

Road-killed wild animals in the North of Paraná State, Brazil: first reports of an underestimated environmental problem

Animais silvestres atropelados no Norte do Paraná, Brasil: primeiros relatos de um problema ambiental subestimado

Gabriel Brambila Milleo^{1*}; Andressa Maria Rorato Nascimento de Matos²; Eloiza Teles Caldart³; Fernanda Pinto-Ferreira³; Aline Ticiani Pereira Paschoal²; Mariana de Mello Zanim²; Ricardo Luís Nascimento de Matos⁴; Italmir Teodorico Navarro³; Ana Paula Vidotto Magnoni⁵

Highlights

The order with the highest number of road kills was Didelphimorphia.

We found some disturbing records, including three species threatened with extinction.

PR-445 in Mauá da Serra-PR had the highest concentration of accidents.

Abstract

Highways are essential for the economic development of a country, however, on the other hand, they are one of the main causes of habitat fragmentation and loss of biodiversity. Wildlife-vehicle collisions are the main cause of mortality on the roads, which can lead to changes in population dynamics and the behavior of species. This study aimed to evaluate the roadkill of medium and large mammals in 4 transects in the north of the state of Paraná, in the southern region of Brazil. The transects studied range from 193 to 242 km in length and were covered once a month between November/2016 and October/2018. In total, 20,592 km were covered, and approximately 330 carcasses were recorded, of which 66 were medium and large mammals with collection conditions adequate for identification. The Order with the highest incidence was Didelphimorphia, followed by Carnivora and Cingulata. The highest concentration of roadkill animals occurred on the PR-445, between the municipalities of Mauá da Serra

¹ PhD Student in the Postgraduate Program in Biological Sciences, Universidade Estadual, UEL, Londrina, PR, Brazil. E-mail: gabriel.brambila@uel.br

² PhD Students in the Postgraduate Program in Animal Science, UEL, Londrina, PR, Brazil. E-mail: andressa.rorato@uel.br; aline.paschoal@adapar.pr.gov.br; marianamzanim@gmail.com

³ Profs. Drs., Department of Veterinary Preventive Medicine, UEL, Londrina, PR, Brazil. E-mail: eloizacaldart@uel.br; fferreira@uel.br; italmir@uel.br

⁴ PhD Student in the Postgraduate Program in Chemistry, UEL, Londrina, PR, Brazil. E-mail: ricardolnm@uel.br

⁵ Profª Drª, Department of Animal and Plant Biology, UEL, Londrina, PR, Brazil. E-mail: anavidotto@uel.br

* Author for correspondence

and Tamarana, both in the state of Paraná. The mortality of endangered species such as *Leopardus wiedii*, *Leopardus guttulus*, and *Puma concolor* remains a concern. These results can help managers and government agencies responsible for roads to mitigate road accidents, with radars and wildlife signage and crossings in critical areas, such as the stretch with the highest number of roadkill records.

Key words: Wildlife crossings. Endangered species. Atlantic Forest. Habitat Fragmentation.

Resumo

As rodovias são essenciais para o desenvolvimento econômico de um país, em contrapartida são uma das principais causas de fragmentação de habitat e perda de biodiversidade. A colisão de animais silvestres com veículos é a principal causa de morte nas estradas, podendo acarretar na alteração das dinâmicas populacionais e no comportamento das espécies. O objetivo deste estudo foi avaliar os atropelamentos de mamíferos de médio e grande porte em quatro transectos nas estradas do norte do Paraná, Sul do Brasil. Os transectos variaram de 193 a 242 km de extensão e foram percorridos uma vez por mês, entre novembro de 2016 a outubro de 2018. Após 20.592 km percorridos foram registradas aproximadamente 330 carcaças, sendo que destas, 66 mamíferos de médio e grande porte apresentaram condições de coleta. A Ordem com maior incidência foi Didelphimorphia, seguido por Carnivora e Cingulata. A maior concentração de atropelamentos ocorreu na PR-445, entre os municípios de Mauá da Serra e Tamarana, ambos no estado do Paraná. A mortalidade de espécies ameaçadas de extinção, como *L. wiedii*, *L. guttulus* e *P. concolor* é preocupante. Nossos resultados podem ajudar gestores e órgãos competentes responsáveis pelas estradas a mitigar os atropelamentos, com placas de sinalização de vida silvestre, radares e passagens de fauna em áreas críticas, como o trecho de maior número de registros.

Palavras-chave: Passagens de fauna. Espécies ameaçadas de extinção. Mata Atlântica. Fragmentação de Habitats.

Introduction

Roads and highways are essential for the economic and social development of a country (Bager & Fontoura, 2013); however, they are also one of the main causes of habitat fragmentation (Pinto et al., 2022). Brazil has an extensive road network, totaling 1,720,909 km (Confederação Nacional do Transporte [CNT], 2022). The State of Paraná alone has a road network comprising 10,473.56 km of paved roads and 1,448.56 km of unpaved roads (Departamento de Estradas de Rodagem [DER], 2021). At

least 25 million kilometers of new roads are planned worldwide by 2050, representing a 60% increase in the total length of roads compared to 2010 (Laurance et al., 2014). This huge expansion has the potential to cause serious direct (habitat loss and fragmentation) and indirect (noise, air, and visual pollution) environmental impacts (Laurance et al., 2009; Bager, 2012; Laurance et al., 2014). Another direct negative impact on fauna is road mortality (Van der Ree et al., 2015). In Europe alone, it is estimated that approximately 194 million birds and 90 million mammals are killed on roads annually (Grilo

et al., 2020), while in Brazil, it is estimated that 475 million vertebrates are lost annually (CENTRO BRASILEIRO DE ECOLOGIA DE ESTRADAS [CBEE, 2020]).

The Atlantic Forest has a great richness of mammal species, 321 species (Paglia et al., 2012; Graipel, 2017), of which 94 are medium and large mammals (Souza et al., 2019). This group is extremely vulnerable to the effects of defaunation (Bogoni et al., 2018) and to the effects of roads (Fahrig & Rytwinski, 2009; Ascensão et al., 2017). It is estimated that approximately 9 million medium and large mammals are killed on roads annually, which represents serious risks for the conservation of mammals (Pinto et al., 2022). Moreover, these species are particularly sensitive to the adverse effects of roads (Ascensão et al., 2017) due to their need for expansive habitats, high mobility, and movement between forest patches (Fahrig & Rytwinski, 2009).

The north of Paraná is mostly comprised of seasonal semi-deciduous forests (Roderjan et al., 2002), one of the most deforested phytogeographies of the Atlantic Forest (Bogoni et al., 2018). Studies on the road-killed fauna in the state are scarce, particularly in the northern region (Pereira et al., 2021). However, it is increasingly necessary to establish mitigation measures to reduce road mortality, and the first step is to identify and quantify the species most susceptible to being killed on roads (Barger & Rosa, 2011; Grilo et al., 2020). The current work aims to identify and quantify medium and large mammals killed on roads in northern Paraná, as well as to carry out spatial analysis and characterize the locations of crossings.

Material and Methods

Study area and collecting road-killed animals

Animals were collected as roadkill from urban and intermunicipal roads in the North Central and Pioneer North mesoregions of Paraná State (Roderjan et al., 2002) over a 24-month period (November 2016 to October 2018). Transects were systematically designed to cover the main municipalities within these regions (Figure 1, Table 1). One transect was covered per week, with the start and end points always located in the municipality of Londrina (23.2927°S, 51.1732°W). The average speed of the car ranged from 40 to 60 km/h, with three researchers always present: the driver and two researchers (one observing the right side and the other the left side of the highway) (Secco et al., 2018). Opportunistic records were also made through communication of the roadkill incidents by the 2nd Company of the Environmental Police or 2nd Road Police Company, even when outside the transects (OOT samples). The animals were collected dead, preferably in rigor mortis (less than 24h after death), without evisceration (Pugliares et al., 2007), and with the absence of fly larvae. Carcasses were placed in bags for biological material samples, transported to the Laboratory of Animal Pathology of the State University of Londrina, and stored in a cold room (-4 °C) for further study (Caldart et al., 2021; Silva et al., 2021). Species identification (Reis et al., 2011) and photo documentation were conducted in the laboratory.

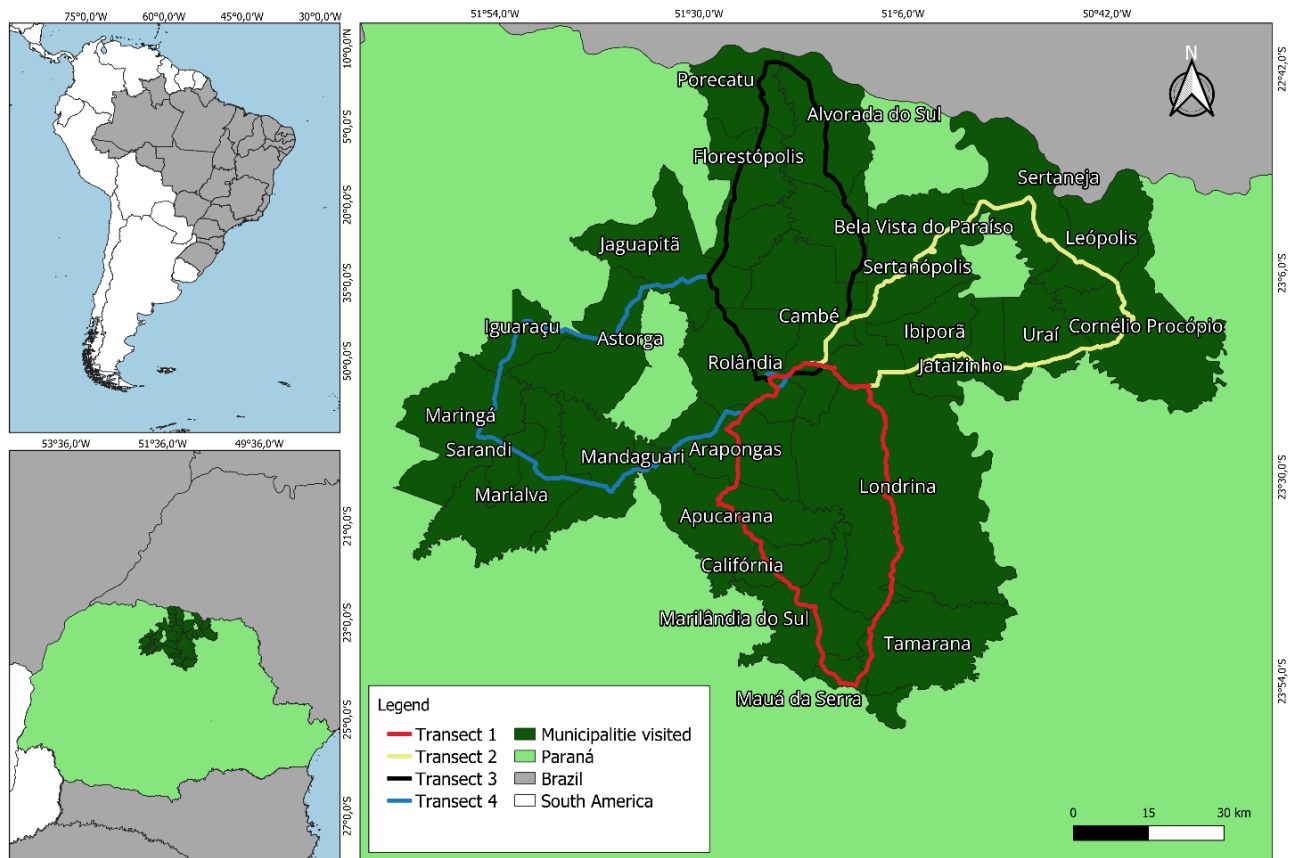


Figure 1. Transects traveled monthly in the active search for road-killed wild animals in municipalities in the North of Paraná, from November/2016 to October/2018.

Table 1

Characterization of transects by municipalities, total population (according to Instituto Brasileiro de Geografia e Estatística [IBGE], 2023), length, and characteristics of the roads

Transect	Municipalities	Total population (IBGE, 2023)	Length (km)	Characteristics of the roads
T1 (South transect)	Londrina, Cambé, Rolândia, Araongas, Apucarana, Califórnia, Marilândia do Sul, and Mauá da Serra	1,010,857 inhabitants	193	PR-445, BR-376, BR-369 Simple paved, double paved, no wildlife signs, no radar.
T2 (East transect)	Londrina, Ibiporã, Cornélio Procópio, Jataizinho, Leopólis, Sertaneja, Sertanópolis, and Cambé	940,438 inhabitants	228	BR-369, PR-160, PR-323, PR-445 Simple paved and double paved, with wildlife signs, no radar.
T3 (North transect)	Londrina, Rolândia, Jaguapitã, Florestópolis, Porecatu, Alvorada do Sul, Bela Vista do Paraíso, and Cambé	798,166 inhabitants	195	PR-445, PR-090, PR-170, Simple paved and double paved, without wild animal signs, with radar.
T4 (West transect)	Londrina, Mandaguari, Maringá, Astorga, and Jaguapitã	1,042,907 inhabitants	242	BR-369, PR-444, PR-317, PR-218, PR-454, PR-170, PR-444 Single pavement, double pavement, no wildlife signs, no radar.

Georeferencing and epidemiological research

The coordinate reference system used was EPSG:31982, and all trampling points of the study were mapped using the Global Positioning System (GPS). The spatial analysis of the occurrences was conducted in the QGIS program, version 3.30, using the Kernel intensity estimator (QGIS Development Team, 2023).

For the epidemiological investigation, a form was completed for each animal collected, with the purpose of obtaining data on the season, killing site (single or double road, presence/absence of electronic speed monitoring, presence/absence of signage to: wild animals, allowed velocity), and characteristics of the killed animal (sex, sexual maturity, and status of conservation of the species according to International Union for Conservation of Nature [IUCN] (2023) and Portaria MMA nº 148 Ministério do Meio Ambiente [MMA] (2022).

Ethical aspects

The project received approval from the Ethics Committee on Animal Use (CEUA) of the State University of Londrina on October 2017 (number 30/2017) and was approved by the System of Authorization and Information on Biodiversity (SISBIO) in October 2016 (number 55384-1).

Statistical analyses

The rate of wildlife killed by collision with vehicles per 100 km per year was calculated for the specimens collected during systematic monitoring, following the methodology outlined by Sadleir and Linklater (2016). To assess differences between the habits of the groups, we conducted non-parametric Kruskal-Wallis tests, with the total number of individuals of the collected species as the predictor variable and their habits, such as diet, activity period, locomotion, and group composition as the response variables. All analyses were performed using R software version 4.2.1 (R Core Team [R], 2022). The functional traits table was developed based on the works of Paglia et al. (2012), Wilman et al. (2014), and Abreu et al. (2021).

Results and Discussion

We registered 17 species of medium and large-sized mammals, with a large proportion of carnivores, represented by eight species. Among them, *Leopardus wiedii* (margay), *Leopardus gutullus* (southern oncilla), and *Puma concolor* (puma) are classified as vulnerable, according to the MMA (2022) (Figure 2).

A total of 20,592 km was monitored over the 24 months of sampling. We registered approximately 330 carcasses, with 66 (20%) in an adequate condition for collection. The order with the highest occurrence was Didelphimorphia (N=25; 37.88%), followed by Carnivora (N = 20; 30.30%), and Rodentia (N = 7; 10.61%), Cingulata, Pilosa, and Primates accounted for 6.06% (N=4), and Cetartiodactyla and Lagomorpha for 1.52% (N= 1).

The most commonly collected species was *Didelphis albiventris* (white-eared opossum), representing 37.88% of the total (N =25), eight specimens of which were found within the campus of the State University of Londrina (Table 2).

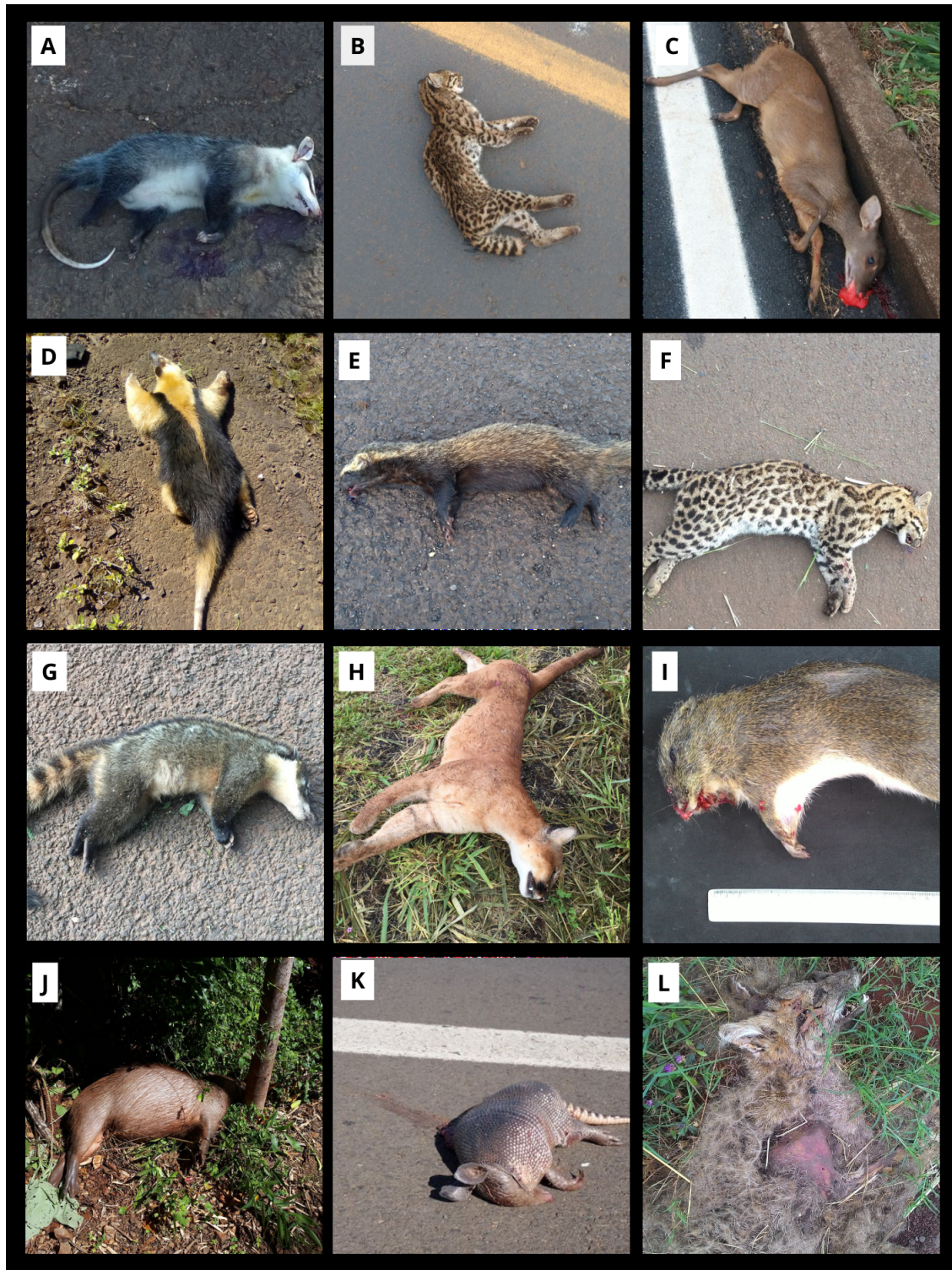


Figure 2. Specimens of A) *Didelphis albiventris*, B) *Leopardus guttulus*, C) *Subulo gouazoubira*, D) *Tamandua tetradactyla*, E) *Galictis cuja*, F) *Leopardus pardalis*, G) *Nasua nasua*, H) *Puma concolor*, I) *Cavia aperea*, J) *Hydrochoerus hydrochaeris*, K) *Dasypus novemcinctus*, and L) *Cerdocyon thous*.

Table 2
Scientific name, frequency, and transect of collection of the road-killed wild animals collected in municipalities in the North of Paraná from November/2016 to October /2018

<i>Taxa</i>	T	%	Locomotion	Activity	Comp	Diet	Threat	status
							IUCN	MMA
Didelphimorphia								
<i>Didelphis albiventris</i>	25	37.88	ARBO	NOT	SOL	Fr/On	LC	LC
Cingulata								
<i>Dasybus novemcinctus</i>	4	6.06	TER	NOT	SOL	In/On	LC	LC
Pilosa								
<i>Tamandua tetradactyla</i>	4	6.06	TER	CRE/NOT/DIU	SOL	Myr	LC	LC
Primates								
<i>Sapajus nigritus</i>	4	6.06	ARBO	DIUR	GREG	Fr/On	LC	LC
Lagomorpha								
<i>Lepus europaeus</i>	1	1.52	TER	NOT	SOL	Hb	LC	LC
Rodentia								
<i>Cavia aperea</i>	3	4.55	TER	CRE	GREG	Hb	LC	LC
<i>Hydrochaeris hydrochaeris</i>	2	3.03	AQUA	DIUR	GREG	Hb	LC	LC
<i>Coendou spinosus</i>	2	3.03	ARBO	NOT	SOL	Fr/Fo	LC	LC
Carnivora								
<i>Cerdocyon thous</i>	5	7.58	TER	NOT	SOL	In/On	LC	LC
<i>Galictis cuja</i>	1	1.52	TER	CRE/NOT	SOL	Ca	LC	LC
<i>Leopardus guttulus</i>	4	6.06	TER	NOT	SOL	Ca	VU	VU
<i>Leopardus pardalis</i>	1	1.52	TER	NOT	SOL	Ca	LC	LC
<i>Leopardus wiedii</i>	1	1.52	TER	CRE/NOT	SOL	Ca	NT	VU
<i>Nasua nasua</i>	5	7.58	TER	DIUR	GREG	Fr/On	LC	LC
<i>Procyon cancrivorus</i>	1	1.52	SC	NOT	SOL	Fr/On	LC	LC
<i>Puma concolor</i>	2	3.03	TER	NOT	SOL	Ca	LC	VU
Cetartiodactyla								
<i>Subulo gouazoubira</i>	1	1.52	TER	DIUR	SOL	Fr/Hb	LC	LC
Total:	66	100%						

T: Total numbers of occurrences. %: Frequency of occurrence by species. Activity pattern: Nocturnal (NOT); Diurnal (DIUR); Crepuscular (CRE). Locomotion: ARBO (Arboreal); TE (Terrestrial); AQUA (Aquatic); SC (scansorial). Behavior: Gregarious (GREG); Solitaire (SOL). Diet: Carnivore (Ca); Frugivore (Fr); Insectivore (In); Herbivore (Hb); Folivore (Fo); Omnivore (Om). IUCN: Least Concern (LC); Near Threatened (NT). MMA: Least Concern (LC); Vulnerable (VU).

Regarding activity patterns, nine species were nocturnal, four species were diurnal, and four species were predominantly crepuscular. Four modes of locomotion were recorded: terrestrial (10 species), arboreal (three species), aquatic, and scansorial (one species each). Concerning the diet of the species, six were predominantly frugivores, five were carnivores, three were herbivores, two were insectivores, and one was myrmecophagous. Most of the species collected exhibit solitary behavior (13 species), while four have a gregarious habit.

The majority of the animals were collected in autumn (34.85%), followed by spring (28.79%), winter (25.76%), and summer (10.61%). Of the collected animals, 58.54% were male; 57.32% were adults, 36.59% young, and 6.10% infants. The cause of death for 75.61% of the collected animals was a single contusion. Only 3.85% of the roadkill sites had signs indicating the presence of wild animals on the highway and 53.25% had speed limit signs, considering a 2 km radius for both variables.

Regarding the transects, 15 animals were collected in T1, nine in T2, four in T3, and none in T4. Most of the animals, 57.58% (N = 38), were collected outside transects, mainly due to communication between co-participating police battalions and project team members. The rate of wild animals with a medium or large size killed by collision with vehicles was 0.231 animals/100 km/year in T1, 0.138 in T2, 0.081 in T3, and 0.0541 in T4. When considering all animals collected (within and outside transects), the Kernel map indicates the highest intensity of registers in the Londrina municipality (Figure 3). However, when considering only registers within transects (excluding those outside the transects), the highest intensity of registers in the Kernel occurred in Mauá da Serra municipality (Figure 4).

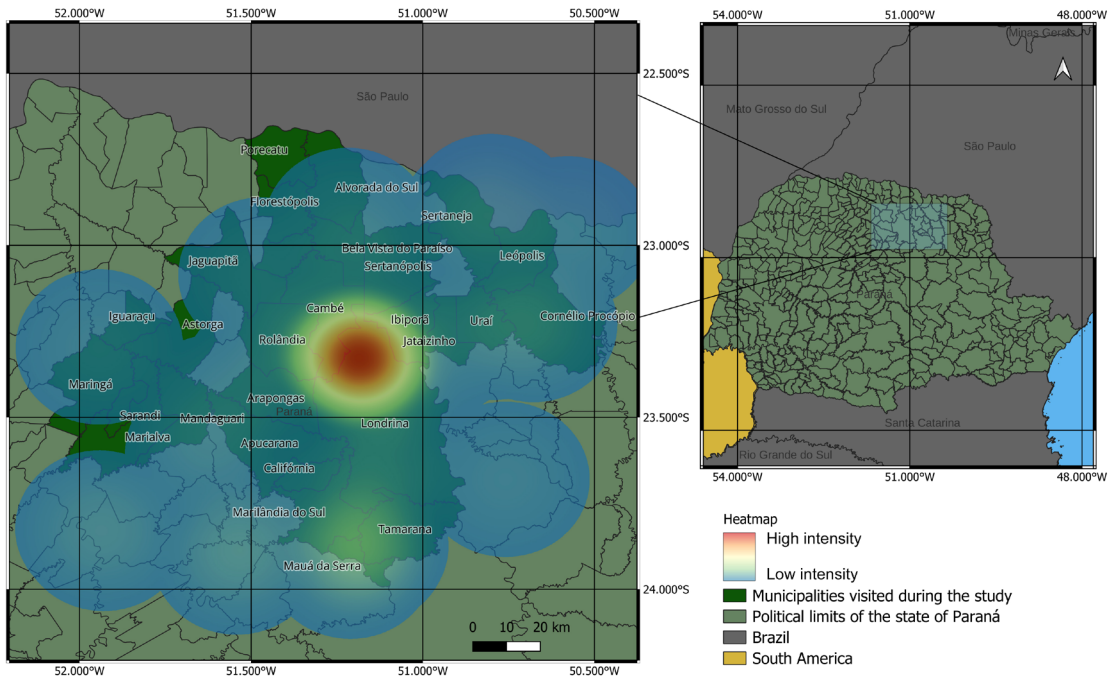


Figure 3. Kernel map demonstrating the concentration of road-killed wild animals collected in 24 municipalities of the state of Paraná, from November/2016 to October/2018, considering registers within transects and outside of transects (OOT).

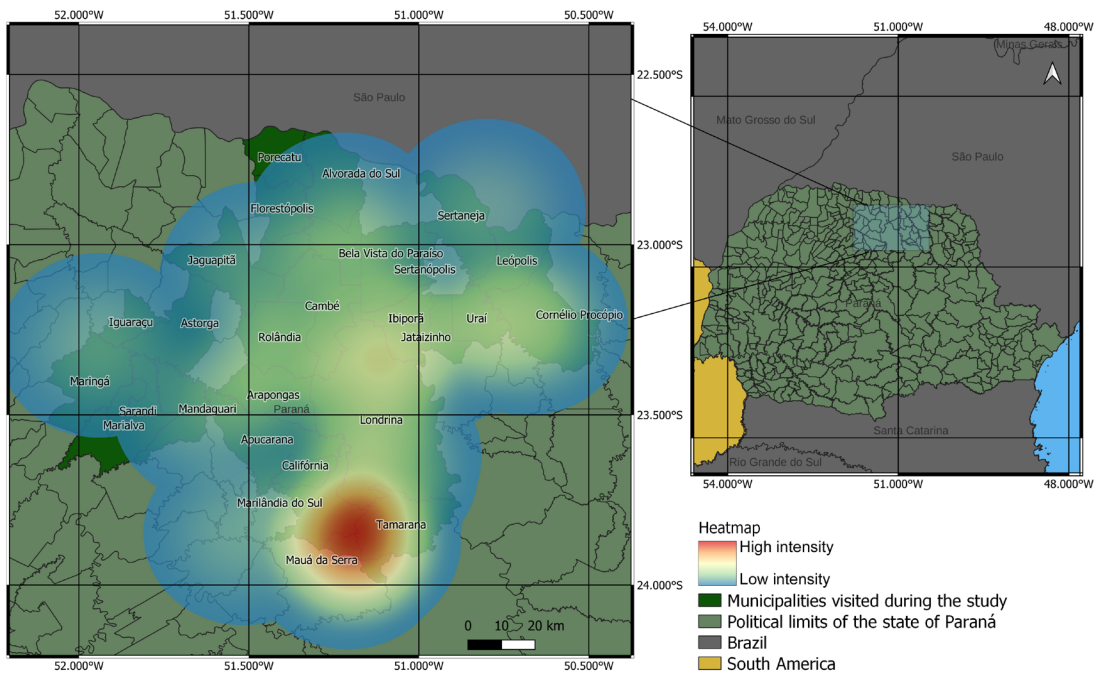


Figure 4. Kernel map demonstrating the concentration of road-killed wild animals collected in 24 municipalities of the state of Paraná, from November/2016 to October/2018, considering only registers within the transects.

In an attempt to understand whether differences in habits possibly influence killing rates, no significant differences were observed between the groups for locomotion (chi-squared = 3.1478, df = 4,

p-value = 0.5334), daily activity (chi-squared = 3.2507, df = 4, p-value = 0.5168), diet (chi-squared = 7.5967, df = 6, p-value = 0.2692), and composition (chi-squared = 1.3657, df = 1, p-value = 0.2425) (Figure 5).

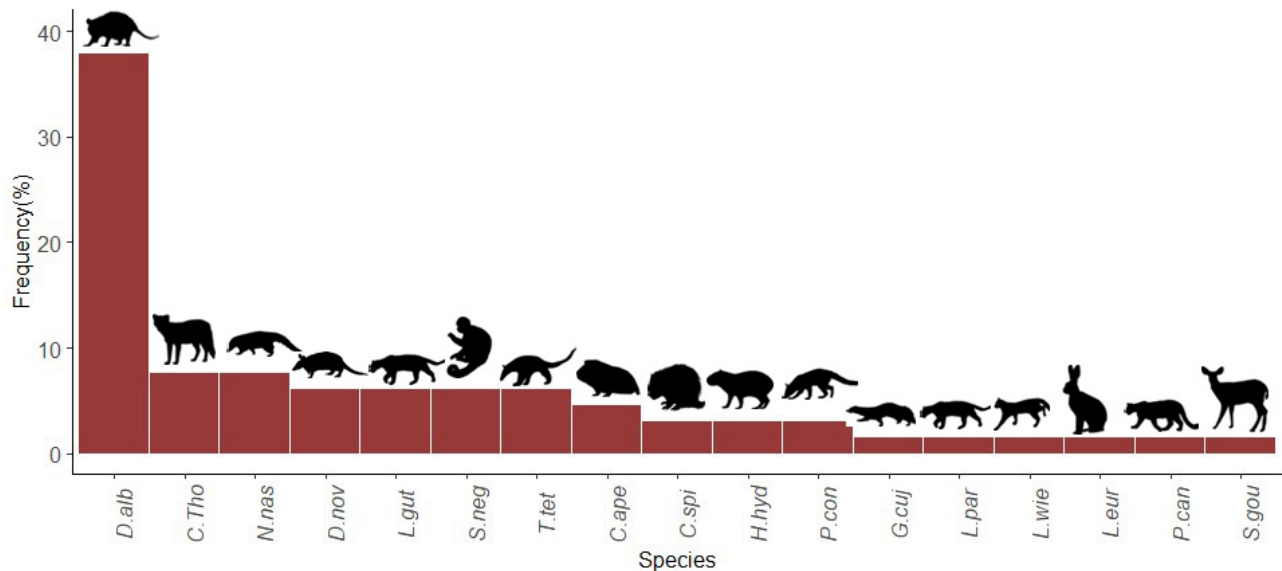


Figure 5. Frequency of medium and large-sized mammals killed by collision in the Northern state of Paraná.

Fragmentation of habitats is one of the major causes of species loss and decline. The construction of roads fragments habitats and creates obstacles to the natural movement of animals (Bischof et al., 2017). Many stretches were built or duplicated without the provision of wildlife passages and fences to guide and facilitate animal movement, as observed by Plante et al. (2019), which compromises the long-term persistence of populations.

The number of medium and large-sized mammal species recorded was similar to that observed in other studies, such as those by Zanzini et al. (2018), Pereira et al. (2021), and Ferreguetti et al. (2020). However, studies encompassing larger temporal and spatial scales registered a significantly greater number of species. For example, Abra et al. (2021) documented 32 species of road-killed animals in Sao Paulo State, between the years of 2009 and 2014.

Among the species detected, there was no distinction in collision rates with patterns of locomotion, activity, diet, and taxonomic composition, mirroring findings observed by Pereira et al. (2021). In other words, collisions occurred randomly, regardless of the animals' activities and behavioral patterns. Animals struck by cars may predominantly belong to generalist species (Abra et al., 2021), due to their larger trophic range and ability to explore a wider variety of environments, resulting in greater proximity to the road when foraging for anthropogenic resources left by drivers (Vizcaíno et al., 2022). In the state of Rio de Janeiro, arboreal mammals tolerant of human disturbance, such as opossums and some primates, were also the most abundant species (Secco et al., 2023). In the state of Espírito Santo, Ferregueti et al. (2020) also found that generalist species of the genus *Didelphis* were the most frequently run over, consistent with the findings of Vizcaíno et al. (2022) in a compilation of 85 articles on bird and mammal roadkill, in which the order *Didelphidia* was the most common mammal order found.

The mortality of species that are highly endangered and vulnerable to extinction or those with declining populations, such as the medium-sized felids, *L. wiedii* and *L. guttulus*, and the large felid, *P. concolor*, is worrisome (MMA, 2022; IUCN, 2023). *Puma concolor* needs a large home range size, associated with territoriality, feeding, and reproduction (Nielsen et al., 2015). The occurrence of this species in the current work reinforces that some have higher requirements from the environment, being sensitive to modifications in the landscape

structure (Pereira et al., 2021). In addition, the state of Paraná proposed, by decree, a Conservation Program for large felid species (*Panthera onca* and *Puma concolor*) (Decreto Nº 6.040/2024 [PARANÁ, 2024]), reinforcing the importance for conservation strategies. Considering smaller and endangered felid species, *Leopardus wiedii* and *L. guttulus* have low reproduction rates and can suffer due to competition with other conspicuous felid species, such as the ocelot (*L. pardalis*), known as the "ocelot effect" (Oliveira et al., 2015, Oliveira et al., 2016).

We detected low rates of roadkill compared to other locations, such as those reported by Zanzini et al. (2018) (roadkill rate of 0.009 per km) but similar to those observed by Secco et al. (2023) (roadkill rates between 0.0002 and 0.025 per km). This result may have been underestimated because we did not collect information about inviable carcasses 264 (330-66), which could compromise the calculation of the rates. This discrepancy may also be related to the low identification rate of deteriorated animals and the infrequent transect visits (once a month), or because smaller species may die instantly, resulting in a higher registration rate, while larger species may sustain injuries and continue moving, dying nearby (Bruinderink & Hazebroek, 1996). Additionally, high road temperatures, the presence of carnivores, traffic, and climate can contribute to the rapid degradation of carcasses, leading to their swift disappearance (Bager & Rosa, 2011; Santos et al., 2011).

The south transect (T1) had the highest roadkill rate. This transect passes through the municipalities of Londrina, California, and Mauá da Serra, an area with

a large human population and intense traffic flow. However, Londrina municipality had the highest intensity of registers outside the transects, possibly related to the proximity to the starting point of the trips and to the laboratory.

The T1 borders some important forest fragments, such as State Park Mata dos Godoy, a protected area covering more than 690 hectares of preserved semideciduous stational forest, along with several privately protected areas located in Mauá da Serra. This result was also observed by Pereira et al. (2021), and urgent policies are needed to reduce these rates of roadkill in the region.

Conclusion

From the data found, it can be concluded that there was high diversity of species killed on the road. However, no correlations were observed between the rate of roadkill and the functional traits of the mammal species collected in the study.

The construction of roads in Paraná State has led to significant habitat fragmentation, posing a threat to the populations of many medium and large mammal species. The mortality of endangered species, such as *L. wiedii*, *L. guttulus*, and *P. concolor* remains a concern. These findings underscore the urgent need for public policies to alleviate the impact of road construction on wildlife in the state of Paraná, including the construction of wildlife crossings and other mitigation measures in critical areas for animal survival.

Acknowledgments

The Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for granting a doctoral scholarship to GBM; and the commanders of the 2nd Company of Environmental Police and the 2nd Company of Traffic Police.

References

- Abra, F. D., Huijser, M. P., Magioli, M., Bovo, A. A., & Ferraz, K. M. P. M. de B. (2021). An estimate of wild mammal roadkill in São Paulo state, *Brazil. Heliyon*, 7(1), e06015. doi: 10.1016/j.heliyon.2021.e06 015
- Abreu, E. F., Casali, D., Costa-Araújo, R., Garbino, G. S. T., Libardi, G. S., Loretto, D., Loss, A. C., Marmontel, M., Moras, L. M., Nascimento, M. C., Oliveira, M. L., Pavan, S. E., & Tirelli, F. P. (2021). Lista de mamíferos do Brasil (2021-2). [Data set]. *Zenodo*. doi: 10.5281/zenodo.5802047
- Ascensão, F., Desbiez, A. L. J., Medici, E. P., & Bager, A. (2017). Spatial patterns of road mortality of medium-large mammals in Mato Grosso do Sul, Brazil. *Wildlife Research*, 44(2), 135-146. doi: 10.1071/wr16108
- Bager, A. (2012). *Ecologia de estradas* (vol. 1). Empreendedor Acadêmico.
- Bager, A., & Fontoura, V. (2013). Evaluation of the effectiveness of a wildlife roadkill mitigation system in wetland habitat. *Ecological Engineering*, 53(1), 31-38. doi: 10.1016/j.ecoleng.2013.01.006
- Bager, A., & Rosa, C. A. da. (2011). Influence of sampling effort on the estimated richness of road-killed vertebrate wildlife.

- Environmental Management*, 47(5), 851-858. doi: 10.1007/s00267-011-9656-x
- Bischof, R., Steyaert, S. M. J. G., & Kindberg, J. (2017). Caught in the mesh: roads and their network-scale impediment to animal movement. *Ecography*, 40(12), 1369-1380. doi: 10.1111/ecog.02801
- Bogoni, J. A., Pires, J. S. R., Graipel, M. E., Peroni, N., & Peres, C. A. (2018). Wish you were here: how defaunated is the Atlantic Forest biome of its medium- to large-bodied mammal fauna? *Plos One*, 13(9), e0204515. doi: 10.1371/journal.pone.0204515
- Bruinderink, G. W. T. A. G., & Hazebroek, E. (1996). Ungulate traffic collisions in Europe. *Conservation Biology*, 10(4), 1059-1067. doi: 10.1046/j.1523-1739.1996.10041059.x
- Caldart, E. T., Pinto-Ferreira, F., Matos, A. M. R. N. de, Pascoal, A. T. P., Bertão-Santos, A., Mitsuka-Breganó, R., & Navarro, I. T. (2021). Evaluation of an active and early surveillance methodology for visceral leishmaniasis by molecular detection in road-killed wild fauna. *Revista Brasileira de Parasitologia Veterinária*, 30(2), 1-11. doi: 10.1590/s1984-29612021026
- Centro Brasileiro de Ecologia de Estradas (2020). CBEE. <https://ecoestradas.com.br/>
- Confederação Nacional do Transporte (2022). Anuário do CNT Transporte. CNT. <https://anuariodotransporte.cnt.org.br/2022/Rodoviario/1-3-1-1-1-/Inicial>
- Decreto Nº 6.040/2024 de 05 de junho. *Diário Oficial do Estado do Paraná n.º 11673*. Governo do Estado do Paraná.
- Departamento de Estradas de Rodagem (2021). *Sistema Rodoviário Estadual 2020*. DER. <https://www.der.pr.gov.br>
- Fahrig, L., & Rytwinski, T. (2009). Effects of roads on animal abundance: an empirical review and synthesis. *Ecology and Society*, 14(1), 1-21. doi: 10.5751/es-02815-140121
- Ferreguetti, Á. C., Graciano, J. M., Luppi, A. P., Pereira-Ribeiro, J., Frederico, C., & Bergallo, H. G. (2020). Roadkill of medium to large mammals along a Brazilian road (BR-262) in Southeastern Brazil: spatial distribution and seasonal variation. *Studies on Neotropical Fauna and Environment*, 55(3), 216-225. doi: 10.1080/01650521.2020.1719006
- Graipel, M. E., Cherem J. J., Monteiro, E. L. A., Jr., & Carmignotto, A. P. (2017). Mamíferos da Mata Atlântica. In E. L. A. Monteiro F^o, & C. E. Conte (Orgs.), *Revisões em zoologia: Mata Atlântica* (pp. 391-482). Curitiba.
- Grilo, C., Koroleva, E., Andrášik, R., Bíl, M., & González-Suárez, M. (2020). Roadkill risk and population vulnerability in European birds and mammals. *Frontiers in Ecology and the Environment*, 18(6), 323-328. doi: 10.1002/fee.2216
- Instituto Brasileiro de Geografia e Estatística (2023). *Censo demográfico 2022: população e domicílios: primeiros resultados*. IBGE.
- International Union for Conservation of Nature (2022). *The IUCN red list of threatened species*. IUCN. <https://www.iucnredlist.org>
- Laurance, W. F., Clements, G. R., Sloan, S., O'Connell, C. S., Mueller, N. D., Goosem, M., Venter, O., Edwards, D. P., Phalan, B., Balmford, A., Van Der Ree, R., & Arrea, I. B. (2014). A global strategy for road building. *Nature*, 513(7517), 229-232. doi: 10.1038/nature13717

- Laurance, W. F., Goosem, M., & Laurance, S. G. W. (2009). Impacts of roads and linear clearings on tropical forests. *Trends in Ecology & Evolution*, 24(12), 659-669. doi: 10.1016/j.tree.2009.06.009
- Nielsen, C., Kelly, M., Lopez-Gonzalez, C., & Thompson, D. (2015). *IUCN red list of threatened species: puma concolor*. IUCN. <https://www.iucnredlist.org/species/18868/97216466>
- Oliveira, T., Paviolo, A., Schipper, J., Bianchi, R., Payan, E., & Carvajal, S.V. (2015). *Leopardus wiedii*. *The IUCN Red List of Threatened Species 2015*: e.T11511A50654216. doi: 10.2305/IUCN.UK.2015-4.RLTS.T11511A50654216.en
- Oliveira, T., Trigo, T., Tortato, M., Paviolo, A., Bianchi, R. de C., & Pitman, R. L. (2016). *Leopardus guttulus*, Margay. *The IUCN Red List of Threatened Species 2015*: e.T11511A50654216. doi: 10.2305/IUCN.UK.2015-4.RLTS.T11511A50654216.en
- Paglia, A. P., Fonseca, G. A. B. da, Rylands, A. B., Herrmann, G., Aguiar, L. M. S., Chiarello, A. G., Leite, Y. L. R., Costa, L. P., Siciliano, S., Kierulff, M. C. M., Mendes, S. L., Tavares, V. da C., Mittermeier, R. A., & Patton J. L. (2012). *Annotated checklist of Brazilian mammals* (2nd ed., n.6). Occasional Papers in Conservation Biology, Conservation International.
- Pereira, A. D., Hideki, M., Geller, I. V., Lehn, C. R., Vidotto-Magnoni, A. P., Bogoni, J. A., & Orsi, M. L. (2021). Don't speed up, speed kills: mammal roadkills on highway sections of pr-445 in the south of Brazil. *Oecologia Australis*, 25(1), 34-46. doi: 10.4257/oeco.2021.2501.04
- Pinto, F. A. S., Cirino, D. W., Cerqueira, R. C., Rosa, C., & Freitas, S. R. (2022). How many mammals are killed on Brazilian roads? Assessing impacts and conservation implications. *Diversity*, 14(10), 835. doi: 10.3390/d14100835
- Plante, J., Jaeger, J. A. G., & Desrochers, A. (2019). How do landscape context and fences influence roadkill locations of small and medium-sized mammals? *Journal of Environmental Management*, 235(1), 511-520. doi: 10.1016/j.jenvman.2018.10.093
- Portaria MMA no 148, de 7 de junho de 2022. *Ministério do Meio Ambiente* (2022). https://www.icmbio.gov.br/cepsul/images/stories/legislacao/Portaria/2020/P_mma_148_2022_altera_anexos_P_mma_443_444_445_2014_atualiza_especies_ameacadas_extincao.pdf
- Pugliares, K. R., Bogomolni, A. L., Touhey, K. M., Herzig, S. M., Harry, C. T., & Moore, M. (2007). *Marine mammal necropsy: an introductory guide for stranding responders and field biologists*.
- QGIS Development Team (2023). *Open source geospatial foundation project*. QGIS Geographic Information System. <http://qgis.osgeo.org>
- R Core Team (2023). *R: A language and environment for statistical computing*. R Foundation
- Reis, N. R., Peracchi, A. L., Pedro, W. A., & De, I. P. (2011). *Mamíferos do Brasil*. Edur.
- Roderjan, C., Galvão, F., Kuniyoshi, Y. S., & Hatschbach, G. G. (2002). As unidades fitogeográficas do Estado do Paraná. *Ciência & Ambiente*, 24, 75-92.

- Sadleir, R. M., & Linklater, W. L. (2016). Annual and seasonal patterns in wildlife road-kill and their relationship with traffic density. *New Zealand Journal of Zoology*, 43(3), 275-291. doi: 10.1080/03014223.2016.1155465
- Santos, S. M., Carvalho, F., & Mira, A. (2011). How Long Do the Dead Survive on the Road? Carcass Persistence Probability and Implications for Road-Kill Monitoring Surveys. *PLoS One*, 6(9), e25383-e25383. doi: 10.1371/journal.pone.0025383
- Secco, H., Farina, L. F., Oliveira, V., Beiroz, W., Guerreiro, M., & Gonçalves, P. R. (2023). Identifying roadkill hotspots for mammals in the Brazilian Atlantic Forest using a functional group approach. *Environmental Management*, 73(2), 365-377. doi: 10.1007/s00267-023-01844-7
- Secco, H., Melo, F. R., Talebi, M., Gordo, M., Morais, M. M., Jr., & Bager, A. (2018). Diagnóstico dos impactos de rodovias sobre primatas no Brasil. In A. Bager (Org.), *Infraestrutura viária & biodiversidade: métodos e diagnósticos* (pp. 35-40). Lavras. https://www.researchgate.net/publication/330292109_Diagnostico_dos_impactos_de_rodovias_sobre_primatas_no_Brasil
- Silva, A. C. dos S., Paschoal, A. T. P., Bernardes, J. C., Matos, A. M. R. N. de, Balbino, L. S., Santomauro, R. A., Viana, J. G. N., Caldart, E. T., Lacerda, L. H., Oliveira, C. de, Chrysafidis, A. L., Garcia, J. L., Navarro, I. T., Mitsuka-Breganó, R., & Pinto-Ferreira, F. (2021). Parasites in road-killed wild felines from North of Paraná state, Brazil. *Revista Brasileira de Parasitologia Veterinária*, 30(1), e016320. doi: 10.1590/S1984-296120201090
- Souza, Y., Gonçalves, F., Lautenschlager, L., Akkawi, P., Mendes, C., Carvalho, M. M., Bovendorp, R. S., Fernandes-Ferreira, H., Rosa, C., Graipel, M. E., Peroni, N., Cherem, J. J., Bogoni, J. A., Brocardo, C. R., Miranda, J., Zago da Silva, L., Melo, G., Cáceres, N., Sponchiado, J., & Ribeiro, M. C. (2019). Atlantic Mammals: a data set of assemblages of medium- and large-sized mammals of the Atlantic Forest of South America. *Ecology*, 100(10), e02785. doi: 10.1002/ecy.2785
- VanderRee, R., Gagnon, J. W., & Smith, D. (2015). Fencing. *Handbook of Road Ecology*, (1), 159-171. doi: 10.1002/9781118568170.ch20
- Vizcaíno, P. M., Grilo, C., Campos, F., Douglas, W., Ramiro Dario Melinski, Schultz, E. D., & González-Suárez, M. (2022). Roadkill patterns in Latin American birds and mammals. *Global Ecology and Biogeography*, 31(9), 1756-1783. doi: 10.1111/geb.13557
- Wilman, H., Belmaker, J., Simpson, J., Rosa, C. de la, Rivadeneira, M. M., & Jetz, W. (2014). EltonTraits 1.0: species-level foraging attributes of the world's birds and mammals. *Ecology*, 95(7), 2027-2027. doi: 10.1890/13-1917.1
- Zanzini, A. C. da S., Machado, F. S., Oliveira, J. E. de, & Oliveira, E. C. M. de. (2018). Roadkills of medium and large-sized mammals on highway BR-242, Midwest Brazil: a proposal of new indexes for evaluating animal roadkill rates. *Oecologia Australis*, 22(3), 248-257. doi: 10.4257/oeco.2018.2203.04