; epidemiology; fauna road-kill; Iguana iguana; roadkill; wild animals*

Resumen

Antecedentes: A pesar de la mejoría en la conectividad y movilidad de las carreteras nacionales, la infraestructura vial promueve importantes perturbaciones ambientales, incluidas colisiones entre vehículos y fauna silvestre. **Objetivo:** Estimar la mortalidad vial directa de especies silvestres durante la construcción de la Autopista al Mar 1 de dos carriles, Antioquia (Colombia), 2018-2021. **Métodos:** Se realizó un estudio observacional retrospectivo, utilizando fuentes primarias de información. Se utilizaron estadísticas descriptivas para estimar la mortalidad directa en carretera para cada grupo taxonómico y se calcularon dos tasas de atropellos (atropellados/día y atropellados/km/día). Los eventos y las relaciones mes/año se exploraron usando ANOVA, los puntos críticos se identificaron usando un análisis de conglomerados y la estadística K de Ripley, y se realizó una regresión logística para determinar qué parámetros ambientales predijeron mejor los atropellos de vida silvestre a lo largo de la carretera. **Resultados:** Se registraron un total de 295 eventos, siendo *Didelphis marsupialis* (18,3%) e *Iguana iguana* (13,6%) las especies atropelladas con mayor frecuencia en el área de estudio. El número de atropellamientos/día fue de 0,7553 y el número de atropellamientos/km/día fue de 0,0218, es decir que estimamos una mortalidad de al menos 275,68 animales silvestres cada año en esta carretera. Se encontró un mayor valor de agregación espacial en ocho puntos críticos de la carretera, pero no estuvieron asociados a ninguno de los parámetros ambientales explorados. **Conclusión:** Zarigüeyas e iguanas representaron más de un tercio de la mortalidad animal registrada en la vía de interés. Los factores ambientales y temporales no explicaron esta mortalidad, pero se plantean hipótesis sobre factores explicativos asociados con la pandemia de COVID-19. Los datos de mortalidad vial presentados pueden servir en la gestión de los accidentes viales, principalmente en la implementación de medidas de mitigación que puedan reducir la mortalidad de especies silvestres en la vía.

Palabras clave: *atropellamiento; atropellamiento de fauna; Boa imperator; Coragyps atratus; Didelphis marsupialis; epidemiología; fauna silvestre; Iguana iguana.*

Resumo

Antecedentes: Despeito das melhorias na conectividade e mobilidade das rodovias nacionais, a infraestrutura rodoviária promove perturbações ambientais significativas, incluindo colisões entre veículos e animais selvagens. **Objetivo:** Este estudo teve como objetivo estimar a mortalidade rodoviária direta de espécies silvestres durante a construção da rodovia de duas pistas Autopista al Mar 1, Antioquia (Colômbia), 2018-2021. **Métodos:** Foi realizado um estudo observacional retrospectivo, utilizando fontes primárias de informação. Estatísticas descritivas foram usadas para estimar a mortalidade rodoviária direta para cada grupo taxonômico e duas taxas de atropelamentos foram calculadas (atropelados/dia e atropelados/km/dia). Os eventos e as relações mês/ano foram explorados usando ANOVA, os pontos críticos foram identificados usando uma análise de conglomerados e a estatística K de Ripley. Uma regressão logística foi realizada para determinar quais parâmetros ambientais previam melhor os atropelamentos de animais selvagens ao longo da estrada. **Resultados:** Foram registrados 295 eventos, sendo *Didelphis marsupialis* (18,3%) e *Iguana iguana* (13,6%) as espécies atropeladas com maior frequência na área de estudo. O número de atropelamentos/dia foi de 0,7553 e o número de atropelamentos/km/dia foi de 0,0218; pelo menos 275,68 animais selvagens morrem todos os anos nesta rodovia. Maiores valores de agregação espacial foram encontrados em oito pontos críticos da rodovia, mas não foram associados a nenhum dos parâmetros ambientais explorados. **Conclusão:** Gambás e iguanas representaram mais de um terço da mortalidade animal registrada na estrada de interesse. Fatores ambientais e temporais não explicaram esta mortalidade, porem são hipotetizados fatores explicativos associados à pandemia por COVID-19. Os dados de mortalidade rodoviária apresentados podem servir para a gestão de acidentes rodoviários, principalmente para a implementação de medidas de mitigação que possam reduzir a mortalidade de espécies selvagens nas estradas.

Palavras-chave: *atropelar; Boa imperator; Coragyps atratus; Didelphis marsupialis; epidemiologia; fauna silvestre; Iguana iguana; vida selvagem atropelada.*

Introduction

Roads promote urban development but also disconnect areas that are part of the distribution range of the species (Carvalho and Mira, 2011). This lack of connectivity has drastic consequences on the dispersion and migration of animals, as it may reduce population size and genetic diversity (Riggio and Caro, 2017). Both invertebrates and vertebrates are vulnerable to these effects —irrespective of their dimensions, which can result in population declines (Fahrig and Rytwinski, 2009).

In Colombia there is a new national program on road infrastructure that involves the construction and operation of more than 8,000 km of highways. The objective of that program, known as Fourth Generation Highways (4G), is to improve the country's competitiveness by reducing the cost and time of transportation of cargo and people, particularly from manufacturing points to export ports. The highway Autopista al Mar 1 is one of the 4G road infrastructure projects in the country, this will modify an existing road into a two-lane highway, including the construction of a second tunnel and its accesses (Devimar, 2022).

Several Latin American countries have compiled databases documenting wildlife mortality on roads, which have contributed to the emerging field of road ecology (Gallina and Badillo, 2013; Morantes-Hernández, 2017). Medrano-Vizcaíno *et al.* (2022) conducted the first evaluation of roadkill impacts on birds and mammals in the region, while Medrano-Vizcaíno *et al.* (2023) provided the initial national assessment of wildlife mortality on Ecuadorian roads. In Colombia, however, the data collection on wildlife road mortality is still incipient, and there is a need to develop studies covering the different geographical regions in terms of vertebrate roadkill observation and patterns (Rojano-Bolaño and Ávila-Avilán, 2021).

Therefore, given that the construction phase or road improvement phase has the greatest direct negative impact on wildlife (Parra and Rincón, 2016), in this study we wanted to characterize the vertebrate’s road mortality during the two-lane highway construction along the Autopista al Mar 1 highway (province of Antioquia, Colombia) between 2018 and 2021. Specifically, we quantified the vertebrate wildlife species and evaluated how it varied through time and space. Also, we analyzed some landscape and road variables that may be associated to the vertebrate roadkill in the area of interest.

Materials and Methods

Ethical considerations

This study is based on the use of wildlife roadkill records collected by Devimar S.A. concessionaire, the Autopista al Mar 1 two-lane highway construction company. The authorization for the use of the database for research purposes has been obtained from the concessionaire. Manipulation or direct interventions on animals was not needed.

Study type and area

A retrospective observational study was conducted using primary sources of information (concessionaire's records). The study area was constrained to the two-lane highway construction of the Autopista al Mar 1 highway, approximately 34.7 km long, and located between the municipalities of Santa Fe de Antioquia (6°30’44.8” N and75°49’04.6” W) and Medellín (6°17’28.4” N and 75°38’59.9682” W) (Figure 1) in the province of Antioquia (Colombia). The highway is subdivided into five functional units (FU), having as a characteristic a layout made up of two-lane primary roads. The roads of interest in the present study correspond to FU 1, 2.1, and 3. These are located between the municipalities of Medellín, San Jerónimo, Sopetrán, and Santa Fe de Antioquia (Figure 1). The zones surrounding the roads of interest correspond to a tropical dry forest life zone as the predominant ecosystem. The altitude varies from 450 (Santa Fe de Antioquia) to 1 950 (Medellín) m.a.s.l. surrounded by grasslands, agriculture, dry forest, and urban settlements (Cortes, 2012; Osorio, 2016; Arango-Lozano and Patiño-Siro, 2020) (Figure 2).

Field registration methods and database

Two surveys were carried weekly by the concessionaire between January 2018 and December 2021. Two people in a vehicle (at 60 km/h) searched for vertebrate wildlife roadkill events, carrying out a total of 400 field route surveys. All mortality events were registered in an Excel database, according to the environmental license guidelines granted to the concessionaire by the national environmental licenses authority (Autoridad Nacional de Licencias Ambientales, ANLA). When possible, the following information was collected for each wildlife roadkill event: date (day, month, year), geographic coordinates (latitude, longitude), taxonomic classification (class —Amphibia, Reptilia, Aves, Mammalia, scientific name, common name, stage of development —juvenile, subadult, adult), and type of vegetation cover (forest, cultivation, wetland, paddock, river/stream, urbanized). Carcasses were removed from the road and buried away to avoid sampling duplicates (Parra and Rincón, 2016) and to reduce the probability of new roadkill due to scavenging animals (Carvalho-Roel *et al*., 2019; De Araújo *et al*., 2019; Medrano-Vizcaíno *et al*., 2022). When the identification of the wildlife subject was not possible, it was registered as unidentified.

Statistical analysis

The number of roadkilled amphibians, reptiles, birds, and mammals was summarized by month and year, by descriptive statistics using Stata v.16.1 (StataCorp, 2020, College Station, Texas, USA). Roadkill rates were also calculated (roadkills/day, roadkills/km/day, roadkills/year) using Siriema® Road Mortality Software v. 2.0 (NERF UFRGS, 2014, Porto Alegre, BR), according to previous reports (McNish, 2007; Santos *et al*., 2011). This software automatically adjusts raw data on the observed number of roadkilled individuals to consider the probability of detection. This adjustment was done for each group of vertebrates using the following mathematical model:

$$N = \sum_{i=0}^{n-1} N_i = \lambda T_R p \sum_{i=0}^{n-1} \left(1 - \sum_{j=1}^{i} e^{-\frac{jT_S}{T_R}} p(1-p)^{j-1}\right)$$

N = total number of carcasses recorded during the study (295), λ = the number of carcasses per day. p = probability of detection (where the upper limit for each group was 0.59; Teixeira *et al.*, 2013). TR = time that elapses from the moment of the shock until the complete disappearance of the carcass (in days; Santos *et al.*, 2011), resulting in 1 day for amphibians, birds and reptiles (<500 g of average weight), bats, and squirrels; 2 days for reptiles (>500 g of average weight); 3 days for birds (>500 g of average weight), turtles, stifles, and mammals (400-900 g of average weight); and 4 days for mammals (1 000-5 000 g of average weight). TS = sampling interval between surveys set at 3.5 (representing an approximate range of 3 to 4 days among samplings), totaling 400 surveys in the 4 years-period. i = a given point on the road, and J = represents each roadkill event.

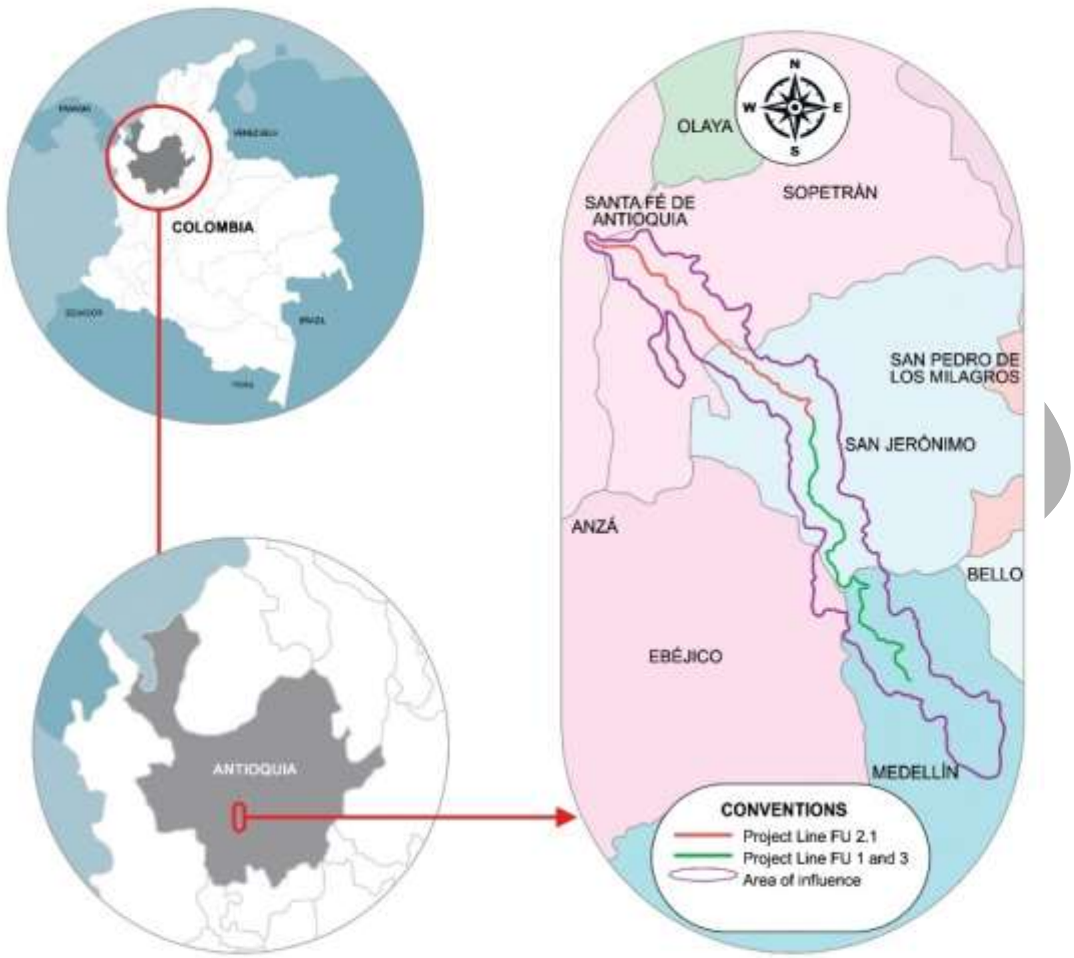

Figure 1. Study location: Autopista al Mar 1 highway, between the municipalities of Santa Fe de Antioquia and Medellín, central Andean region of Colombia.

To understand whether mortality events varied over time (month or year), an estimated mortality rate data was set for the most representative species, replacing *TS* with the number of weeks in each month of sampling. Transformed data were used to carry out a mixed linear (additive) and bilinear (multiplicative) models, using time by month (for the 12 months) and by year (2018, 2019, 2020, 2021) as independent variables. Each analysis was performed independently after normal distribution was assessed, using Shapiro-Wilk test. Statistical differences were detected with ANOVAs (analysis of variance) on R® environment, v. 2023.03.0 (R Foundation for Statistical Computing, 2023), considering a significance level of $P < 0.05$.

Spatial analysis

A conglomerates analysis of the roadkill events was carried out. A 2-D Ripley's K statistic was performed to test for a non-random spatial distribution of the events, considering the sinuosity of the road through multiple scales (Coelho *et al*., 2008; Arango-Lozano and Patiño-Siro, 2020). In addition, a 2-D hotspot analysis was carried out to differentiate spatial aggregation of vertebrate roadkill events with a statistical significance which also made it possible to generate graphic descriptions of the results (Coelho *et al*., 2008; Magioli *et al*., 2019), following a radius of 100 m, a 1,000 number of simulations, and a 95% confidence limits (CL). The Siriema® Road Mortality Software v. 2.0 (NERF UFRGS, 2014. Porto Alegre, BR) was used (Coelho *et al.*, 2014).

Landscape and road variables analysis

A logistic regression was carried out to identify environmental parameters best-predicting wildlife roadkill events along the road, using R® software, v. 2023.03.0 (R Foundation for Statistical Computing, 2023), considering a significance level of $P < 0.05$. The explanatory variables corresponded to spatial information according to the cartographic data supplied by Devimar S.A.S concessionaire) of the area of influence, were: 1. the surrounding land cover (forests/natural areas, watercourses —rivers, lakes, streams, croplands, modified terrain — urban, extraction lands) and 2. the road geometry (curved or straight road segments). In addition, the road was divided into 1-km segments and spatial aggregation (hotspots) of each km (hotspot presence = 1 and no hotspot presence = 0). Land cover parameters were determined as the number of square meters surrounding 1-linear road km (1 km buffer). In addition, road geometry was reduced as the summarized linear meters of curved and straight segments, as previously defined by Rojano-Bolaño and Ávila-Avilán (2021).

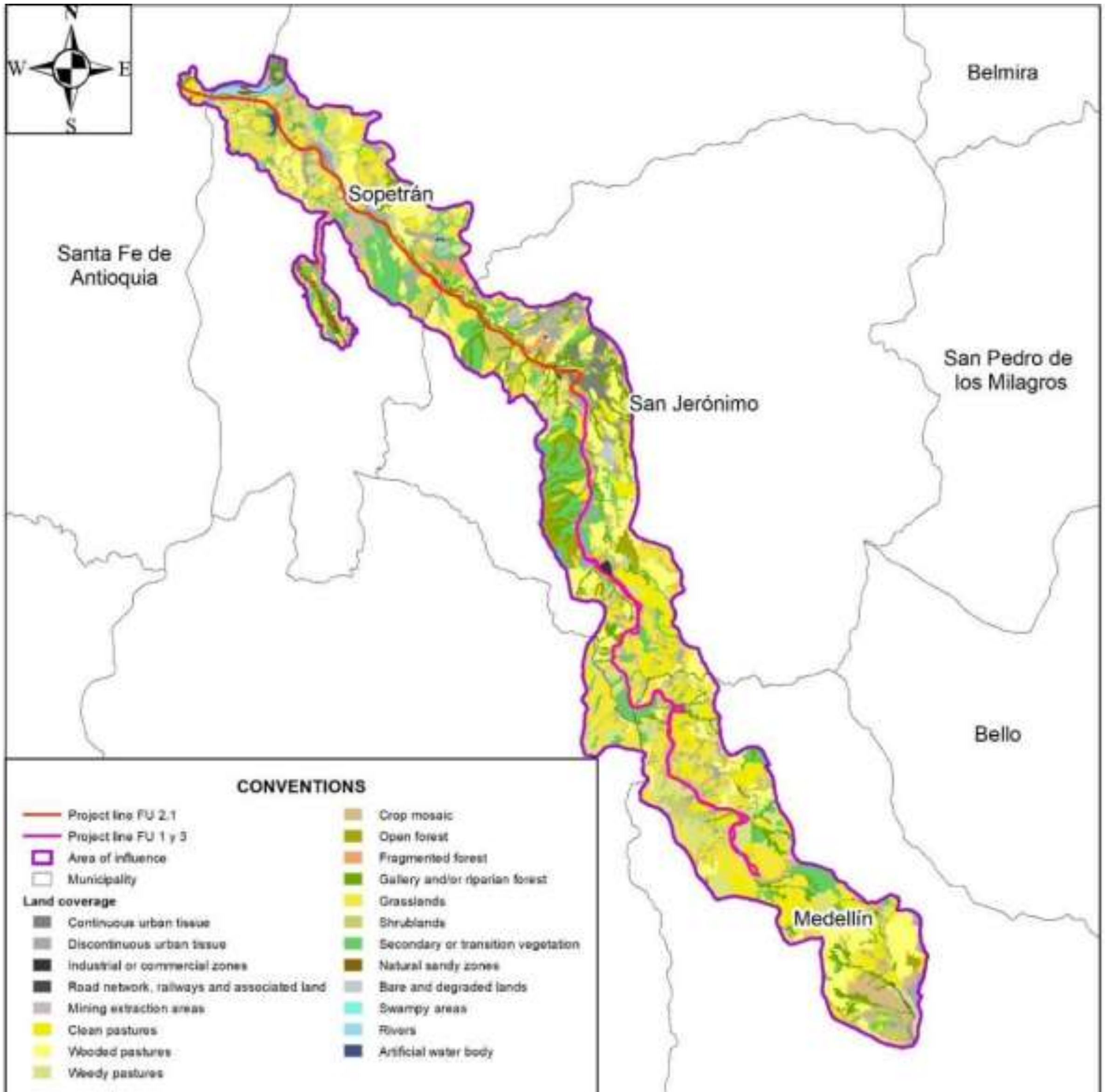

Figure 2. Vegetation cover in the Autopista al Mar 1 highway influence area (Antioquia, Colombia), 2021.

Results

A total of 295 wildlife roadkill events were reported in 4 years (from January 2018 to December 2021). The class with the highest mortality were the mammals (n = 94), followed by reptiles and birds (n = 89 each), amphibians (n = 9), and 14 roadkilled individuals were unidentified. The mortality corresponded to 76 different species between amphibians (3%), reptiles (32.9%), birds (31.9%), and mammals (32.2%). The species with the highest incidence of vertebrate roadkill events were *Didelphis marsupialis* (n = 54; 18.3%), *Iguana*

iguana (n = 40; 13.6%), *Boa imperator* (n = 16; 5.4%), *Coragyps atratus* (n = 15; 5.1%), *Cerdocyon thous* (n = 12; 4.1%), *Megascops choliba* (n = 10; 3.4%), and 15 were classified as unidentified at the genus an/or species level. The highest number of events occurred in 2021 (39.7%), followed by 2018 (24.1%), 2019 (20%) and 2020 (16.3%) (

Table **1**).

The total number of roadkills/day was 0.7553, the total amount of roadkills/km/day of follow-up was 0.0218, and by year was 275.68 road kills. Table 2 presents the roadkill rates, according to taxonomic groups, with reptiles being the animal class with the highest roadkill probability per day and per km/day.

Table 1. Vertebrate wildlife roadkill events frequency and distribution, according to the taxonomic group, in the Autopista al Mar 1 highway (Antioquia, Colombia), 2018-2021.

Family	**Scientific name**	**Common name (in Spanish)**	**Red list category (IUCN)**	**Frequency (% for the 4 years)**
		Class Amphibia		
Bufonidae	*Rhinella horribilis*	Sapo común	LC	7 (2.37)
Leptodactylinae	*Leptodactylus* sp.	Rana terrestre	LC	2 (0.68)
		Class Reptilia		
Alligatoridae	*Caiman crocodilus*	Babilla	LC	2 (0.68)
Boidae	*Boa imperator*	Boa cazadora	LC	16 (5.42)
	Atractus sp.	Serpiente tierrera	DD	2 (0.68)
	Clelia equatoriana	Cazadora negra	LC	1 (0.34)
	Dendrophidion bivittatus	Guardacaminos corredora	LC	1 (0.34)
Colubridae	Dipsadinae sp.	Culebra	LC	1 (0.34)
	Lampropeltis micropholis	Falsa coral	LC	1 (0.34)
	Mastigodryas pleii	Guardacaminos	LC	7 (2.37)
	Mastigodryas sp.	Culebra guardacaminos	LC	1 (0.34)

	Oxyrhopus petolarius	Falsa rabo de ají	NE	5 (1.69)
	Pliocercus euryzonus	Falsa coralilla	LC	1 (0.34)
	Pseudoboa neuwiedii	Candelilla	LC	4 (1.35)
	Spilotes pullatus	Serpiente toche	LC	1 (0.34)
Dactyloidae	*Anolis* sp.	Lagartija	DD	1 (0.34)
Emydidae	*Trachemys* sp.	Hicotea	DD	1 (0.34)
Iguanidae	*Iguana iguana*	Iguana verde	LC	40 (13.56)
	Polychrus gutturosus	Camaleón andino	LC	1 (0.34)
Kinosternidae	*Kinosternon leucostomum*	Tortuga tapaculo	DD	1 (0.34)
Teiidae	*Ameiva ameiva*	Lagarto terrestre	LC	1 (0.34)
	Ameiva sp.	Lagarto	LC	1 (0.34)
	Holcosus festivus	Lobito	LC	1 (0.34)
Scincidae	*Mabuya mabouya*	Lagarto mabuya	NE	1 (0.34)
Unidentified				6 (2.03)
		Class Aves		
Accipitridae	*Rupornis magnirostris*	Gavilán caminero	LC	1 (0.34)
Ardeidae	*Bubulcus ibis*	Garza garrapatera	LC	1 (0.34)
Cathartidae	*Coragyps atratus*	Gallinazo común	LC	15 (5.08)
Corvidae	*Cyanocorax affinis*	Carriquí pechiblanco	LC	1 (0.34)
Cuculidae	*Crotophaga ani*	Garrapatero ani	LC	2 (0.68)
	Crotophaga sp.	Garrapatero	LC	1 (0.34)
	Piaya cayana	Cuco ardilla	LC	1 (0.34)
Fringillidae	*Euphonia laniirostris*	Eufonia gorgiamarilla	LC	4 (1.35)
Galliformes	*Ortalis columbiana*	Guacharaca común	LC	7 (2.37)
Icteridae	*Psaracolius decumanus*	Oropéndola crestada	LC	1 (0.34)
Hirundinidae	*Stelgidopteryx ruficollis*	Golondrina gorgirrufa	LC	1 (0.34)
Strigidae	*Megascops choliba*	Búho currucutú	LC	10 (3.39)
Picidae	*Colaptes puntigula*	Carpintero moteado	LC	1 (0.34)
Psitacidae	*Forpus conspicillatus*	Perico de anteojos	LC	1 (0.34)
Thraupidae	*Ramphocelus dimidiatus*	Toche pico de plata	LC	1 (0.34)

	Ramphocelus flammigerus	Tángara flamiguera	LC	1 (0.34)
	Sporophilla sp.	Arrocero	LC	1 (0.34)
	Stilpnia vitriolina	Tángara rastrojera	LC	1 (0.34)
	Tangara gyrola	Tángara cabecibaya	LC	1 (0.34)
	Thraupis episcopus	Azulejo	LC	4 (1.35)
	Thraupis palmarum	Azulejo de palmeras	LC	2 (0.68)
Trochilidae	*Amazilia* sp.	Colibrí	LC	2 (0.68)
	Amazilia tzacalt	Amazilia de cola rufa	LC	1 (0.34)
Troglodytidae	*Campylorhynchus griseus*	Cucarachero común	LC	1 (0.34)
Turdidae	*Catharus ustulatus*	Zorzal	LC	1 (0.34)
	Turdus ignobilis	Mirla común	LC	4 (1.35)
	Turdus sp.	Mirla	LC	3 (1.02)
Tyrannidae	*Elaenia flavogaster*	Fiofío ventriamarillo	LC	1 (0.34)
	Machetornis rixosa	Picabuey	LC	1 (0.34)
	Myiodynastes maculatus	Bienteveo rayado	LC	1 (0.34)
	Myiozetetes similis	Benteveo mediano	LC	1 (0.34)
	Tyrannus melancholicus	Sirirí común	LC	9 (3.05)
	Tyrannidae sp.	Atrapamoscas	-	1 (0.34)
Vireonidae	*Vireo flavoviridis*	Vireo verdeamarillo	LC	1 (0.34)
	Vireo olivaceus	Vireo ojirrojo	LC	1 (0.34)
Unidentified				8 (2.71)
		Class Mammalia		
Aotidae	*Aotus cf lemurinus*	Marteja	VU	2 (0.68)
Canidae	*Cerdocyon thous*	Zorro perro	LC	12 (0.04)
Not reported	*Chiroptera* sp.	Murciélago	-	1 (0.34)
Dasyproctidae	*Dasyprocta punctata*	Ñeque	LC	1 (0.34)
Didelphidae	*Caluromys lanatus*	Chucha lanuda	LC	1 (0.34)
	Didelphis marsupialis	Zarigüeya común	LC	54 (18.30)
	Didelphis sp.	Zarigüeya	LC	8 (2.71)
Myrmecophagidae	*Tamandua mexicana*	Oso hormiguero	LC	6 (2.03)

Sciuridae	*Syntheosciurus granatensis*	Ardilla roja	LC	2 (0.68)
Phyllostomidae	-	Murciélago	-	3 (1.02)
Procyonidae	*Potos flavus*	Perro de monte	LC	1 (0.34)
	Procyon cancrivorus	Mapache	LC	2 (0.68)
Vespertilionidae	*Myotis* sp.	Murciélago negro	LC	1 (0.34)
Unidentified				1 (0.34)

NE = Not evaluated, DD = Data deficient, LC = Least concern, VU = Vulnerable, - = Unidentified species.

The mortality of *D. marsupialis* was similar in all four years (P = 0.56) and months (P = 0.26). Similarly, the mortality of *I. iguana* was similar in all years and months (P = 0.61 and P = 0.19, respectively), however we found a significant interaction month by year (P = 0.006) which corresponded to a higher mortality recorded in January 2021 (n=16).

The 2-D Ripley's K statistic (300 m radius and 95%CL) found a greater value of spatial aggregation of L(r) > 6.5, between the km 15.5 and 17.9 of the highway. The same corresponds to a curved segment in the main entrance of the San Jerónimo municipality (6°26'42.2698" N. 75°43'51.9356" W). The 2-D hotspots analysis identified eight road segments with statistically significant spatial aggregation (i.e. K1+8, K3+5, K6+2, K7+1, K13+5, K15+5, K9+5, and K26) (**¡Error! No se encuentra el origen de la referencia.**3). There was no statistical relation between the roadkill events aggregation and environmental parameters associated to the road (Table 3).

Table 2. Vertebrate roadkill rates according to the taxonomic group in the Autopista al Mar 1 highway (Antioquia, Colombia), 2018-2021.

Taxonomic group	**Roadkilled individuals (n)**	**Roadkill rates in 34.7 km-long route**				
		p (detectability)	**TR (in days)**	**Roadkills/day**	**Roadkills/km/day**	**Roadkills/km/year**
Amphibians	9	0.59	1.1378	0.0344	0.0009	0.3957
Reptilians	97	0.59	1.3318	0.3260	0.0094	3.7970
Birds	94	0.59	2.7147	0.1795	0.0051	2.3752
Mammals	95	0.59	19.1436	0.0819	0.0023	1.3769
Total	295	0.59	24.3279	0.6220	0.0258	9.4389

p = probability of detection (where the upper limit for each group was 0.59) (Teixeira *et al.*, 2013); TR = time that elapses from the moment of the roadkill until the complete disappearance of the carcass.

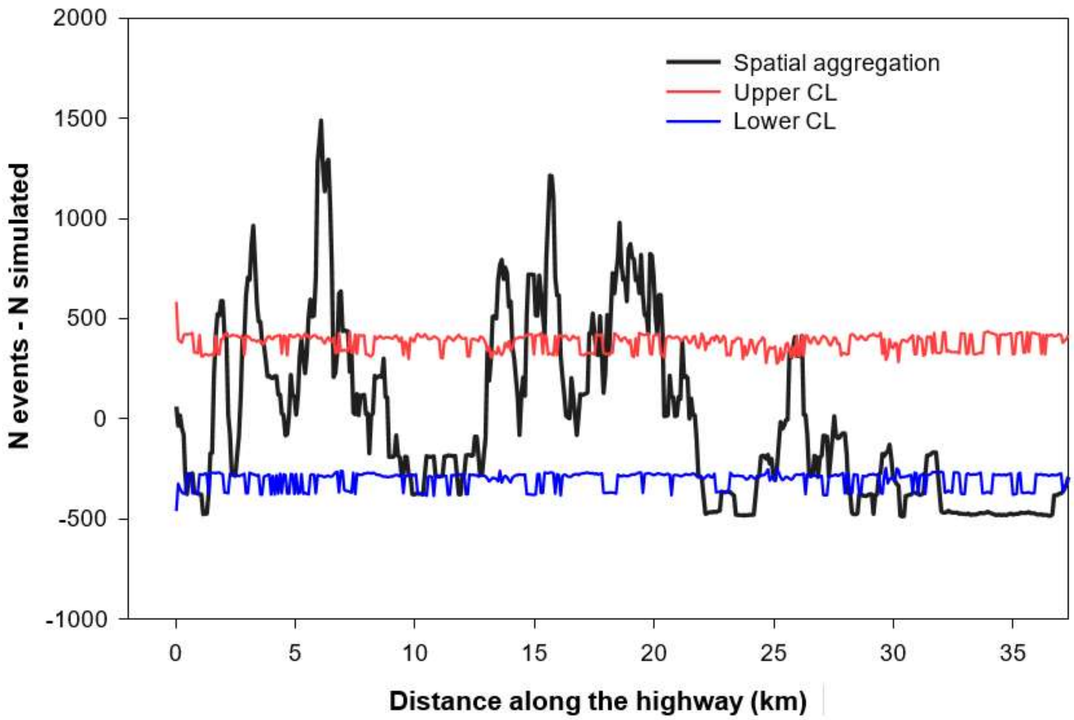

Figure 3. Spatial aggregation of the vertebrate wildlife roadkill events (black line) in the Autopista al Mar 1 highway (Antioquia, Colombia), 2018-2021. The blue and red lines represent the lower and upper 95%CL, respectively.

Table 3. Odds estimated from the surrounding land covers and road geometry aiming to explain the spatial aggregation of the vertebrate wildlife roadkill events in the Autopista al Mar 1 highway (Antioquia, Colombia), 2018-2021.

Environmental parameters	**Coefficients**	**Estimate**	**SE**	**Z-value**	**P-value**
	Intercept	**-3.954**	**2.071**	**-1.909**	**0.056**
Surrounding land cover (1 km radius)	Forest/natural areas	0.001	0.001	1.445	0.148
	Watercourse	0.0006	0.006	0.097	0.922
	Agriculture	0.001	0.001	0.912	0.362
	Modified lands	0.001	0.001	1.375	0.169
Road geometry	Curve	-0.001	0.002	-0.482	0.630
	Straight	NA	NA	NA	NA

SE = Standard error; NA = Not applicable.

Discussion

This study aimed to estimate the direct road mortality of wildlife species during the construction of the two-lane highway Autopista al Mar 1 (2018-2021).

The common black-eared opossum (*D. marsupialis)* and the green iguana (*I. iguana)* were the species with the highest roadkill events, representing more than 18 and 13% of the total of events, respectively. These species adapt easily to urbanized habitats and they are frequently reported as road mortality events in Colombia (Rodda and Grajal, 1990; Delgado-Vélez, 2014; Castillo *et al*., 2015; De La Ossa and Galván-Guevara, 2015; Arango-Lozano and Patiño-Siro, 2020). These findings could also be associated to higher population densities in natural and modified habitats relative to the other species (Rodda and Grajal, 1990; Pinowski, 2005; Castillo *et al*., 2015; Arango-Lozano and Patiño-Siro, 2020).

The higher mortality of opossums and iguanas was observed homogenously through the years and months, with only a peak in the mortality of iguanas observed in January 2021. This year was the last one of the highway construction and many new roadways (approx. 200 km) were opened to the public, which potentially contributed to increase the vehicle flow, as previously reported (Clevenger *et al*., 2003; de Freitas *et al.*, 2015). By January 2021 the COVID-19 confinement had finished in Colombia, and January coincides with the national holiday periods in Colombia (Oxley *et al*., 1974; Arévalo *et al*., 2017). No association was found for the other months, years, and their combinations, which may be due in part to the limited observation time.

The roadkill rates observed herein are low when compared to other reports in different regions of Colombia (Diaz-Pulido and Benítez, 2013; Pallares and Joya, 2018; Rojano-Bolaño and Ávila-Avilán, 2021; Arana-Rivera and Gutiérrez-Quintero, 2022). Regarding mammals, the characterization conducted by Meza *et al.* (2019) in the municipality of Barrancabermeja (province of Santander, Colombia), reported a higher roadkill rate but agreed with the most affected species, the opossum. On the other hand, (Quintero-Ángel *et al.*, 2012) reported a lower roadkill rate for reptiles (78.8/year) over 5 months. The differences found in these studies can be attributed to different factors, such as vehicular traffic, road geometry, adjacent vegetation, weather conditions, and elevation (Rincón-Aranguri *et al*., 2015). Hence the importance of segmentalizing the characterization of wildlife roadkill as a matrix for proposing strategic solutions given these particularities.

Eight roadkill hotspots were identified in the 34.7 km of the Autopista al Mar 1 highway. Despite the apparent non incidence of the types of land covers and road geometries to roadkill aggregations —possibly by the high variation of the land use in the spatial matrix surrounding 1 lineal km of the road, all roadkill hotspots were found in curved segments of the road and may be interpreted as a more dangerous geometry for wildlife in this specific circumstances, as previously reported by (Arango-Lozano and Patiño-Siro, 2020).

The 2-D Ripley's K statistic found a greater value of spatial aggregation in a curved segment in the main entrance of the touristic San Jerónimo municipality. In this sense, it is important to highlight that social dynamics of the region also play a crucial role in road ecology. The tourism activity in this municipality has grown in recent years, evidenced by the construction of inns, hotels, urbanizations, and recreational hostels (Alcaldía de San Jerónimo, 2022). In such cases, public awareness and traffic control could be effective strategies to mitigate wildlife road mortality (Fernández-López *et al.*, 2022).

A statistical significance for the relationship of environmental parameters and the wildlife roadkill was not identified herein. Nevertheless, addressing the ecology and biology of the species may become relevant in the mortality of wildlife on the road of interest (Cáceres, 2011). Ecological aspects such as those associated to natural and modified green covers surrounding roads and human infrastructures, use of road as source of heath surface for thermoregulation and basking of iguanas (Townsend *et al.*, 2003; Colino-Rabanal and Lizana, 2012), and the use of the road as a source for scavenging diets (opossums). In addition to biological, physiological-behavioral aspects such as a greater body area occupying lane of iguanas crossing the roads, and the remaining still when a car approaches behavior of opossums, make these species more sensitive to get hit by vehicles (Delgado-Vélez, 2007; Castillo *et al.*, 2015).

Our results for iguanas revealed that the roadkill rate of this species peaks during the months of November to January, which agrees with their breeding season (Campos and Desbiez, 2013). In addition, nesting season is reported between January and June (Muñoz *et al.*, 2003), under Colombian specific tropical conditions. It is well known that during the breeding season many species increase their activity and move in search of a partner and suitable territories. This exposes them to a greater risk of being hit by vehicles when crossing the road (Ritchie and Johnson, 2009; Fernández-López *et al.*, 2022). As for the opossum, anthropic effects such as deforestation and urbanization in rural areas have led to direct contact with peri-urban and urban populations (Delgado-Vélez, 2007; Jaramillo *et al*, 2017). These observations are of great importance as it suggests a possible relationship between the biology of this species and the timing of traffic accidents.

This study presented some limitations, since a relatively small sampling period was considered, incomplete morphometric data of the animals, and a limited availability of censuses due to the global pandemic confinement were observed. However, there were certain strengths, such as the profound analysis of information that allows the suggestion of strategic mitigation measures associate the results with the biology and ecology of the species. On the other hand, data on wildlife that survives the run-over event and the monitoring of such cases over time was not available from the database, leaving a gap in understanding the impact on the health and well-being of the affected individuals. However, no report in the country has considered this type of information so far.

The science of road ecology is still in the early stages of discovery regarding the actual needs to ensure the survival of wildlife in road constructions at the national level. Therefore, the focus of studies is to expand scientific knowledge on strategic interventions for the conservation of Colombian biodiversity, considering factors such as thermal floors, topography, human cultures, among others.

This study highlights the impact of road construction on wildlife. A total of 295 cases of wildlife roadkill were recorded over four years on Autopista al Mar 1 (Antioquia, Colombia). Opossums and iguanas were most affected species during the study period. A mortality rate of 275.68 roadkills/year for all classes of vertebrate animals was observed. There was also an interaction between the variables of month and year in iguana mortality for January 2021. This analysis identified eight road segments with statistically significant spatial clustering, including the entrance of San Jerónimo municipality. Finally, among the evaluated environmental parameters, none showed significance in predicting roadkill along the route of interest, highlighting the need to explore the ecological and biological factors as possible predictors of vertebrate wildlife roadkill. Our methodology can be useful on any national and international highway, adapting the tools to the need. As a method of optimizing existing resources, it is possible to combine preventive vertical traffic signs regarding the passage of wildlife, as well as the use of vertical and horizontal electronic signs installed on the

Highway, prioritizing the use of information on the presence of wild animals, mainly during reproductive seasons or times of greatest wildlife transit.

From the perspective of environmental impact assessment, the present results could significantly contribute to the design of measures to prevent and minimize the environmental impact on fauna during both the construction and operation phases of highways. In the construction phase, our findings can guide the strategic placement of wildlife crossing structures, such as underpasses or overpasses, in identified hotspots to facilitate safe animal movement and reduce roadkill incidents. During the operation phase, the continuous monitoring of wildlife roadkill and the adaptive management of signage and other mitigation measures can help in maintaining the effectiveness of these interventions. Implementing these measures can not only enhance road safety by reducing wildlife roadkill but also conserve biodiversity by protecting wildlife populations from the adverse effects of road networks.

Declarations

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Conflict of interest

The author declares that he has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

The authors confirm contribution to the paper as follows: study conception and design: KA and NC; data collection: KA; analysis and interpretation of results: KA, CC, and CR; draft manuscript preparation: KA and NC. All authors reviewed the results and approved the final version of the manuscript.

Use of AI

No AI or AI-assisted technologies were used during the preparation of this work.

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