


Seasonality and paving affect roadkill rates on a highway in Cerrado biome, Brazil

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ABSTRACT

Wildlife roadkill poses a significant global threat to biodiversity, with heightened concerns in Protected Areas, such as the Panga Ecological Station (PES) located in the Cerrado biome, Minas Gerais state, Brazil. Throughout this research, the adjacent highway to the PES, MGC-455 highway, underwent paving. To assess roadkill rates and explore the potential impact of seasonality and road pavement type, bimonthly monitoring was conducted along the highway. The survey spanned 35 km of paved and 10 km of unpaved road from April 2012 to April 2013, and 50 km of paved and 30 km of unpaved road from May 2013 to April 2015, covering a total distance of 4,820 km. Despite the extended survey period, progress in the highway's paving remained minimal. A total of 87 individuals were identified, comprising 38 birds, 24 reptiles, 17 mammals, and eight amphibians. Across the documented species (37 in total), there were 15 bird species, 12 reptile species, eight mammal species, and two amphibian species. The roadkill rate for wild vertebrates was 0.008 individuals/km/day, likely underestimated. Reptile roadkill was notably prevalent during the rainy season. Roadkill rates were approximately six times higher on paved stretches (0.006 individuals/km/day) compared to unpaved sections (0.001). The presence of endangered species in the area emphasizes the ongoing need for monitoring roadkill, particularly with the anticipated rise in roadkill rates due to the continued highway paving. Future comparisons can be drawn between our data and post-paving roadkill rates to assess potential increases.

Keywords: Road Ecology, Dirt road, Paved road, Wildlife-vehicle accidents, Panga Ecological Station.

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SIGNIFICANCE STATEMENT

Roadkill stands as a significant menace to wildlife conservation, particularly in the remaining protected areas within the Cerrado biome, Brazil, recognized as a hotspot for biodiversity conservation. Despite its necessity, there is a scarcity of studies conducted before or during highway paving. Our objectives were to: 1) quantify the number of animals falling victim to roadkill during highway paving, and 2) assess whether roadkill rates exhibit variations among seasons and between paved and unpaved highway segments. We present roadkill data from three consecutive years of surveys, encompassing 69 field trips and covering 4,820 km. Our dataset can serve as a foundation for forthcoming studies seeking to comprehend the impact of highway paving on roadkill rates and, consequently, in local wildlife within the Panga Ecological Station and its surroundings.

INTRODUCTION

Highways play a crucial role in human life, facilitating the movement of people and goods, and contributing to the development and progress of more remote populations (Percoco 2016). Despite their benefits, highways also generate negative impacts, including habitat fragmentation, deforestation, edge effects, facilitation of hunting, barrier effects, and wildlife roadkill (Forman and Alexander 1998; Laurance *et al.* 2009; Pinto *et al.* 2020; Van der Ree *et al.* 2015; Trombulak and Frissell 2000). Among these impacts, wildlife roadkill stands as the second leading cause of biodiversity loss caused by human activity worldwide (Hill *et al.* 2019). In the USA, around one million animals die on roads each day. In the Netherlands, 159 thousand mammals and 653 thousand birds are killed annually. Bulgaria records seven million bird fatalities per year, and in Australia five million amphibians and reptiles fall victim to roadkill each year (Forman and Alexander 1998). In Europe, about 194 million birds and 29 million mammals are killed on roads annually (Grilo *et al.* 2020), while in Brazil, the numbers reach eight million birds and two million mammals per year (González-Suárez *et al.* 2018).

In Brazil, highways are the main mode of transportation, which has led to large investments in paving and modernizing the existing road network (Teixeira *et al.* 2016). As road networks grow and vehicular traffic increases, wildlife roadkill emerges as a growing concern (Pinto *et al.* 2020; Teixeira *et al.* 2016). Studies on Brazilian highways have aimed to estimate the number and rates of wildlife roadkill, addressing factors such as spatial patterns, seasonality, and road characteristics (Brum *et al.* 2018; Carvalho-Roel *et al.* 2023; Distrito Federal 2013; Prada 2004; Prado *et al.* 2006; Santos *et al.* 2012; Turci and Bernarde 2009). For instance, it appears that roadkill rates vary with season (Carvalho *et al.* 2017; Ferrugueti *et al.* 2019; Santos *et al.* 2012). During dry seasons, animals may need to travel further in search of food, increasing their chances of being hit by vehicles (Melo and Santos-Filho 2007). Roadkill involving reptiles rises during the rainy and hot season when these animals are more active (Andrews *et al.* 2015). Sea-

sonal increases in roadkill rates are also reported for other species, particularly during reproductive seasons or recruitment periods (Grilo *et al.* 2009). Roadkill is also associated with factors facilitating higher vehicle speeds, such as lane width and pavement type (Distrito Federal 2013; Grilo *et al.* 2010; Gunson *et al.* 2010). Paved roads generally have higher roadkill rates than unpaved ones because they allow vehicles to travel faster, reducing the reaction time for both animals and drivers (Distrito Federal 2013; Figueiredo *et al.* 2013; Grilo *et al.* 2010; Ribeiro 2016; Smith-Patten and Patten 2008).

Roadkill rates tend to be higher near preserved areas (Prado *et al.* 2006), likely because these areas support greater biodiversity and larger animal populations (Bager 2003; Lima 2013). Highways strongly impact protected areas, with over half (62%) of Brazil's federal protected areas intersected by highways, and 72% indirectly affected by roads (Lima 2013). A preserved area in the municipality of Uberlândia, Minas Gerais state, is the Panga Ecological Station (PES). It was acquired by the Federal University of Uberlândia (UFU) in 1985 and later designated a Private Natural Heritage Reserve. Since then, its native vegetation has been naturally regenerating (Ranal 2003). PES covers 403.85 hectares of native Cerrado vegetation and stands as one of the last extensive remnants of this biome in the region. Regarding size, it is a significant conservation unit in Triângulo Mineiro, used for educational and research purposes (Marçal Júnior *et al.* 2009). PES borders the MGC-455 highway to the east (Cardoso *et al.* 2009). At the time of this study, the MGC-455 highway, a state road, was undergoing paving, which allowed us to monitor both paved and unpaved sections.

Despite the importance of such infrastructure projects, few studies are conducted before or during the paving of a highway. Before-after and before-after-control-impact studies are essential to: 1) comprehend whether an impact, such as roadkill, attains a level of concern, and for which species persistence, 2) determine if road mortality warrants attention for driver safety, 3) provide information about the species involved to inform decision-makers about the advised mitigation structures, and 4) identify locations and

times of high mortality rates (Kindel *et al.* 2017). This study was conducted exactly during the highway paving process, allowing us to document roadkill under both conditions. With the completion of paving, we anticipate increased vehicle speed and flow, which may lead to higher wildlife mortality.

Therefore, the objectives of this study were to identify the animal taxa involved in roadkill, classify them at the lowest possible taxonomic level, estimate roadkill rates, and assess the influence of seasonality and pavement type (paved or unpaved) on roadkill. We propose two main hypotheses to be tested in this study:

1) We hypothesize that roadkill rates will differ between seasons, particularly for "reptiles" (as observed by Carvalho *et al.* (2017)), with higher mortality during the rainy season when these ectothermic animal exhibit increased activity levels and movement in response to warmer temperatures and greater availability of resources (Andrews *et al.* 2015). In contrast, we expect roadkill rates for birds and mammals to remain relatively constant across seasons (Carvalho *et al.* 2017; Santos *et al.* 2012), as these groups are generally less dependent on climatic conditions to regulate their activity patterns. Many birds and mammals maintain regular movement for foraging or territorial behavior throughout the year in Tropical countries (Pough *et al.* 2008), which may lead to more stable roadkill rates over time.

2) We hypothesize that roadkill rates will be higher on paved sections of the highway compared to unpaved sections (Correia Junior and Corrêa 2013; Distrito Federal 2013; Figueiredo *et al.* 2013; Ribeiro 2016). This expectation is based on the observation that paved roads facilitate higher vehicle speeds and greater traffic volumes, both of which are positively associated with increased wildlife mortality (Gunson *et al.* 2011; Rendall *et al.* 2021). After the highway paving is completed, our findings can be used to investigate the impact of asphalt and the resulting increase in vehicle flow and speed on wildlife roadkill rates.

MATERIAL AND METHODS

Study area

The MGC-455 is a state highway that extends over 137.3 km and borders the PES on its eastern side (Figure 1). This road connects the city of Uberlândia, in Minas Gerais state, to the BR-364 highway, situated in the municipality of Planura near the São Paulo state border (Ministério dos Transportes 2011). It is a two-way, single-lane road without shoulders, artificial lighting, slopes and protective barriers. In comparison to other highways in the region, MGC-455 has lower vehicular traffic. Throughout most of its length, the

road is mostly straight without significant curves, uphill or downhill stretches. As of 2015, approximately 50% of the studied stretch had been paved. The remaining unpaved kilometers were in poor condition, making vehicular traffic difficult. This highway is located in Cerrado biome, one of the hotspots for biodiversity conservation (Myers *et al.* 2000). The surrounding vegetation includes pastures (39%, within a 10 km buffer), fragments of savannahs and wetlands (28%), and monocultures (21%).

Monitoring

We collected data bimonthly over three years. From April 2012 to April 2013, we covered 45 km, which included 35 km of paved road and 10 km of unpaved road. From May 2013 to April 2015, we expanded the route to 80 km, 50 km of paved, and 30 km of unpaved segments (Figure 2). There was minimal progress in paving throughout the monitoring period. Surveys began at 7:30 am, we took the necessary time to complete the entire route, averaging about five hours per session. In total, we carried out 69 field surveys, covering 4,820 km. Sixty percent of the data was collected during the seven-month rainy season in the study area (Rosa *et al.* 1991).

The monitoring was executed by car, maintaining a speed of 30 km/h on unpaved and difficult sections, and 50 km/h on paved segments. Each survey included two observers and the driver. The second observer and the driver did not impact carcass detection. We examined both sides of the highway, and only animal recorded on the route from Uberlândia city to Rio do Peixe village were considered systematic and used to calculate roadkill rates. Animals found in the vicinity or on the return route (from Rio do Peixe village to Uberlândia city) were categorized as occasional and excluded from roadkill rate calculations. This methodology follows the protocol of the Brazilian Center for Studies in Road Ecology, ensuring standardization for future comparisons (Rosa *et al.* 2012). We photographed all carcasses for accurate identification. After each record, carcass were removed from the road to avoid duplicate counts and to prevent the roadkill of scavengers (Rosa *et al.* 2012). For seasonal analyses, the months from October to April were considered the rainy and hot season, while May to September were considered the dry and cold season (Rosa *et al.* 1991). Conservation status for animals recorded as roadkill was based on state, national, and global lists (CO-PAM 2010; IUCN 2022; Ministério do Meio Ambiente 2022).

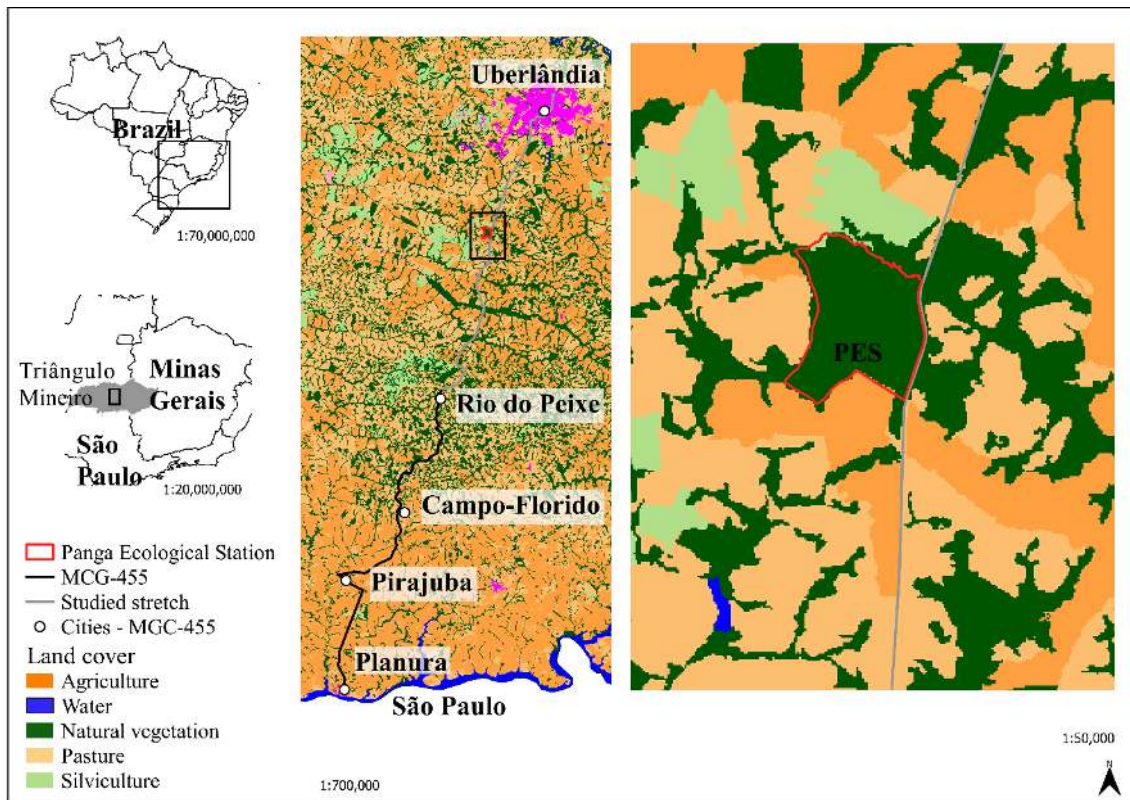


Figure 1. MGC-455 highway, Cerrado biome, Brazil, highlighting the Panga Ecological Station (PES) and the land cover in its surroundings.



Figure 2. Unpaved (on the left) and paved stretch (on the right) of the MGC-455 highway, Cerrado biome, Brazil.

Data analyses

We calculated roadkill rates using only systematic records of wildlife, dividing the total number of carcasses by the sampling effort measured in kilometers. We did not apply any correction factor, as no specific protocol was used to estimate carcass removal and persistence. Therefore, a priori, the reported rates are likely underestimated values. Although important, few studies have calculated removal and persistence rates, mainly due to logistic challenges. For the com-

parison between paved and unpaved sections, we used all records (including systematic, non-systematic, and domestic animals). To test for monthly differences in roadkill rates between the dry and rainy seasons, and to compare rates between paved and unpaved sections, we used the Mann-Whitney (U) test, due to the non-normal distribution of the data. All analyses were performed using R 3.6.1 software (R Core Team 2023).

RESULTS

We recorded 171 roadkill, 126 wild animals (73.7%), and 45 domestic (26.3%), averaging 57 animals per year (Table 1, Additional File 1). Of the 126 wild animals, 87 (66%) were systematic records. Eleven species had only occasional records, namely: *Falco femoralis* (aplomado falcon), *Herpetotheres cachinnans* (laughing falcon), *Crotophaga ani* (smooth-billed ani), *Athene cunicularia* (burrowing owl), *Furnarius rufus* (rufous hornero), *Pitangus sulphuratus* (great kiskadee), *Tyrannus melancholicus* (tropical kingbird), *Gallus gallus domesticus* (chicken), *Columba livia* (common pigeon), *Lygophis lineatus* (lined ground snake), and *Microtus arvalis* (common vole). Among the systematic records, 38 individuals (43.7%) were birds, 24 (27.6%) reptiles, 17 (19.5%) mammals, and eight (9.2%) amphibians (Table 1). We documented 37 systematized species as roadkill victims, including 15 bird species (40.6%), 12 reptile species (32.4%), eight mammal species (21.6%), and two amphibian species (5.4%).

The roadkill rate for wild vertebrates was 0.008 individuals/km/day, with a minimum of one and a maximum of 12 animals observed per visit. Among taxonomic groups, the roadkill rate was 0.003 individuals/km/day for birds, 0.002 for reptiles and 0.001 for wild mammals. *Cariama cristata* (red-legged seriema) was the most frequently recorded wild species and bird in this study (0.002 individuals/km/day). *Caracara plancus* (crested caracara) was the second most recorded bird (0.001 individuals/km/day). Among reptiles, *Erythrolamprus aesculappi* (South American false coral snake) (0.002 individuals/km/day), *Amphisbaena alba* (red worm lizard) (0.001 individuals/km/day), and *Crotalus durissus* (South American rattlesnake) (0.001 individuals/km/day) were the most affected species. The most common mammal in this study was *Cerdocyon thous* (crab-eating fox) (0.001 individuals/km/day). Concerning conservation status, *Myrmecophaga tridactyla* (giant anteater) (0.001 individuals/km/day) is classified as vulnerable in the state of Minas Gerais, Brazil, and globally. *Lycalopex vetulus* (hoary fox) (0.0004 individuals/km/day) is classified as near-threatened globally and vulnerable in Brazil, and *Sylvilagus brasiliensis* (common tapeti) (0.0004 individuals/km/day) is classified as endangered globally.

We observed no difference in the monthly roadkill rates between the rainy and dry seasons for wild vertebrates ($U = 214.5$; $p = 0.125$), wild birds ($U = 166.500$; $p = 0.974$), and wild mammals ($U = 171.000$; $p = 0.845$). However, for reptiles, there was a higher roadkill rate for this group during the rainy seasons ($U = 239.000$; $p = 0.009$) (Figure 3). Overall, the roadkill rate was approximately six times higher on

paved sections compared to unpaved ones ($U = 348.5$; $p < 0.000$) (Figure 4), 0.006 and 0.001 individuals/km/day, respectively.

Table 1. Vertebrates roadkill on MGC-455 Highway, Cerrado biome, Minas Gerais state, Brazil (2012-2015).

<i>Taxa</i>	<i>Common name</i>	<i>IUCN</i> ¹ <i>Global</i>	<i>MMA</i> ² <i>National</i>	<i>COPAM</i> ³ <i>State</i>	<i>N</i>
Birds					
Cariamiformes					
Cariamidae					
<i>Cariama cristata</i> (Linnaeus, 1766)	Red-legged seriema	LC	LC	LC	9
Falconiformes					
Falconidae					
<i>Caracara plancus</i> (Jacquin, 1784) *	Crested caracara	LC	LC	LC	6
<i>Milvago chimachima</i> (Vieillot, 1816)	Yellow-headed caracara	LC	LC	LC	1
<i>Falco femoralis</i> (Temminck, 1822) "	Aplomado falcon	LC	LC	LC	1
<i>Herpotheres cachinnans</i> (Linnaeus, 1758) "	Laughing falcon	LC	LC	LC	1
Cuculiformes					
Crotophaginae					
<i>Guira guira</i> (Gmelin, 1788) *	Guira cuckoo	LC	LC	LC	2
<i>Crotophaga ani</i> (Linnaeus, 1758) "	Smooth-billed ani	LC	LC	LC	1
Gaubuliformes					
<i>Galbula ruficauda</i> (Levaillant, 1801)	Rufous-tailed jacamar	LC	LC	LC	1
Strigiformes					
Strigidae					
<i>Athene cunicularia</i> (Molina, 1782) "	Burrowing owl	LC	LC	LC	1
Caprimulgiformes					
Caprimulgidae					

to be continued...

<i>Taxa</i>	<i>Common name</i>	<i>IUCN</i> ¹	<i>MMA</i> ²	<i>COPAM</i> ³	<i>N</i>
		<i>Global</i>	<i>National</i>	<i>State</i>	
<i>Nyctidromus albicollis</i> (Gmelin, 1789) *	Pauraque	LC	LC	LC	3
Piciformes					2
Picidae					
<i>Colaptes campestris</i> (Vieillot, 1818) *	Campo flicker	LC	LC	LC	2
Passeriformes					6
Furnariidae					2
<i>Furnarius rufus</i> (Gmelin, 1788) "	Rufous hornero	LC	LC	LC	2
Tyrannidae					2
<i>Pitangus sulphuratus</i> (Linnaeus, 1766) "	Great kiskadee	LC	LC	LC	1
<i>Tyrannus melancholicus</i> (Vieillot, 1819) "	Tropical kingbird	LC	LC	LC	1
Emberizidae					4
<i>Volatinia jacarina</i> (Linnaeus, 1766) *	Blue-black grassquit	LC	LC	LC	2
<i>Sporophila</i> sp.					1
<i>Zonotrichia</i> sp.					1
Passeridae					2
<i>Passer domesticus</i> (Linnaeus, 1758) *	House sparrow	LC	NA	NA	2
Thraupidae					2
<i>Sicalis citrina</i> (Pelzeln, 1870) *	Stripe-tailed yellow finch	LC	LC	LC	2
Galliformes					1
Phasianidae					1
<i>Gallus gallus domesticus</i> (Linnaeus, 1758) " ~	Chicken				1
Columbiformes					1
Columbidae					1
<i>Columba livia</i> (Gmelin, 1789) "	Common pigeon	LC	NA	NA	1

to be continued...

<i>Taxa</i>	<i>Common name</i>	<i>IUCN</i> ¹ <i>Global</i>	<i>MMA</i> ² <i>National</i>	<i>COPAM</i> ³ <i>State</i>	<i>N</i>
Cathartiformes					3
Cathartidae					3
<i>Coragyps atratus</i> (Bechstein, 1793)	Black vulture	LC	LC	LC	3
Camprimulgiformes					2
Camprimulgidae					2
<i>Chordeiles acutipennis</i> (Hermann, 1783)	Lesser nighthawk	LC	LC	LC	2
Apodiformes					1
Trochilinae					1
<i>Chlorostilbon lucidus</i> (Shaw, 1812)	Glittering-bellied emerald	LC	LC	LC	1
Not-identified					2
Reptiles					
Amphisbaenidae					3
<i>Amphisbaena alba</i> (Linnaeus, 1758) *	Red worm lizard	LC	LC	LC	3
Teiidae					1
<i>Salvator merianae</i> (Duméril e Bibron, 1839) *	Black and white tegu	LC	LC	LC	1
Boidae					2
<i>Boa constrictor amarali</i> (Stull, 1932) *	Common boa	LC	LC	LC	2
Viperidae					8
<i>Bothrops moojeni</i> (Hoge, 1966)	Brazilian lancehead	LC	LC	LC	2
<i>Bothrops pauloensis</i> (Amaral, 1925)	jararaca-pintada	LC	LC	-	1
<i>Crotalus durissus collilineatus</i> (Amaral, 1926)	South American rattlesnake	LC	LC	LC	5
Anguidae					1
<i>Ophiodes striatus</i> (Spix, 1825)	Striped worm lizard	LC	LC	LC	1
Colubridae					9

to be continued...

<i>Taxa</i>	<i>Common name</i>	<i>IUCN</i> ¹	<i>MMA</i> ²	<i>COPAM</i> ³	<i>N</i>
		<i>Global</i>	<i>National</i>	<i>State</i>	
<i>Chironius</i> sp.					1
<i>Philodryas mattogrossensis</i> (Grazziotin et al. 2012)	Miranda green racer	LC	LC	LC	1
<i>Dipsas mikanii</i> (Myers, 2003)	Dormideira	LC	LC	LC	2
<i>Spilotes pullatus</i> (Linnaeus, 1758)	Chicken snake	LC	LC	LC	2
<i>Erythrolamprus aesculapii</i> (Linnaeus, 1766) *	South American false coral snake	LC	LC	LC	4
<i>Lygophis lineatus</i> (Linnaeus, 1758) "	Lined ground snake	LC	LC	LC	1
Mammals					
Carnivora					
Canidae					
<i>Canis familiaris</i> (Linnaeus, 1758) * ~	Domestic dog				25
<i>Cerdocyon thous</i> (Linnaeus, 1758)	Crab-eating fox	LC	LC	LC	5
<i>Lycalopex vetulus</i> (Lunda, 1842) *	Hoary fox	NT	VU	NA	2
Felidae					
<i>Felis catus</i> (Linnaeus, 1758) * ~	Domestic cat				5
Mephitidae					
<i>Conepatus semistriatus</i> (Boddaert, 1785)	Striped hog-nosed skunk	LC	LC	LC	1
Procyonidae					
<i>Procyon cancrivorus</i> (Cuvier, 1798)	Crab-eating raccoon	LC	LC	LC	1
Pilosa					
Myrmecophagidae					
<i>Myrmecophaga tridactyla</i> (Linnaeus, 1758)	Giant anteater	VU	VU	VU	2
Cingulata					
Dasypodidae					
<i>Euphractus</i> sp. (Linnaeus, 1758) *	Armadillo	LC	LC	LC	3

to be continued...

<i>Taxa</i>	<i>Common name</i>	<i>IUCN</i> ¹ <i>Global</i>	<i>MMA</i> ² <i>National</i>	<i>COPAM</i> ³ <i>State</i>	<i>N</i>
Peryssodactyla					1
Equidae					
<i>Equus caballus</i> (Linnaeus, 1758) ~	Horse				1
Rodentia					1
Cricetidae					
<i>Microtus arvalis</i> (Pallas, 1778) "	Common vole	LC	LC	LC	1
Artiodactyla					2
Bovidae					
<i>Bos taurus taurus</i> (Linnaeus, 1758) ~	Cattle				2
Lagomorpha					1
Leporidae					1
<i>Sylvilagus brasiliensis</i> (Linnaeus, 1758)	Common tapeti	EN	LC	LC	1
Didelphimorphia					1
Didelphidae					
<i>Lutreolina crassicaudata</i> (Desmarest, 1804)	Big lutrine opossum	LC	LC	LC	1
Anura					
Bufonidae					
<i>Rhinella schneideri</i> (Wemer, 1894)	Cope's toad	LC	LC	LC	4
Ranidae					
<i>Leptodactylus</i> sp.	Frog				3
Not-identified					1
Total					171

Legend: ¹International Union for Conservation of Nature. ²Ministério do Meio Ambiente. ³COPAM – Conselho Estadual de Política Ambiental. LC – least concern, VU – vulnerable, NT – near threatened, EN – endangered, NA – not available. * Groups with occasional records. " Groups only with occasional records. ~ Domestic animals.

DISCUSSION

When considered out of context, the roadkill rates from this study may appear low compared to other findings. For instance, Brum *et al.* (2018) recorded a roadkill rate of 0.035 ind./km/day along a 105 km stretch (including 55 km within indigenous land) with an average traffic volume of 1,900 vehicles/day. Carvalho-Roel *et al.* (2023) reported a higher rate, 0.059 ind./km/day, on BR-050, also located in Triângulo Mineiro region, where traffic volume reaches approximately 12,000 vehicles/day. Neto *et al.* (2015) documented a rate of 0.058 ind./km/day near Chapadão do Céu (Goiás state) on a stretch with up to 12.33 vehicles passing every five minutes. In contrast, our study was conducted in an area with significantly lower vehicle flow, where during five hours of monitoring, only 15 vehicles were recorded, which is an extremely low value when compared to the studies mentioned above. This low traffic volume, combined with a large unpaved road portion, likely contributed to the lower roadkill rates observed.

It is worth noting that none of the above-cited studies, including ours, considered carcasses removal and detection probability to calculate a correction rate for roadkill. As a result, roadkill rates from this research and most studies in the field are underestimated due to various factors (Rosa *et al.* 2012). One major limitation relates to the reduced carcass visibility inherent to the methodology. Additionally, many species exhibit high removal rates due to their small biomass (Barrientos *et al.* 2018; Teixeira *et al.* 2013). Climatic factors such as wind and rain can also act as removal agents for carcasses (Santos *et al.* 2016), along with the high speed of heavy vehicles, which can, due to friction, accelerate the carcass removal process from the highway (Teixeira *et al.* 2013).

The impact of these removal and detectability biases can be substantial. For instance, after applying correction factors, Santos *et al.* (2016) estimated roadkill rates that were ten times higher for small animals (<100 g) and twice as high for larger-bodied species (>100 g) compared to uncorrected values. Similarly, actual roadkill rates for reptiles and birds have been shown to be significantly underestimated without such adjustments (Teixeira *et al.* 2013). Although logistical constraints prevented the implementation of correction measures in our study, such as carcass persistence trials and observer efficiency assessments, this limitation likely resulted in an underestimation of actual mortality, particularly for small-bodied species that are less detectable and more rapidly removed (Barrientos *et al.* 2018; Santos *et al.* 2016; Teixeira *et al.* 2013). Consequently, comparisons with studies that applied correction factors should be avoided, as lower roadkill rates in our results may reflect methodological limi-

tations rather than genuinely lower mortality. Future research should prioritize the integration of correction protocols to enhance the accuracy and comparability of roadkill estimates (Barrientos *et al.* 2018; Teixeira *et al.* 2013).

Another reason supporting that the roadkill rates may be underestimated is the identification of 39 individuals on the return route. Due to the method used and the characteristics of the highway, some animals near the shoulder on the opposite side of the observer went unnoticed and were only recorded on the return route, thus considered occasional records. Other studies have reported similar issues; for example, mice on road shoulders persisted longer than those on the traffic lanes (Santos and Ascensão 2019). If these individuals were counted, our roadkill rate would be at least 34% higher.

Moreover, all other roadkill rates presented here were conducted on paved roads, while approximately 50% of the road in this study was still unpaved. Santos and Ascensão (2019) showed that carcass persistence varies between dirt, two and four-lane roads, with mice carcasses lasting longer on four-lane roads. This may also explain why our roadkill rates are low when compared to other studies, scavengers likely have easier access to carrion on dirt and two-lane roads (Santos and Ascensão 2019). In addition, on dirt roads, carcasses are more frequently removed by scavengers at night (Ratton *et al.* 2014). As we always started our monitoring early in the morning, higher nighttime carcass removal could also have influenced our roadkill rates compared to those on two and four-lane roads.

Other authors also have observed lower roadkill rates for dirt roads. Similarly, Ribeiro (2016) and Figueiredo *et al.* (2013) found lower roadkill rates for unpaved road stretches: 0.029 ind./km/day and 0.01 ind./km/day, respectively. Ribeiro (2016) monitored the GO-239 highway, which is the main access route to the Chapada dos Veadeiros National Park, while Figueiredo *et al.* (2013) surveyed roadkill fauna near 11 Conservation Units in the Federal District. These values are closer to the rates obtained in this study, where roadkill rates on unpaved stretches were approximately six times lower than on paved stretches. This difference reinforces that the reduced vehicle speed on unpaved and poorly maintained roads contributes to fewer roadkills. Therefore, it is necessary to consider that during the monitoring, a large portion of the road was still unpaved and in poor condition. This situation forced vehicles to travel at a very low speed, approximately 30/40 km/h. The roadkill rate in unpaved stretches may be even lower than it appears, as slower monitoring speeds likely increased the chance of detecting a carcass compared to paved sections.

In addition to roadkill rates, another factor highlighting the importance of the study area is the pres-

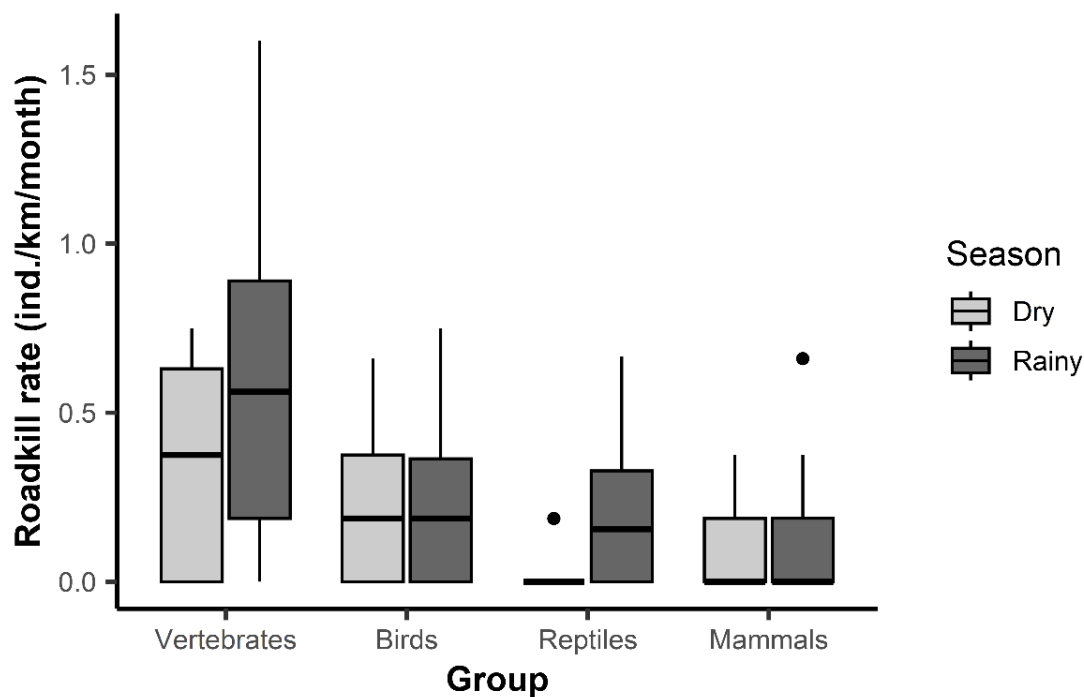


Figure 3. Wildlife roadkill rate according to the season, MGC-455 highway, Cerrado biome, Minas Gerais state, Brazil (2012-2015).

ence of endangered species. *Lycalopex vetulus*, an endemic species of the Cerrado biome, easily approaches human settlements and inhabits modified areas such as pastures (Marinho *et al.* 2022), increasing its risk of roadkill. Considering the extensive loss of the original Cerrado vegetation and threats such as roadkill, predation by domestic dogs, diseases, retaliation for alleged predation of domestic animals, and high mortality of offspring/juveniles, the population is estimated to have declined by at least 30% in the last 15 years. This canid is expected to experience a similar decline in the next 15 years (Lemos *et al.* 2013). A recent study showed that in an agroecosystem area, this species has an 80% chance of extinction over the next 100 years (Carvalho-Roel *et al.* 2024). Another endangered species present in the area is *Myrmecophaga tridactyla*. In the Cerrado, its population has undergone a drastic reduction due to habitat loss and expansion of road infrastructure (Desbiez *et al.* 2020; Miranda *et al.* 2015). For this species, mortality from roadkill is by far the most serious threat to its long-term persistence (Diniz and Brito 2013). Therefore, given the ecological importance and vulnerability of these species, research and conservation actions aimed at reducing roadkill in the area is essential.

Only about 8% of the birds listed for PES (Marçal Júnior *et al.* 2009) were found as roadkill. Their low number likely reflects the study's methodology, suggesting that other species may also be victims of road-

kill in the area. Car surveys detect only 7% of small animal carcasses, animal weighing less than 100 grams typically remain on the road for an average of only two days (Santos *et al.* 2016). About 50% of the birds carcass weighed more than 100 grams. Even during the time taken to complete the monitoring route and return, several small-sized bird species (around 100 grams each) were found dead. For medium and large-sized mammals, only 25% of the species listed for the PES (Bruna *et al.* 2010) were identified as roadkill. However, around 39% of the species listed for the reserve are small-sized, making carcass detection challenging. Regarding amphibians, their numbers are likely underestimated, as their small bodies disappear from the road surface quickly (Barrientos *et al.* 2018). Although there are challenges in detecting these carcasses, Turci and Bernarde (2009) found Amphibia as the group most affected by roadkill. This may be because their study used motorcycles and lower speeds (around 40 km/h), improving carcasses visibility. Also, it was conducted in the Amazon biome, where landscape characteristics may also contribute to higher detection rates.

Regarding seasonality, Santos *et al.* (2012) and Carvalho *et al.* (2017) also found that roadkill of reptiles is concentrated in summer months. This group appears to be the most influenced by seasonal and climatic variables. This pattern is ecologically driven, as during rainy months the warmer and wetter condi-

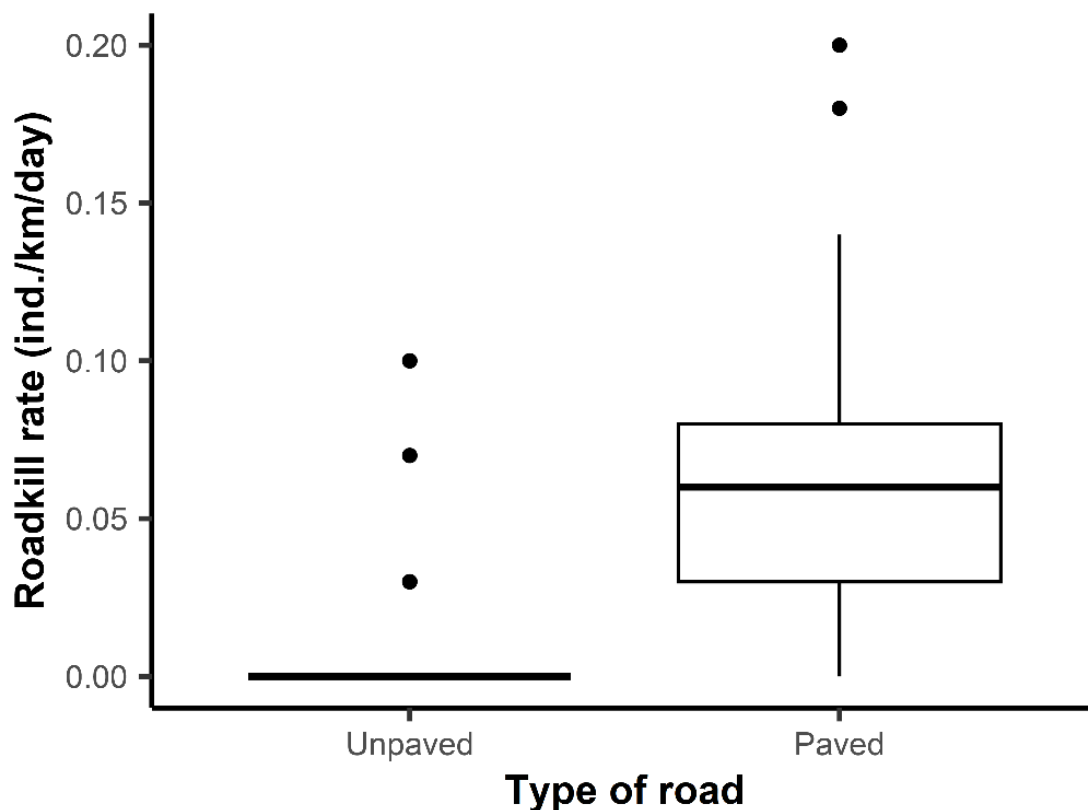


Figure 4. Wildlife roadkill rate on unpaved and paved stretches, MGC-455 highway, Cerrado biome, Minas Gerais state, Brazil (2012-2015).

tions are more appropriate for reptiles to forage, regulate their body temperature and reproduce (Pough *et al.* 2008). As a result, they become more active and tend to move across open areas, including roads, making them more vulnerable to collisions (Andrews *et al.* 2015). For birds, seasonal patterns are harder to detect due to the large diversity within the group and the need for greater sampling effort compared to other groups (Bager and Rosa 2011). Mammals generally do not exhibit seasonal variation in roadkill (Coelho *et al.* 2008). However, when pressured by resource scarcity, they may move across larger areas (Almeida 2007), increasing the change of crossing roads. Amphibians roadkill usually coincides with major migration events, which occur during breeding or juvenile dispersal periods, similar to reptiles (Garrah *et al.* 2015; Mazerolle 2004).

Some studies have been conducted aiming to compare roadkill rates based on the pavement type of the road (Correia Junior and Corrêa 2013; Distrito Federal 2013; Figueiredo *et al.* 2013; Ribeiro 2016). Figueiredo *et al.* (2013) reported the following roadkill rates for wildlife concerning pavement type: 0.09 ind./km/day for paved highways and 0.01 ind./km/day for unpaved highways. Distrito Fed-

eral (2013) states that the average number of roadkill records per 1 km segment on paved roads is 6.28, while on unpaved roads, it is 1.56. Similar to the aforementioned studies, roadkill rates in the present study are six times higher on paved sections of the highway, probably because paved highways allows drivers to adopt higher speeds and a greater flow of vehicles (Gunson *et al.* 2011; Smith-Patten and Patten 2008).

Considering the ecological importance of the area, the presence of threatened species, and the significant roadkill rates observed, with a probable increase in the coming years, it is recommended to implement mitigation measures aimed at reducing wildlife-vehicle accidents. Among available strategies, fencing with or without wildlife crossing structures is the most effective method for reducing mortality (Rytwinski *et al.* 2016). However, fences alone create a barrier to movement, reducing connectivity and increasing population isolation. That is why it is advocated to implement fences combined with crossing structures (Jakes *et al.* 2018). Additionally, when constructing these structures is not feasible, existing bridges and culverts can be adapted by installing dry ledges to facilitate wildlife passage (Brunen *et al.* 2020).

To be truly effective, mitigation strategies need

to be grounded in research that's aligned with clear goals, whether it's reducing wildlife deaths on roads or keeping natural habitats connected (Teixeira *et al.* 2016). Also important is the development of long-term monitoring programs to assess the effectiveness of mitigation measures and inform adaptive management (van der Grift *et al.* 2015). In addition, fostering collaboration among stakeholders, practitioners and researchers is essential. Working together in this way helps generate shared knowledge and builds the capacity needed to carry out environmental impact assessments (EIAs) that genuinely protect ecological connectivity (Gonçalves *et al.* 2022).

CONCLUSION

Our results indicate that reptiles are more frequently involved in roadkill incidents during the rainy season, and paved sections exhibit higher roadkill rates than unpaved ones. This study could contribute to the development and implementation of mitigation measures. The presence of endangered species in the area underscores the importance of preserving Cerrado remnants such as the PES, which stands as one of the crucial areas for the conservation of wildlife and flora in the Triângulo Mineiro region.

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DATA AVAILABILITY

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

CONTRIBUTION STATEMENT

Conceived of the presented idea: WGP, AEIC, CFCR.
Carried out the experiment: WGP, JRA, ISB.
Carried out the data analysis: CFCR.
Wrote the first draft of the manuscript: WGP.
Review and final write of the manuscript: WGP, AEIC, CFCR.
Supervision: AEIC, CFCR.

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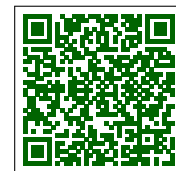
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Additional Files



Add File 1. Some examples of animals victim of roadkill on MGC-455, Cerrado biome, Minas Gerais state, Brazil (2012-2015). a) *Crotalus durissus collilineatus*, b) *Erytrolamprus aesculapii*, c) *Bothrops moojeni*, d) *Salvator merianae*, e) *Amphisbaena alba*, f) *Chironius* sp., g) *Athene cunicularia*, h) *Guira guira*, i) *Cariama cristata*, j) *Colaptes campestris*, k) *Procyon cancrivorus*, l) *Euphractus* sp., m) *Myrmecophaga tridactyla*, n) *Cerdocyon thous*, o) *Rhinella schneideri*.