



Research Article

Analysis of patterns related to wildlife roadkill in the Humid Chaco of Paraguay

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Abstract

Paved roads are a solution for communication between human societies, but at the same time, their expansion is detrimental to wildlife. In this work, vertebrate mortality events due to traffic collisions on National Route N° 5, in a 50 km stretch from the town of Pozo Colorado to the east, are evaluated. Vehicle journeys were carried out at a constant speed of 40 km/h every two months, from November 2020 to May 2022. All findings of roadkill were recorded by photograph. To analyse spatial patterns, the Kernel density was estimated, assessing the randomness of Ripley's K collisions and standardised roadkill rates were assessed for each species. A total of 272 individuals were recorded, corresponding to 87 amphibians, 38 birds and 35 mammals. In terms of the number of species, the composition was as follows: reptiles with 20 species, birds with 13 species, mammals with 11 and amphibians with at least 12 species. The species with the highest number of dead individuals was the common toad (*Rhinella diptycha*), followed by snakes. According to the standardised roadkill rates, the most affected animals are *Cerdocoyon thous*, *Rhinella diptycha*, *Caiman yacare* and *Dryophylax hypoconia* with more than 200 individuals per kilometre per year. A bat, *Lasiurus ega*, was identified for the first time for

Paraguay. Climatic conditions seems to have no strong effect on the occurrence pattern of the different taxa, with the exception of birds that decrease with stronger winds. The greatest coincidences occurred in three sections: km 5–5.5, km 33.5–34.5 and km 40–43. There was a correlation with crossroads areas, watercourses and forest islands. In view of the road development policy in the region, it is necessary to carry out studies of its impacts in the longer term.

Keywords

highway ecology, KAI, faunal mortality, road safety, South America, vertebrates

Introduction

Paraguay is a country located in the south-central portion of South America, geopolitically divided into two large regions: Eastern and Western, separated by the river of the same name that runs from north to south. Since pre-Columbian times, the Western Region has been locally known as the "Chaco" (Clay et al. 2008). The Gran Chaco Americano is a semi-arid biome with marked climatic seasonality (Prado 1993), extending from south-eastern Bolivia to central Argentina and occupying more than 60% of Paraguay's territory, where both the Dry and Humid Chaco are present, comprising ecoregions vastly distinct from each other (Olson et al. 2001). The latter is exceptionally valuable due to the abundant fauna it harbours, making it one of the ecoregions in Paraguay with the highest abundance of wild vertebrate populations, particularly mammals (del Castillo and Clay 2005, Rumbo 2010, De La Sancha et al. 2017) and birds (del Castillo 2019). This biodiversity richness mainly arises from the environmental heterogeneity, where natural elements of savannahs, estuaries and gallery forests intermingle (Rumbo 2010), along with its intermediate location in ecotonal regions between the Gran Chaco, the Cerrado, the Atlantic Forest and the Mesopotamian and Pampa Grasslands (Clay et al. 2008).

The Western Region stands as one of the least populated areas with the least developed road network in Paraguay (Da Ponte et al. 2021). According to the country's statistical data, the Western Region exhibits the lowest population density, ranging between 0.2 and 1.7 inhabitants per km². It is characterised by extensive livestock fields, with this productive activity being the predominant one in this ecoregion (Gill et al. 2020). Presently, the focus of socioeconomic development is on the colonisation of the Western Region, which has experienced one of the highest deforestation rates globally, with losses of up to 45% of its entire extent until 2019, primarily due to livestock activities (de la Sancha et al. 2021). However, agricultural activities in the Chaco have been on the rise in recent years, thus altering the ecoregion's landscape (Da Ponte et al. 2021). These impacts entail the expansion of the road network within the territory. Globally, the density of paved roads correlates directly with the economic growth of a region and the exploitation of natural resources, leading to landscape degradation with issues such as habitat fragmentation, deforestation and the disappearance of natural habitats (Wilkie et al. 2008, Spencer et al.

2023), as well as the decline in evolutionary potential and an increase in extinction risks (Aulsebrook et al. 1987).

Road ecology studies in Paraguay remain scarce and in their infancy (Cartes et al. 2010, Ortega and Weiler 2018). To date, only a few sections of the country's 22 national routes have been studied, with two routes located within the Humid Chaco being the subject of research. Wildlife vehicle collision fatalities (WVCF) are increasingly becoming a major cause of mortality amongst threatened species (Collins and Kays 2011, Grillo et al. 2021). Therefore, by systematising these data and undertaking long-term monitoring efforts, critical conservation strategies can be developed (Schwartz et al. 2020).

The Humid Chaco is characterised by several species of armadillos (*Tolypeutes matacus*, *Dasyus novemcinctus*, *Euphractus sexcinctus*, *Chaetophractus vellerosus* and *C. villosus*), the coati (*Nasua nasua*), jaguar (*Panthera onca*), maned wolf (*Chrysocyon brachyurus*), Azara's night monkey (*Aotus azarai*), black howler monkey (*Alouatta caraya*), giant anteater (*Myrmecophaga tridactyla*), small anteater (*Tamandua tetradactyla*), puma (*Puma concolor*) and lowland tapir (*Tapirus terrestris*) being amongst the most characteristic mammals (Rumbo 2010, Huck et al. 2017, Caballero et al. 2020). Aquatic birds and waders are also common, such as storks, herons, ibexes, coots and ducks, along with the characteristic rhea (*Rhea americana*) and, amongst the threatened species, the crowned eagle (*Buteogallus coronatus*) (Narosky et al. 2022). Additionally, the area harbours characteristic amphibians and reptiles, including species associated with humid environments, such as frogs of the genus *Leptodactylus* and some snakes like the ñacaniná (*Hydrodynastes gigas*), cuttlefish snakes (*Dryophylax* spp.) and the black-green snake *Erythrolamprus poecilogyrus* (Brusquetti and Lavilla 2006, Weiler et al. 2013, Cacciali et al. 2016). Amongst this rich vertebrate diversity, some species (e.g. Cathartiformes, Cariamiformes, Pilosa and Cingulata) are considered priorities for conservation in road ecology due to the high susceptibility to roadkills (Medrano-Vizcaíno et al. 2023).

Given the scarcity of research of this nature in the region and virtually none in the country, the objective of this study was to analyse WVCF associated with vertebrate mortality caused by accidents on a paved road in the Humid Chaco. The study was conducted along a 50 km section of National Route PY05 "General Bernardino Caballero," from the City of Pozo Colorado, at the progressive KM 269+800 (23°29'38.11"S, 58°47'29.32"W), to the progressive marked as Km 318+920 (23°30'04.04"S, 58°18'55.16"W), as a specific monitoring effort of fauna affected by WVCF in traffic over a year and a half, coinciding with road reconditioning works. Data on dead animals during the study period, fatal collisions across all vertebrate groups, spatial patterns and species composition and structure were collected and analysed. This study component was part of the Environmental Management Plan approved by the implementing authority, the Ministry of the Environment and was included as part of public tender No. 571/2020 of the Ministry of Public Works and Communications, awarded to the consulting firm Project Consulting S.A. Given the lack of long-term studies, this experience was documented as comprehensively as possible, recognising the necessity for long-term investigations.

Methods

Study area

The study area corresponds to a plain within the Humid Chaco, situated in the Municipality of Pozo Colorado, in the Department of Presidente Hayes. It features low terrain with a very slight slope ($> 0.1^\circ$) in a southeast direction, ranging from 105 to 90 metres above sea level. Small watercourses are present, such as the Siete Puntas stream, which may dry up seasonally, along with other even smaller seasonal watercourses that serve to connect some of the wetlands during the rainy season. The climate is classified as semi-tropical, with a summer rainfall level ranging from 1,100 to 1,200 mm/year and average temperatures of 24°C , with a tendency to rise. Extreme events such as extreme droughts and forest fires occur, with average maximum temperatures exhibiting anomalies of 1.5°C and flash storms (Grassi 2020).

The predominant vegetation cover consists of a palm savannah of *Copernicia alba*, which develops in hydromorphic savannahs interspersed with forests of *Schinopsis balansae*, particularly in areas with higher soil elevation and lagoons and wetlands in depressions, characterised by a prevalence of aquatic-marsh vegetation (Mereles et al. 2020). Other woody species, such as *Acacia cavens*, *Neltuma ruscifolia*, *N. × vinalillo*, *Mimosa pigra* and *Geoffroea decorticans* are also present, especially in altered environments, accompanied by a herbaceous stratum rich in grasses and aquatic marsh prawns (Salas et al. 2017). Collectively, the landscape can be described as a mosaic of forests, palm groves and wetlands. Soils are generally rich in clays, such as gleysols and vertisols, with the presence of saltwater in the subsoil and can be flooded for periods of six to eight months per year (Mereles et al. 2020).

Wetlands, lagoons and watercourses have formed as a result of the alluvial megafan formation process of the Pilcomayo River, with courses typically orientated from NW to SE, flowing into the Paraguay River (Salas Dueñas 2015). Some maintain their watercourse with minimal current and a high degree of salinity, while the majority have become clogged, forming lagoons and marshes dominated by marsh vegetation, including *Cyperus giganteus*, *Typha dominguensis*, *T. latifolia*, *Thalia geniculata* and *T. multiflora*, along with other genera, such as *Schoenoplectus*, *Paspalum*, *Setaria*, *Distichlis*, *Poa*, *Ludwigia*, *Pontederia*, *Salvinia*, *Limnobiium* and *Azolla* (Peña-Chocarro et al. 2006).

The section of the route included in the study commenced in the urban area of the town of Pozo Colorado ($23^\circ29'38.11''\text{S}$, $58^\circ47'29.32''\text{W}$). For better location reference, this locality was designated as Km 0, extending to Km 50 to the east, with all mentions referring to the distance from Pozo Colorado (Fig. 1). This town encompasses a small urban settlement, with an estimated population of 2,135 inhabitants in 2008, according to official data from the National Institute of Surveys and a military base housing a Nature Reserve (Reserva Natural Privada Coronel Valois Rivarola - RNVR) spanning 981 hectares. The study was conducted from November 2020 to May 2022, encompassing various climatic conditions, ranging from extreme heat in summer (45°C) to cold temperatures in winter (3°C), with both sunny, cloudy and rainy days.

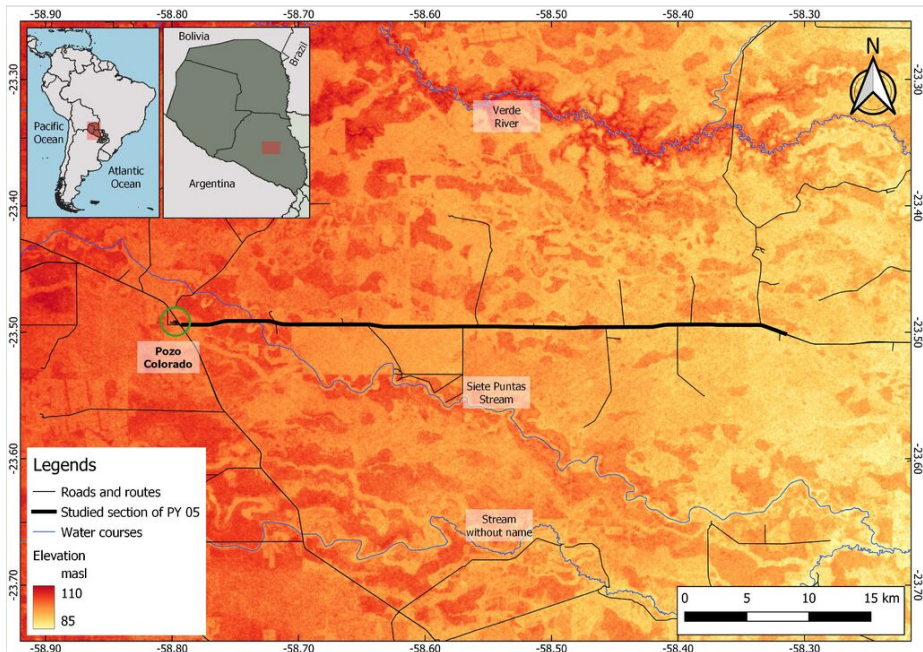


Figure 1.

Studied section of National Route 05 (PY 05), in relation to the coverage of the area. The green circle indicates the town of Pozo Colorado, the nearest urban area associated with the study section and the red dots correspond to the 10 km stretches.

Data Collection

Data collection involved vehicle tours at reduced speeds (40 km/h or less) conducted by a multidisciplinary team, including a specialist from each vertebrate group, to inspect carcasses along the route. These tours were conducted every two months from November 2020 to May 2022, totalling 10 samplings. During each campaign, at least two tours were conducted on consecutive days, covering a total distance of 2500 km, equivalent to approximately 50 routes. Sampling typically occurred between 6:00 a.m. and 11:00 a.m., with an additional session in the late afternoon, between 5:00 p.m. and 8:00 p.m. However, on hot days with temperatures ranging from 28°C to 45°C, a morning sampling session was conducted between 3:00 a.m. and 6:00 a.m. to minimise escapes due to the high activity of birds of prey in the area. Animals weighing less than 500 g, such as frogs, were considered smaller animals and collision areas and extension on the asphalt layer were identified for them. All carcasses encountered were removed to avoid double counting, except for frog collision areas, as they were firmly attached to the asphalt.

Each animal underwent a brief examination for photography, geographical location with GPS coordinates and identification to the species level or as far as the condition of the remains allowed. The time of discovery and any details suggesting an alternative cause of death, such as evidence of hunting, were also recorded. From the third sampling in March

2021 onwards, photographs were taken using a mobile phone with the Timestamp Camera Free application, which records coordinates, date and time for each image. Climatic conditions at the time of each record, including temperature, humidity, wind direction and speed, were also documented. Data were recorded in field spreadsheets.

For information on the conservation status of species at the national level, references such as Motte et al. (2019) for amphibians, Martínez et al. (2020) for reptiles, MADES Resolution No. 254/19 for birds and Saldívar et al. (2017) for mammals were consulted. Global conservation status data were obtained from the IUCN Red List website (www.iucnredlist.org).

Data Analysis

Descriptive comparisons of animal death frequencies due to roadkills were conducted bimonthly from November 2020 to May 2022, summarising 10 samples in total. Results were presented by taxonomic group and by month.

To examine temporal collision patterns, climatic data (temperature and humidity) were taken from the nearest meteorological station in Pozo Colorado, for each sampling tour, correlating these data with the number of roadkills per month, using a coefficient of correlation (R^2). Spatial collision patterns were analysed by identifying areas with a higher concentration of dead animal records using heat maps (Parzen 1962) in QGIS 3.22.7, which utilises Kernel density estimation. This analysis was applied collectively to all taxonomic groups and to each class separately (amphibians, reptiles, birds, mammals). Additionally, Ripley's K statistic was employed to estimate the spatial probability of collision clusters being due to chance (Filius et al. 2020). Values of $L(r)$ above the 99% confidence interval indicated significant clustering, while values below the confidence limit suggested significant random dispersion. Spatial analysis was also conducted to identify hot areas (HotSpot) using a linear Ripley model with a 99% confidence interval, utilising Siriema 2.0.1 software. To associate critical areas with the landscape, we overlap the results of Kernel density and the linear Ripley model with satellite imagery.

The mortality rate in the study section was calculated using the Kilometre Abundance Index (KAI) (Ferry and Frochot 1958), which relates the number of animals collided to the kilometres travelled. The equation used is:

$$KAI = N^{\circ}A / (N^{\circ}km * N^{\circ}R),$$

where " $N^{\circ}A$ " represents the number of animals run over, " $N^{\circ}km$ " indicates the distance travelled and " $N^{\circ}R$ " represents the number of times the section was travelled.

Additionally, roadkill rate per species was estimated by dividing the total number of records of each species by the length (in kilometres) of the surveyed road (50 km) and by the total sampling period (i.e. number of sampling days) which for this work was 21; and, thus, the data generated were converted into a standardised measure of roadkill rates per year, by multiplying by 365 (Medrano-Vizcaino et al. 2023). Considering that survey intervals and body size of the animals may interfere in the detectability of roadkills, a correction factor

was applied following Santos et al. (2011), with which rates of mortality can be better used for comparison. According to this, smaller animals have lower chances to be seen by observers due to the small size and higher probabilities to be removed by a scavenger. To calculate a correction factor, based on the body mass of animals found dead on roads, we classify the records of animals into categories, based on their weight: small (< 2,000 g), medium (2,000 to 20,000 g) and large (> 20,000 g). For each group, we calculate the average persistence time on the road using the Kaplan-Meier estimator (Kaplan and Meier 1958). The correction factor (C_f) for each category can be calculated as $C_f = \text{Total specimens in Small Category} / \text{Total specimens in each category}$. This was applied only to taxa recognised to species level.

Finally, climatic data (temperature, humidity and wind speed) were correlated with collision data to assess whether climatic variables impact taxonomic groups differently. For this, a non-parametric multinomial logistic regression test was performed using average climatic data for each class and a correlation coefficient was taken to assess how the presence of different animal classes is influenced by the climatic data. This was run in R 4.4.1, using the 'ggplot2' (Wickham 2016) and 'dplyr' (Wickham et al. 2023) packages.

Results

Recorded Species

A total of 272 animals were recorded as killed by collisions on the route, with reptiles comprising the majority at 112 individuals, followed by amphibians (87 individuals), birds (38 individuals) and mammals (35 individuals). This highlights the vulnerability of animals with smaller body masses. The complete list of specimens found is provided in Suppl. material 1.

In terms of species diversity, the composition was as follows: 20 reptile species, at least 13 bird species, at least 11 mammal species (including two exotic species) and at least 12 amphibian species. The species most frequently affected by roadkills was the common toad (*Rhinella diptycha*) with 29 individuals, followed by *Dryophylax hypoconia* (26), *Cerdocyon thous* (19), *Helicops leopardinus* (15), *Erythrolamrus poecilogyrus* (13), *Caracara plancus* (12) and *Dryophylax chaquensis* (11), with the rest having fewer than 10 records per species. Notably, *Cerdocyon thous* accounted for more than 50% of mammalian carcass encounters.

Several species were observed only once, including *Mussurana bicolor*, *Xenodon merremii* and *Ophiodes intermedius* amongst the reptiles, *Aramidides ypecaha*, *Cathartes* sp., *Crotophaga ani*, *Myopsitta monachus*, *Ortalis canicolis* and *Paroaria coronata* amongst the birds and *Hydrochaeris hydrochaeris*, *Lasiurus ega*, *Lontra longicaudis*, *Lycalopex gymnocercus*, *Nasua nasua* and *Tamandua tetradactyla* amongst native mammals. It was difficult to ascertain the specific amphibian species recorded by a single individual due to several specimens remaining unidentified. Amongst all recorded species, two mammals belonged to exotic taxa: the domestic dog (*Canis lupus familiaris*) and the bighorn pig (*Sus*

scrofa), each represented by a single individual. Notable records are presented in Fig. 2, with all photographic records available on FigShare (<https://doi.org/10.6084/m9.figshare.c.6819861>).

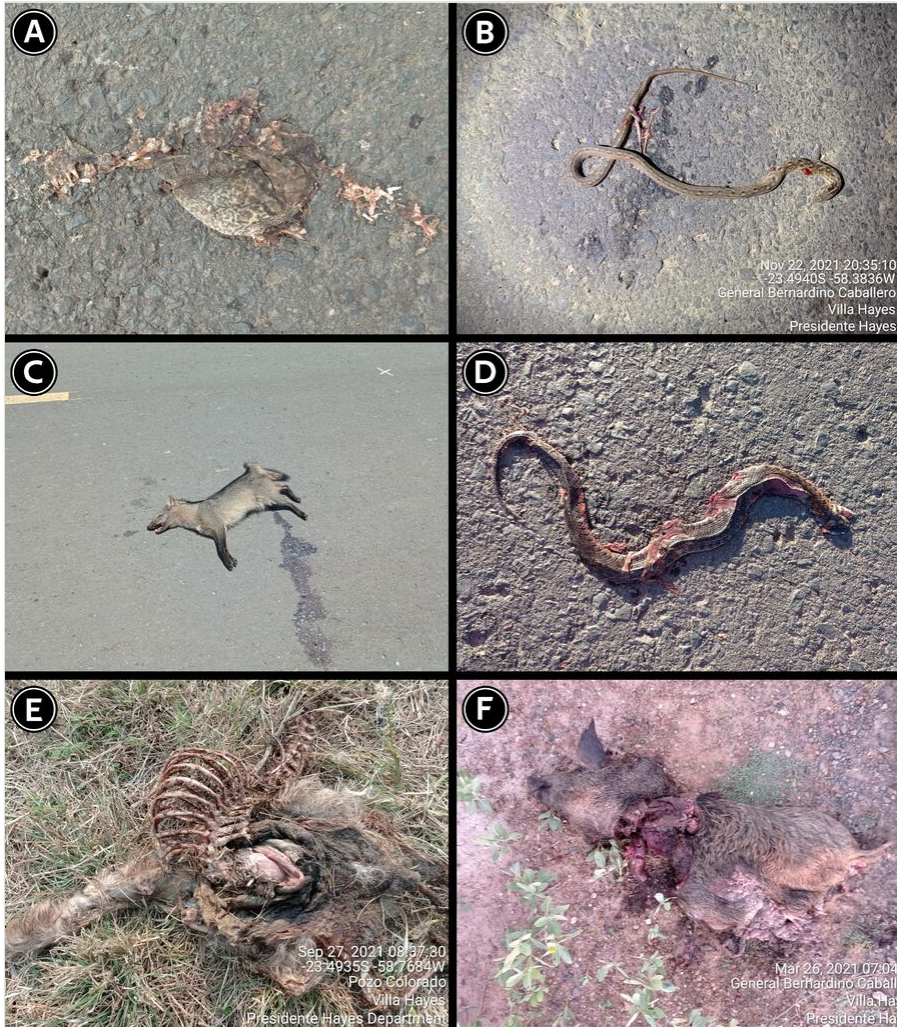


Figure 2.

Animals with the highest frequency of roadkills (A–D) and exotics (E–F). The records correspond to A: *Rhinella diptycha* (specimen 88) with 29 individuals, B: *Dryophylax hypoconia* (specimen 224) with 26 individuals, C: *Cerdocyon thous* (specimen 1) with 19 specimens and D: *Heicops leopardinus* (specimen 124) with 15 individuals. The only exotic species recorded were *Canis lupus familiaris* (E) and *Sus scrofa* (F), each with a single specimen.

An important aspect to consider is that none of the recorded roadkill incidents involved threatened species. All the species affected are relatively common and have wide distribution ranges nationally.

Seasonal Collision Patterns

During the monitoring period, two peaks of high incidence of dead animals were observed along the studied road section: one during the second sampling event (January 2021) and the other during the 7th sampling event (November 2021) (Fig. 3). Despite amphibians being the most affected taxa, as depicted in Fig. 4, the highest numbers of WVCF involving amphibians occurred in January 2020 and November 2021, while other months experienced fewer incidents with these animals. Reptiles exhibited high registration numbers between November 2020 and March 2021, with another peak observed in November 2021. It is noteworthy that reptiles were the only group recorded during all sampling campaigns (Fig. 4). Birds and mammals were observed in relatively consistent numbers during the months they were recorded. When compared to climatic data of average temperature and humidity, a slight correlation was observed between the decrease in these climatic variables and the reduction in the number of animals struck. However, a specific correlation analysis of the data revealed that they did not significantly adjust to temperature variation ($R^2 > 32\%$), although there was a peak of activity observed between 25 and 35°C. Conversely, there was no correlation observed between humidity and the data ($R^2 = 3\%$); however, there was a peak of activity observed between 40 and 80% ambient humidity (Fig. 3).

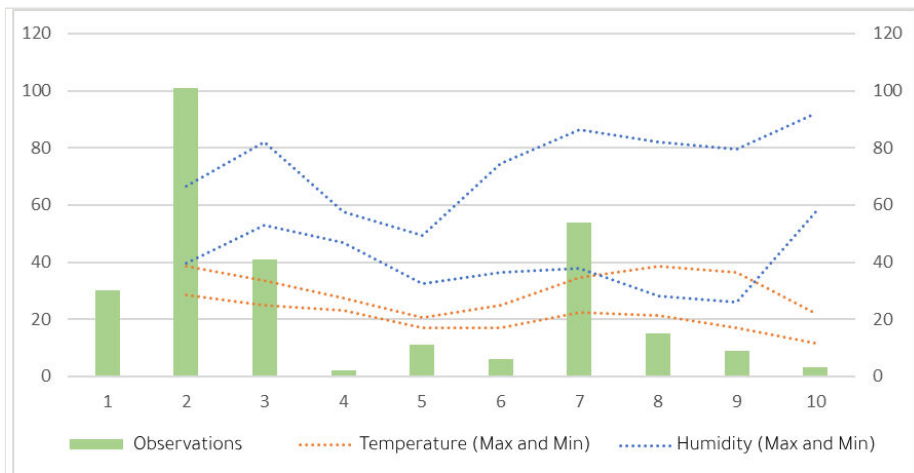


Figure 3.

Contrast of the presence of roadkills (green bars) in relation to the temperature oscillation (red dotted lines) and the minimum and maximum ambient humidity (blue dotted lines).

Climatic values for each animal class is shown in Table 1. Amphibians appear more with higher average humidity (65.84%) and moderate temperature (28.76°C) with moderate wind speed (13.96 km/h) (Fig. 5). Reptiles occur with the highest average temperature (29.79°C) and moderate humidity (61.69%) with higher wind speed (14.92 km/h) (Fig. 5). Birds occur with lower average temperature (26.58°C), humidity (63.18%) and wind speed (11.67 km/h) (Fig. 5). Mammals are present with moderate temperature (27.83°C) and lower humidity (49.89%) with higher wind speed (14.77 km/h) (Fig. 5).

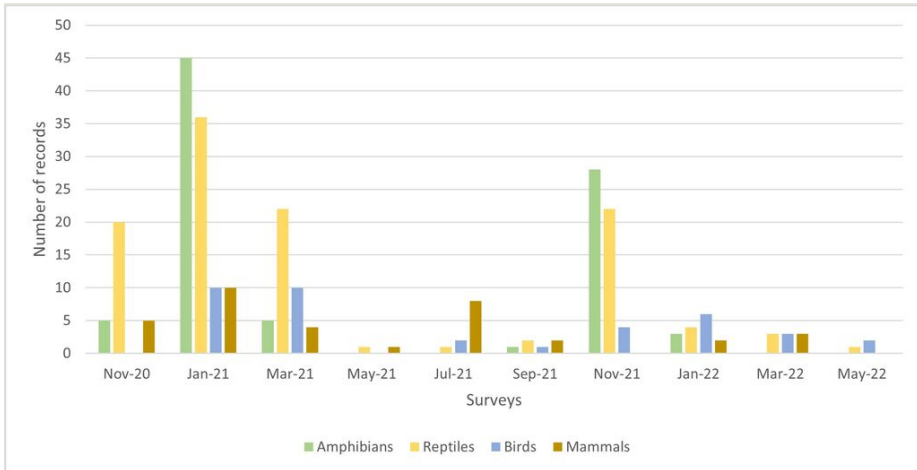


Figure 4. Roadkills per month, classified by taxonomic group.

Table 1. Mean climatic data for each animal class.

Class Climate Distribution			
Class	Temperature (°C)	Humidity (%)	Wind (km/h)
Amphibia	28.75	65.84	13.95
Reptilia	29.79	61.68	14.91
Aves	26.58	63.18	11.67
Mammalia	27.82	49.88	14.77

It is important to note that the study was conducted during a specific period (2020 – 2021) corresponding to the cold current phase of the El Niño Southern Oscillation (ENSO), commonly referred to as the "La Niña" phase. This phenomenon resulted in anomalies of cooler temperatures and relative humidity, with rainfall levels below the patterns considered normal for the climate of the region (Hu et al. 2022, Pereira et al. 2022).

Spatial Patterns of Collisions

The density pattern of all analysed collisions collectively revealed a zone of higher density at km 49 towards the end of the study section, as illustrated in the obtained heat map (Fig. 6). Spatial pattern analysis by taxonomic group (Fig. 7) demonstrated consistent differences amongst the various classes. Amphibians were concentrated at the same high-incidence point, with a significant number (18 specimens). To mitigate any bias in the overall density observed, we conducted an analysis excluding amphibians. However, the results remained largely similar.

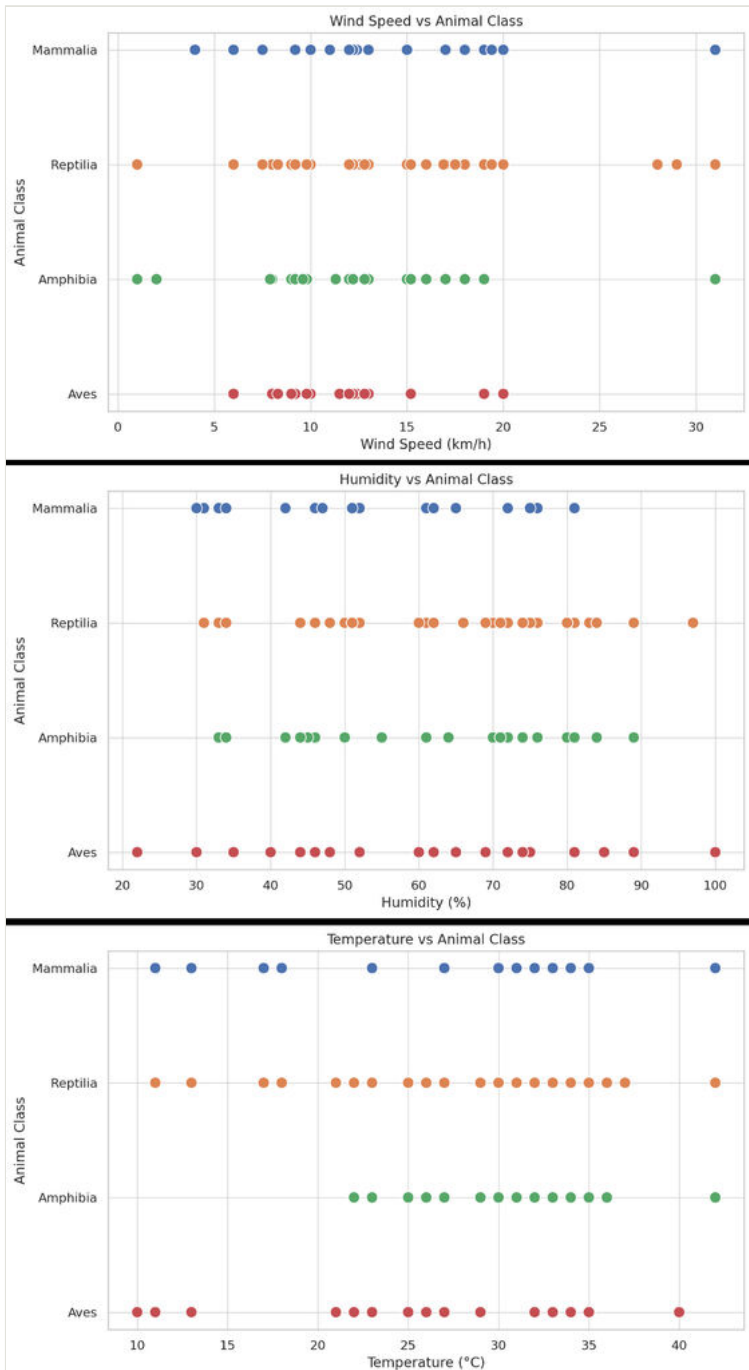


Figure 5. Scatter plots illustrating the relationships between the climatic variables and the different animal classes.

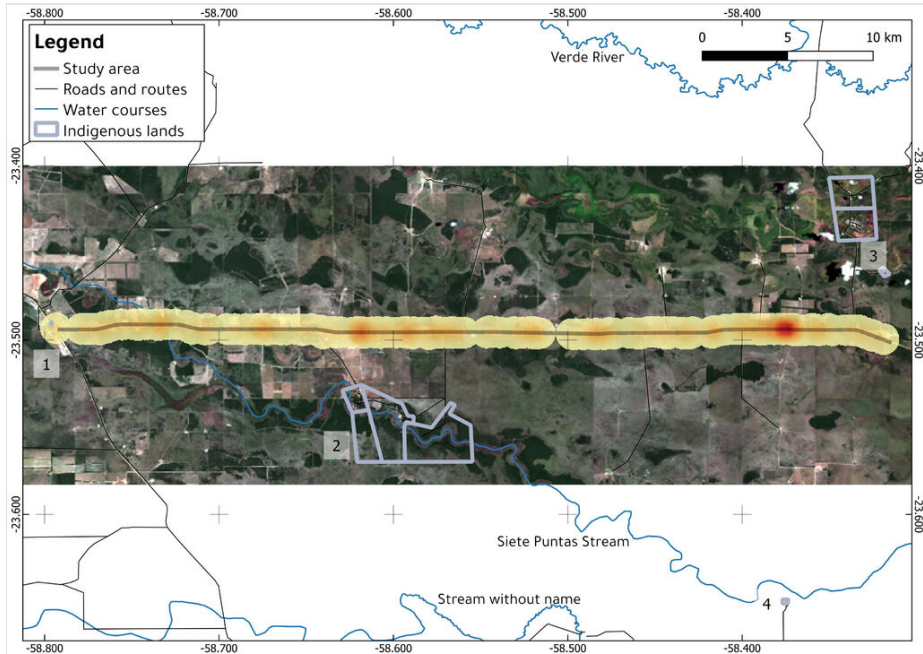


Figure 6.

Heat map according to the Kernel index showing the areas with the highest collision rate, for all taxonomic groups as a whole. A Landsat satellite image dated 21 January 2023 is shown as a baseline. As a reference to the human presence in the area, the surrounding indigenous communities are shown: 1- Pozo Colorado; 2- Yanekyaha; 3- Makhwaya.

The analysis of the Ripley K -statistic indicates that clusters are not randomly distributed between km 11 and 15.5 and between km 43 and 44, with a 99% confidence interval (Fig. 8). The highest concentration of mammals occurs in the section from km 20 to 25, which also coincides with one of the reptile hotspots and a smaller hotspot between km 5 and 7, corresponding to the watercourse. Birds exhibit a distinct pattern compared to other taxa, with hotspots at the beginning of the section (km 5) and towards the end, particularly notable in the final kilometres. However, birds also show a high incidence in the km 15 to 17 section, which is less significant for other classes.

Analysis of critical areas (hotspots) revealed that the highest frequency of roadkills across all classes occurs at three points: between kilometre 5 and 5.5 (Fig. 8), coinciding with the presence of the only permanent stream at the site; another significant point in the section between kilometre 33.5 and 34.5 (Fig. 8), where the largest patch of forest in the vicinity is located, dividing the route into two blocks; and at kilometre 40 to 43, towards the end of the section, bordering the southern flank of a more substantial forest patch than the previous one, spanning 750 hectares (Fig. 9).

Finally, based on 272 records of dead animals in a 50 km stretch, travelled 50 times, the Kilometre Abundance Index (KAI) resulted in 0.109. Additionally, regarding the roadkill rate per species per year, the highest rate is for the fox *Cerdoyon thous* (592 individuals per

kilometre per year), followed by *Rhinella diptycha* (292 ind/km/yr), *Caiman yacare* (287 ind/km/yr) and *Dryophylax hypoconia* (235 ind/km/yr). On the other side, the lower rates go for *Bothrops diporus*, *Crotophaga ani*, *Lasiurus ega*, *Leptodactylus aepyta*, *L. gracilis*, *Micrurus tricolor*, *Myopsitta monacha*, *Ophiodes intermedius*, *Ortalis canicolis*, *Paroaria coronata*, *Pithecopus azureus*, *Rhinella bergi* and *Xenodon merremii*, with less than one specimen per kilometre per year (0.35 ind/km/yr). These data are shown in Suppl. material 2 and Suppl. material 3.

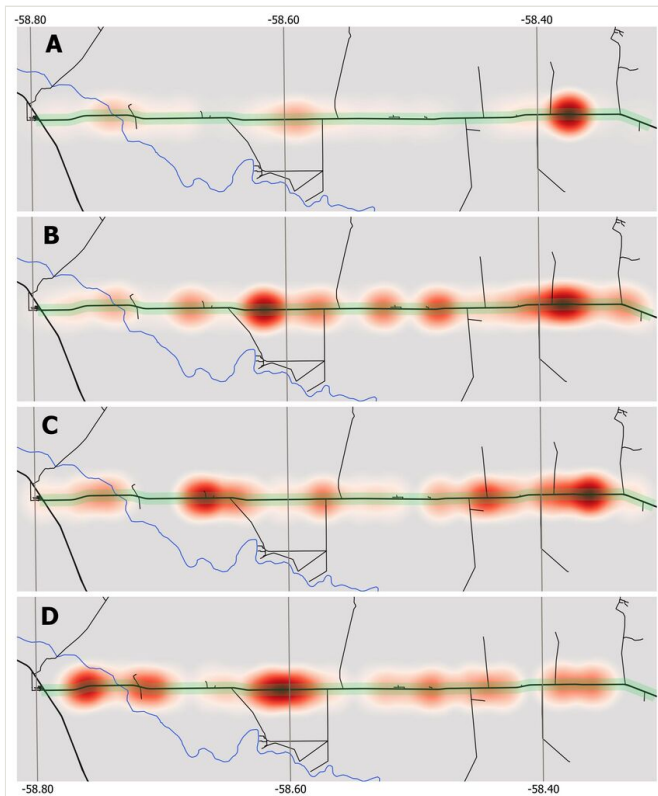


Figure 7.

Heat map according to the Kernel index showing the areas with the highest collision rate, for the different taxonomic groups discriminated into amphibians (A), reptiles (B), birds (C) and mammals (D).

Discussion

The analysis of vertebrates as a whole was challenged by the significant number of amphibians found towards the end of the study section, which introduced a notable bias. Amphibians in the Chaco Region exhibit this type of explosive behaviour during the short wet season for reproductive purposes (Ulloa et al. 2019). This behaviour is crucial to consider in their study and analysis, as they are often not fully accounted for due to the

challenges in counting and identifying them, particularly the small-sized species (Sousa-Guedes et al. 2021). This study marks the first attempt to analyse the areas of Wildlife Vehicle Collision Fatalities (WVCF) associated with these events of massive invasion of the asphalt layer by small amphibians, a phenomenon not previously mentioned in earlier studies (Cartes et al. 2010, Ortega and Weiler 2018).

The study yielded 112 reptiles, 87 amphibians, 38 birds and 35 mammals collisions, totalling 272 individuals. Previous studies (Cartes et al. 2010) indicated that the largest number of species with roadkills were scavenging birds and mammals, including species with conservation concerns, such as the giant anteater (*Myrmecophaga tridactyla*) and the maned wolf (*Chrysocyon brachyurus*). Ortega and Weiler (2018) also found a significant number of roadkills, with scavenging mammals and snakes being prominent. However, there was little or no mention of small animals, such as frogs, in these studies.

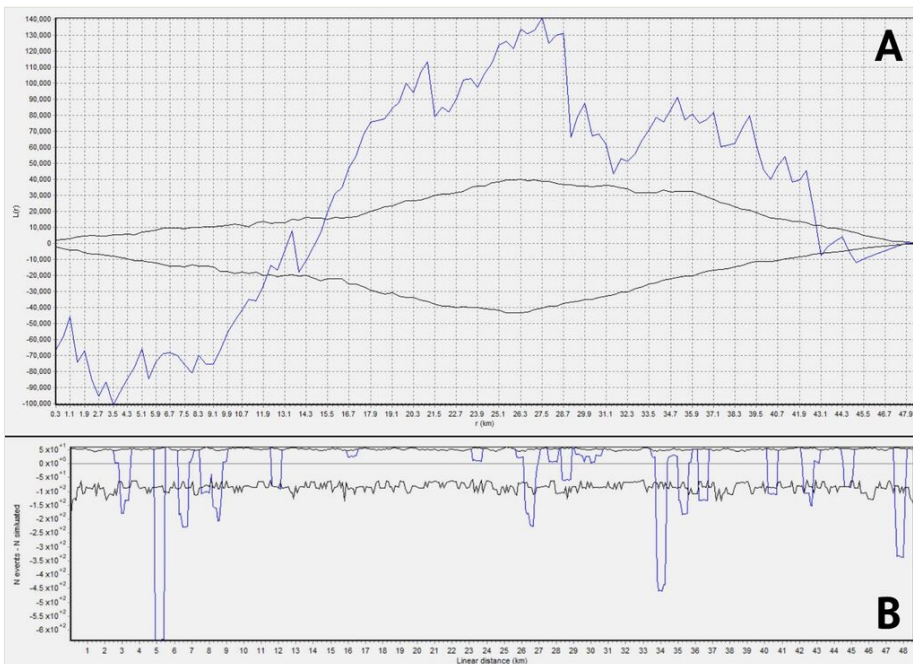


Figure 8.

Statistical analysis of: A- Statistical $L(r)$ (blue line) against the 99% confidence limit (black line) for the collision events of the present study. Values within the confidence limits (black lines) indicate a significant clustering of events not due to chance. B- Analysis of critical points, where the intensity of collisions is shown (blue line) against a confidence limit of 99% (black line). The three highest concentrations are at kilometres 5–5.5, 34 and 47–48 near the end.

Climatic conditions do not have a significant effect on the pattern of the animals in general, with the exception of birds, that seem to be more susceptible to stronger wind, which is not unexpected since the wind can influence the pattern of activity during the flight (McCabe et al. 2017).

Hotspots of fatal wildlife collisions exhibited a pattern associated with two main landscape factors: the presence of relatively significant watercourses and the intersection of the route with forest patches. Previous studies in the country also noted a pattern associated with watercourses (Ortega and Weiler 2018). However, the issue of intersections of roads with forested areas had not been previously reported. This result is deemed relevant in landscapes dominated by palm savannahs and should be considered in future environmental impact studies. In this context, the landscape plays a fundamental role in determining the spatial patterns that influence the locations and rates of roadkills (Clevenger et al. 2003, Medinas et al. 2012).



Figure 9.

Forest present in the study section with 321 hectares, the only one cut by the road and which, according to the analysis of critical points (Fig. 7B), is one of the areas with the highest concentration of collisions due to non-chance. CNES/Airbus satellite image dated 14 May 2023, from Google Earth Pro 7.3.6.

An important finding is the roadkill record of a bat. Bats represent a significant portion of Paraguayan mastofauna (De La Sancha et al. 2017). Similar studies have highlighted the relevance of collision deaths in this group (de Figueiredo Ramalho et al. 2021). This record suggests flaws in the detection ability of these animals and this is why it is necessary for the application of correction factors. The vegetation conditions near the asphalt layer, their activity schedule and the promptness with which traffic, raptors or scavengers can remove carcasses can significantly affect this ability (Bénard et al. 2024). A long-term study found that proximity to high-quality habitats correlates with an increased likelihood of bat impacts (Medinas et al. 2012, Bénard et al. 2024). This is particularly pertinent in the study area, where bat nests were confirmed under the bridge at km 5, in addition to forest patches and

watercourses. However, more data are required in this group, especially considering that road expansions in the area, such as Route PY 09, could increase the risk of bat collisions by up to 12 times (de Figueiredo Ramalho et al. 2021).

The absence of species with conservation concerns is noteworthy, as similar studies in the same ecoregion indicated the vulnerability of several species, such as anteaters (*Myrmecophaga tridactyla*) classified as Vulnerable (VU), maned wolves (*Chrysocyon brachyurus*), river otters (*Lontra longicaudis*) and howler monkeys (*Alouatta caraya*), classified as Near Threatened (NT), on sections of Route PY 09 (Cartes et al. 2010). Studies in neighbouring areas of the Brazilian Cerrado also indicated the presence of roadkills in these species and other threatened species (Costa and Dias 2013, Valadão et al. 2018). Further investigation is required on this topic, particularly with long-term studies.

This study represents the first attempt to analyse patterns of vehicular collisions with wildlife in the Paraguayan Chaco, yielding a Kilometre Abundance Index (KAI) of 0.109. Compared to other data, this value for a Chaco area is lower than those recorded for various regions, such as the Colombian Andes in Cauca (KAI = 0.126–0.317) (Castillo-R et al. 2015), Peruvian Andes in Tambogrande (KAI = 0.13–0.24) (Yesquen Sernaque et al. 2020), Gulf of Mexico (KAI = 0.38) (Pacheco Figueroa et al. 2021) and an ecotonal zone between Monte and Chaco Seco in Córdoba, Argentina (KAI = 0.13) (González-Calderón 2020). It is likely that low vehicular traffic or differences in biodiversity between the studied ecoregions influence the low rate of our index.

The standardised corrected roadkill rates shows that the fox *Cerdocyon thous*, the toad *Rhinella diptycha* and the caiman *Caiman yacare* are the most affected animals. In another study in Ecuador, a toad (*Rhinella marina*) was also amongst the animals with highest rate of roadkill (Medrano-Vizcaino et al. 2023) and it is proved that toads are affected not only in South America, but also in Europe (Kolenda et al. 2018). *Cerdocyon thous* is certainly a species greatly affected by roadkills (Santos et al. 2022, Costa et al. 2022), representing up to 61% of the records in an analysis of Brazilian routes (Pinto et al. 2022) and our data proved the same. We highlight the high number of caimans according to the standardised corrected roadkill rates. Even if we found a high number of these animals, more studies are needed to better understand the impact on populations, not only regarding the number of individuals, but also the broader impact on population dynamics and the reduction of animal movements (Schwartz et al. 2020).

Conclusions

The work carried out was able to obtain consistent data that indicate a high effect of roadkill animals, especially the smaller ones, with amphibians and reptiles as the most affected groups. The section of the Route studied presented several patterns of hot spots, indicating the sites with the highest probability of impact with traffic. For amphibians, that place is located at km 42.

The number of animals run over in a period of two years, for only 50 kilometres of section, is high and we consider it very important considering that the detectability is lower than the real WVFC. This requires much more effort in studies of this type to avoid this impact. Amongst the most recommended measures is the construction of better structures with designs suitable for the passage of wildlife, signage and speed bumps because of high-speed traffic (above 120 km/h), which are issues that must be studied in greater depth. Although compared to routes in other environments in the Americas, the KAI index was low, this value must be monitored permanently, since a higher vehicle load can result in an increase in the index. These results serve as a baseline for future studies.

It is recommended that this type of study should be undertaken in all road constructions to be carried out in the country, in order to gather information regarding the effects of the routes on native fauna. In addition, it is advisable to monitor small animals (invertebrates) for which our method is not very efficient. The under-reporting of animals with reduced body mass is to be expected due to their short duration on asphalt. It is also advisable to initiate long-term studies in order to assess the cumulative impacts.

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Conflicts of interest

The authors have declared that no competing interests exist.

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Supplementary materials

Suppl. material 1: Table S1 [doi](#)

Authors: Cartes P, Santacruz M, Ferreira M, Gómez D, del Castillo H, Sforza L, Cacciali P

Data type: Occurrences

Brief description: Details of the dead specimens found in the section of Route PY 05 during our study.

[Download file](#) (75.26 kb)

Suppl. material 2: Appendix S2. Roadkilled individuals per kilometre per year [doi](#)

Authors: Cartes et al.

Data type: CVS Madrix

Brief description: Matrix containing the species found during our study and specimens for each species, kilometres and days surveyed, body mass and the correction factor. Body mass according to data available in Appndix S3.

[Download file](#) (4.01 kb)

Suppl. material 3: Body mass information [doi](#)

Authors: Cartes et al.

Data type: List of species with body mass information

Brief description: Body mass (in grams) of the species recorded during our sampling, with literature source information.

[Download file](#) (31.60 kb)