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Article

Danger under wheels: mammal roadkills in the threaten lowland Atlantic Forest in southeast Brazil

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ABSTRACT. Understanding the ecological impacts of roads on mammals requires periodic monitoring of roads, and identification of both temporal and spatial distribution of roadkills (i.e., roadkill hotspots). The main aim of the study was to identify the most roadkilled mammals and evaluate the temporal and spatial distribution of roadkills in the RJ-122, a highway that crosses the threaten lowland Atlantic Forest in the state of Rio de Janeiro, southeast Brazil. Between October 2017 and January 2020, an intense monitoring study was conducted, with the carcasses of the roadkilled mammals being collected three times a week. Overall, we recorded 295 roadkilled mammals belonging to 22 species, resulting in 11 roadkills per month for the RJ-122 highway. The black-eared opossum, *Didelphis aurita* (N= 149, 51%) stood out as the most roadkilled mammal, followed by porcupine, *Coendou spinosus* (N= 24, 8%), crab-eating fox, *Cerdocyon thous* (N= 23, 8%), nine-banded armadillo, *Dasypus novemcinctus* (N= 23, 8%), and white-tufted marmoset, *Callithrix jacchus* (N= 20, 7%). Roadkills on the RJ-122 varied throughout the year, being more frequent in the rainy season (N= 180) than in the dry season (N= 115), and were concentrated in two hotspots, indicating some critical points with high roadkill frequency. Hotspots were associated with areas with dense natural vegetation, which can function as forest corridors in this fragmented landscape. Based on our results, several mitigation measures are recommended for the RJ-122 highway.

KEYWORDS. Animal-vehicle collision; roadkill mitigation; road ecology; spatial pattern.

RESUMO. Perigo sob rodas: atropelamentos de mamíferos na ameaçada Mata Atlântica de baixada no sudeste do Brasil. Compreender os impactos ecológicos das estradas sobre a fauna de mamíferos requer o monitoramento periódico das estradas e a identificação da distribuição temporal e espacial dos atropelamentos (ou seja, *hotspots* de atropelamentos). O objetivo principal do estudo foi identificar os mamíferos mais atropelados e avaliar a distribuição temporal e espacial dos atropelamentos na RJ-122, uma rodovia que atravessa a ameaçada Mata Atlântica de baixada no estado do Rio de Janeiro, sudeste do Brasil. Entre outubro de 2017 e janeiro de 2020, foi realizado um intenso estudo de monitoramento, com a coleta três vezes por semana das carcaças dos mamíferos atropelados. Ao todo, registramos 295 mamíferos atropelados pertencentes a 22 espécies, resultando em 11 atropelamentos por mês para a rodovia RJ-122. O gambá-de-orelha-preta, *Didelphis aurita* (N= 149, 51%) destacou-se como o mamífero mais atropelado, seguido pelo ouriço-cacheiro, *Coendou spinosus* (N= 24, 8%), cachorro-do-mato, *Cerdocyon thous* (N= 23, 8%), tatu-galinha, *Dasypus novemcinctus* (N= 23, 8%) e sagui-de-tufo-branco, *Callithrix jacchus* (N= 20, 7%). Os atropelamentos na RJ-122 variaram ao longo do ano, sendo mais frequentes na estação chuvosa (N= 180) do que na estação seca (N= 115), e se concentraram em duas áreas, indicando alguns pontos críticos com alta frequência de atropelamentos. Os *hotspots* foram associados a áreas com vegetação natural densa, que podem funcionar como corredores florestais nesta paisagem fragmentada. Com base em nossos resultados, várias medidas de mitigação são recomendadas para a rodovia RJ-122.

PALAVRAS-CHAVE. Colisão animal-veículo; mitigação de atropelamentos; ecologia de estradas; padrão espacial.

Every second approximately 15 wild animals die on Brazilian roads, and these numbers can reach 1.3 million per day and surpasses 475 million per year (CBEEE, 2020). Nonetheless, the actual number can be even higher since several fatalities are not registered and road impacts go beyond wildlife-vehicle collisions (Van Der Ree *et al.*, 2015). Roads can break habitats apart by reducing their amount, configuration, and quality (Fahrig, 2003); act as barriers to dispersal (Parris & Schneider, 2008; Ware *et al.*, 2015); limit gene flow (Ascensão *et al.*, 2017); affect individual survival, potentially reducing the size of natural populations, and affecting their long-term persistence (Fahrig, 2003; Bueno *et al.*, 2013); and provide humans easy access to previously out of reach areas, thus increasing negative pressure on an already impacted wildlife (Laurance *et al.*, 2009).

Roads have major contribution to the high levels of biodiversity loss worldwide, and roadkills are among the leading causes of direct death of wild vertebrates, surpassing impacts generated by hunting and mortality rates from natural causes (Van Der Ree *et al.*, 2015). Due to the great urgency and growth of the road network worldwide, roadkills and other direct and indirect impacts of roads have received attention in many studies (Bager & Rosa, 2010; Cunha *et al.*, 2010; Santana, 2012; Almeida *et al.*, 2013; Vélez, 2014; Costa *et al.*, 2022). Among the various threats to wildlife are, in addition to collisions with vehicles, the increase in the level of air and noise pollution, increase in temperature, and the emergence of urban settlements on the edge of roads (Bager & Rosa, 2010; Vélez, 2014).

Wildlife roadkills do not occur randomly (Filius *et al.*, 2020). Several factors favor certain species to be roadkilled more often than others, such as biological (body size and diet) (Barthelmess & Brooks, 2010) and landscape characteristics, and the road itself (Bueno *et al.*, 2013, 2015), as well as seasonal variations in temperature and rainfall (Bueno & Almeida, 2010; Santos *et al.*, 2011; Santana, 2012). The vehicular traffic, as well as the vehicle speed, are also important determining characteristics that lead to the collision of the vehicle with the animal (Cunha *et al.*, 2010; Lester, 2015). For mammals, body size has a major influence on roadkills (Barthelmess & Brooks, 2010; Carvalho *et al.*, 2015; Chen & Koprowski, 2019). Small mammals, such as rodents and marsupials, are especially vulnerable because drivers do usually not see them when they cross the road, or when they are in areas surrounding road edges (Laurance *et al.*, 2009; Bueno & Almeida, 2010; Carvalho *et al.*, 2015). Medium and large-sized mammals, on the other hand, require more energy to meet their needs, which results, for many species, in an increase in movement rates in search of food and reproductive partners (Bueno & Almeida, 2010). By moving larger areas, they become more vulnerable to collisions with vehicles.

Although the high rate of roadkills in Brazilian roads is widely discussed in many studies over the years (*e.g.*, Abra *et al.*, 2019, 2021), there is still a paucity of accurate information on the spatial and temporal distribution of roadkills. Understanding the dynamics of wildlife-vehicle collisions allows us to indicate possible solutions to increase safety on the roads, reduce the impacts on humans and wildlife, reduce costs, and invest in mitigation measures aimed at conservation of biodiversity (Rytwinski *et al.*, 2016; Abra *et al.*, 2019).

The objective of this study was to evaluate the impact of roadkill on wild mammals on RJ-122, a highway that crosses the threaten lowland Atlantic Forest in southeast Brazil. Specifically, we (1) identified the most roadkilled mammals between October 2017 and January 2020, (2) evaluated the temporal distribution and the influence of climate seasonality (dry and rainy seasons) on roadkills, and (3) identified whether roadkills are spatially clustered forming roadkill hotspots. We expect (1) the black-eared opossum (*Didelphis aurita*, Wied-Neuwied, 1826) and the crab-eating fox [*Cerdocyon thous*, (Linnaeus, 1766)] to be the most roadkilled species, based on previous studies in Brazil (Coelho *et al.*, 2008; Bueno & Almeida, 2010; Cunha *et al.*, 2010; Zanzini *et al.*, 2018); (2) high roadkill numbers in the dry season (Bueno & Almeida, 2010); and, (3) hotspots located near forested areas, because of the species habits registered in the Atlantic Forest (Paglia *et al.*, 2012).

MATERIAL AND METHODS

Study area. We carried out the monitoring study in the RJ-122 highway, from Km 1 to Km 34, between the municipalities of Guapimirim (UTM 23K 708028/7507913) and Cachoeiras de Macacu (UTM 23K 736352/7507913), in the state of Rio de Janeiro, southeastern Brazil. The highway is immersed in a fragmented landscape of lowland Atlantic Forest, with stretches of native vegetation interspersed by rural and residential properties along the highway. The highway includes two paved lanes, with an average width of 3.5 m each, and a maximum speed of 80 km/h (DER-RJ, 2013). The study area is characterized by a hot and humid tropical climate, with a rainy season from November to April, and a less rainy season from May to October during the study period (INMET, 2021).

Sampling method. We sampled roadkilled mammals three times a week between October 2017 and January 2020. Sampling was not performed in May, October and December of 2019. Roadkilled mammals were identified and collected by car or motorcycle on estimated average speed of 40 km/h by a trained biologist of the NGO SOS Vida Silvestre. For each record, a field form was filled out with the following information: geographic location, kilometer and direction of the road, taxonomic group, and sex, if possible. Fortnightly the specimens collected were taken to the Laboratory of Ecology of the Veiga de Almeida University (RJ), identified, and prepared as study skins, skeletons, or stored whole in the spirits collection depending on the conditions of the material. Species were deposited in the National Museum of Rio de Janeiro collection.

The carcasses of the animals used in this study comply with and are in accordance with the SISBIO License # 30727-9.

Data analysis. We compiled a list of mammal species roadkilled, indicating threatening status at national (ICMBio, 2018) and global (IUCN, 2021) levels, the number of records for each species, and their roadkill rate. The roadkill rate was calculated by dividing the number of recorded individuals/ species by the length of the road (34 km) and the total number of days sampled (N = 165). The roadkill rate was correlated with the climatic season (dry and wet seasons) using the Chi-squared test, in order to assess if seasonality was one of the factors influencing the rate of roadkill. In addition, the Factorial Correspondence analysis was applied to investigate the association between the five most-roadkilled mammals and the months they were recorded. Analyses were conducted in the R environment, version 3.6.1 (R CORE TEAM, 2019).

To evaluate the spatial distribution of mammal roadkill hotspots, we used the heatmap plug-in in QGIS version 3.10.6 (QGIS DEVELOPMENT TEAM, 2019), which uses kernel density estimation, to create a heat map (i.e., a density map) of roadkills. The Kernel density is estimated based on the number of collisions per kilometer of road, with larger numbers of clustered points resulting in larger values. Point density is calculated by adding the values of all overlapping kernel surfaces within any cell of determining size. The bandwidth of the kernel exhibits a strong influence on the estimation results, since it determines the search radius in which roadkills will contribute to the hotspot identification (BÍL *et al.*, 2013). We used a search radius of 100 m to investigate collision clusters, which proved to be appropriate for the region analyzed. To model kernel shape, we used a quartic kernel shape function in the heatmap plug-in (QGIS DEVELOPMENT TEAM, 2019).

RESULTS

We recorded 295 roadkilled mammals belonging to 22 species, resulting in 0.32 recorded individuals per kilometer per month and 11 roadkills per month for the RJ-122 highway (Tab. I). Of the 295 records, 286 were identified at the species level; the other nine could not be identified due to the poor state of conservation. The black-eared opossum **(***Didelphis aurita***)** stood out as the most roadkilled species (N = 149, 51%), followed by the porcupine, *Coendou spinosus* (F. Cuvier, 1823) (N = 24, 8%), the crab-eating fox, *Cerdocyon thous* (N = 23, 8%), the nine-banded armadillo, *Dasypus novemcinctus* Linnaeus, 1758 (N = 23, 8%), and the white-tufted marmoset, *Callithrix jacchus* (Linnaeus, 1758) (N = 20, 7%). These five species concentrated more than 80% of the records, and the opossum alone was responsible for approximately nine roadkills per month. Rodents and bats were the two groups with the highest number of roadkilled species, a total of five each (Tab. I).

Tab. I. Roadkilled mammals along the RJ-122 highway between October 2017 and January 2020, in the state of Rio de Janeiro, Brazil. N = Number of individuals, Roadkill rate = number of individuals/km/day, and Threat status = status of threat of extinction according to IUCN (LC - Least Concern, EN - Endangered) and national list (Vu C1- Vulnerable to extinction, with population numbers declining). Roadkill rates less than 0.001 were designated as 0.

Order	Species	Common name	N	Roadkill rate	Threat status
Carnivora	*Cerdocyon thous* (L., 1766)	Crab-eating fox	23	0.004	LC
	Galictis cuja (Molina, 1782)	Lesser grison	7	0.001	LC
	Herpailurus yagouaroundi (Geoffroy, 1803)	Jaguarundi	1	0	VU C1
	Procyon cancrivorus (G. Cuvier, 1798)	Crab-eating raccoon	2	0	LC
Chiroptera	*Artibeus (Artibeus) lituratus* (Olfers, 1818)	Great fruit-eating Bat	1	0	LC
	Artibeus (Artibeus) fimbriatus Gray, 1838	Fringed fruit-eating bat	1	0	LC
	Artibeus sp.		1	0	LC
	Carollia perspicillata (L., 1758)	Seba's short-tailed bat	4	0	LC
	Desmodus rotundus (Geoffroy, 1810)	Common vampire bat	2	0	LC
	Sturnira lilium (Geoffroy Saint-Hilaire, 1810)	Little yellow-shouldered bat	2	0	LC
	No identification		8	0.001	
Cingulata	*Cabassous unicinctus* (L., 1758)	Southern naked-tailed armadillo	3	0	LC
	Dasypus (Dasypus) novemcinctus L., 1758	Nine-banded armadillo	23	0.004	LC
	Dasypus septemcinctus (L., 1758)	Seven-banded armadillo	2	0	LC
Didelmorphia	*Didelphis aurita* (Wied-Neuwied, 1826)	Black-eared opossum	149	0.026	LC
Lagomorpha	*Sylvilagus brasiliensis* (L., 1758)	Tapeti	7	0.001	EN
Pilosa	*Tamandua tetradactyla* (L., 1758)	Southern tamandua	7	0.001	LC
Primata	*Callithrix jacchus* (L., 1758)	Common marmoset	20	0.003	LC
Rodentia	*Cavia aperea* Erxleben, 1777	Cavie	4	0	LC
	Coendou insidiosus (Olfers, 1818)	Porcupine	24	0.004	LC
	Cuniculus paca (L., 1766)		1	0	LC
	Hydrochoerus hydrochaeris (L., 1766)	Capybara	1	0	LC
	Sciurus aestuans L., 1766	Brazilian squirrel	1	0	LC
	No identification		1	0	

Two mammals are included in the national and global list of threatened species. The jaguarundi, *Herpailurus yagouaroundi* (É. Geoffroy, 1803) (N = 1), was classified as Vulnerable (VU) at the national list and as Least Concern (LC) at the global list. However, the current population trend indicates a decline in population numbers. The tapiti, *Sylvilagus tapetillus* Thomas, 1913 (N = 7), was classified as Vulnerable (VU) at both national and global lists.

The chi-square test revealed a seasonal pattern in roadkills at a significance level of 10% ($p = 0.077$; gl = 11; $\chi 2 = 18.19$), with high number of roadkills in the rainy season (from November to April; N = 180) than in the dry season (from May to October; N = 115). For the five most sampled species (*D. aurita*, *C. spinosus*, *C. thous*, *D. novemcinctus* and *C. jacchus*), there was a higher predominance of roadkills in the rainy season, except for *Cerdocyon thous* that had a higher frequency in the dry months. The months which the five most-roadkilled mammals were recorded differed among species: *Callithrix jacchus* roadkill numbers were higher in September, *Dasypus novemcinctus* in March, and *Cerdocyon thous* in the months of June and July (Fig. 1). No pattern was identified for the opossum, *D. aurita*, and the porcupine, *C. spinosus*.

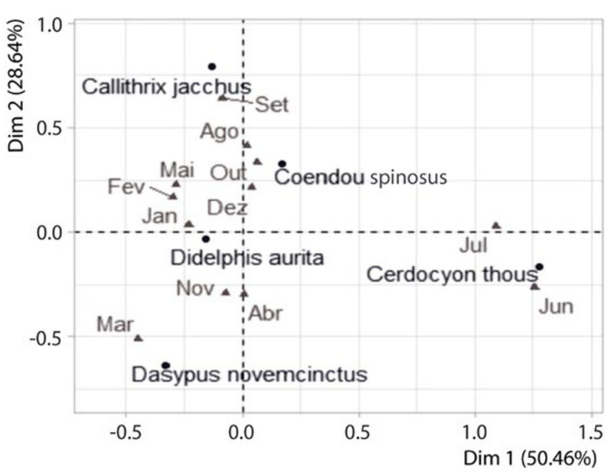

Fig. 1. Biplot of the five most roadkilled species of mammals (*Didelphis aurita*, *Coendou insidiosus*, *Cerdocyon thous*, *Dasypus novemcinctus* and *Callithrix jacchus*) along the RJ-122 highway between October 2017 and January 2020, in the state of Rio de Janeiro, Brazil. Circle: mammal species; Triangle: months of the year.

The distribution of mammals roadkills along the highway was not uniform, indicating some critical points with high roadkill frequency (Fig. 2). Overall, four points had a high roadkill rate: km 23 (N = 22), km 24 (N = 24), km 28 (N = 18) and km 29 (N = 22). Of these, two hotspots were identified in the hotspot map: km 23/24 and km 28/29 (Fig. 3).

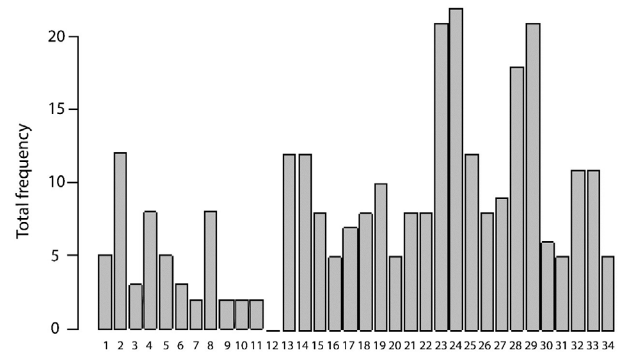

Fig. 2. Frequency of roadkill mammals over every kilometer along the RJ-122 highway between October 2017 and January 2020, in state of Rio de Janeiro, Brazil. Highlights are km 23, km 24, km 28 and km 29.

DISCUSSION

Mammal roadkills at the lowland Atlantic Forest was particularly high (0.32 roadkills per kilometer per month), when compared to other sites in Brazil (Coelho *et al.*, 2008; Cunha *et al.*, 2010). However, comparisons are difficult since this information is not always available or was estimated by different indices (Zanzini *et al.*, 2018). Most estimates are also available for the entire vertebrate clade, not for specific taxonomic groups, or as the number or record per kilometer, which is affected by the duration of the study. Coelho *et al.*, (2008) was one of the few studies that evaluated mammals (and other groups) separately. In this study, vertebrate roadkill rates varied from 0.21 to 0.46 roadkills/km/month in two roads in the sandy and wet restinga, important remnants of Atlantic Forest in the south of Brazil (Coelho *et al.*, 2008). The higher mammal roadkill rate was recorded in the summer (0.36 roadkills/km/month), followed by winter (0.35 roadkills/km/month), autumn (0.33 roadkills/km/month), and spring (0.30 roadkills/km/month). Overall, mammal roadkill numbers in RJ-122 is higher than the total mortality records for the vertebrate groups in other Brazilian roads (Cunha *et al.*, 2010).

As expected, the most roadkilled species was the marsupial, *Didelphis aurita*, which corresponded to more than half of the total number of records. The black-eared opossum is a habitat generalist, locally abundant, and highly mobile species (Cunha & Vieira, 2002; Prevedello & Vieira, 2009; Ferreira *et al.*, 2016). It inhabits forest fragments surrounding the highway (Vieira *et al.*, 2009) and cross fragments frequently in search for new areas, food, or mates (Prevedello & Vieira, 2009). *Didelphis aurita* is probably the most roadkilled mammal in the Atlantic Forest (Bueno & Almeida, 2010), but other species of *Didelphis* are also frequently recorded in road surveys probably due to the high tolerance to anthropized areas (Smith & Dodd Jr, 2003; Pinowski, 2005; Coelho *et al.*, 2008). These high roadkill numbers may affect important ecological functions performed

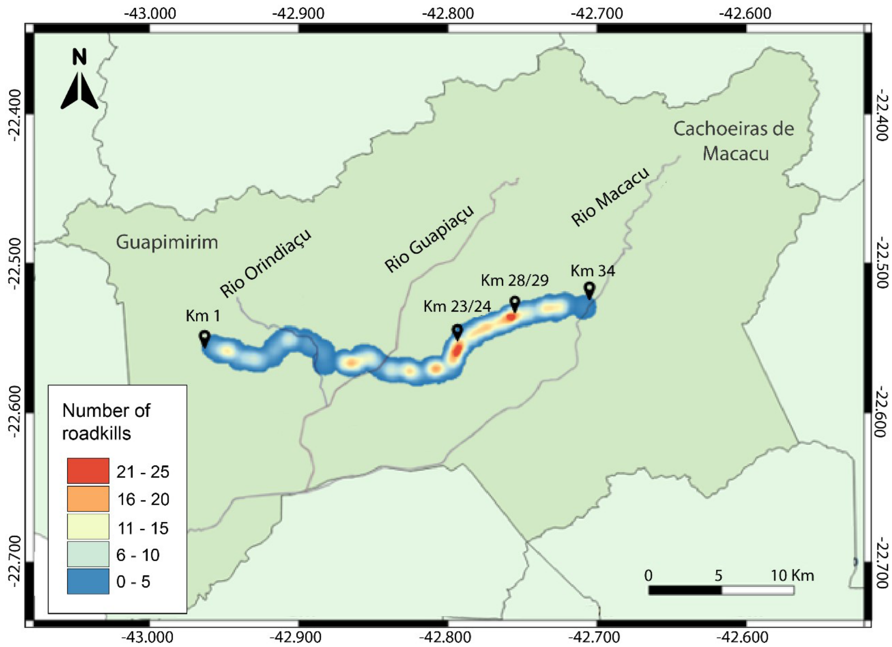

Fig. 3. Roadkill hotspots of mammal species recorded along the RJ-122 highway (from Km 1 to Km 34) between October 2017 and January 2020, in the state of Rio de Janeiro, Brazil. The largest number of roadkills is concentrated in two regions (black location icon), Km 23/24 (N = 22 and N = 24) and Km 28/29 (N = 18 and N = 22).

by these species, such as seed dispersal, regulation of prey population, and pest control (Cáceres & Lessa, 2012).

The porcupine (*Coendou spinosus*), the nine-banded armadillo (*Dasypus novemcinctus*), and the crab-eating fox (*Cerdocyon thous*) had similar mortality numbers, but only *C. thous* is frequently recorded in roadkill surveys (Coelho *et al.*, 2008; Freitas *et al.*, 2015; Orlandin *et al.*, 2015; Cirino *et al.*, 2022; Costa *et al.*, 2022). As *D. aurita*, this medium-sized, insectivorous/omnivorous canid has opportunistic habits, and occupies forests, savannas, and disturbed areas such as cultivated fields, forest fragments, and areas around human settlements (Eisenberg & Redford, 1999). In the Atlantic Forest, the crab-eating fox most commonly inhabiting forested and edge areas, but can also use altered environments (Ferraz *et al.*, 2010; Magioli *et al.*, 2014; Monteiro-Alves *et al.*, 2019). In a nearby highway (BR-040) in the state of Rio de Janeiro, *C. thous* roadkills were more likely to occur near areas with low percentage of crop fields, herbaceous vegetation, and urban areas, reinforcing the forest habits of the species in the region (Costa *et al.*, 2022). However, the high roadkill numbers can also be a consequence of the consumed carcasses of other roadkilled animals found on the side of the road (Gumier-Costa & Sperber, 2009; Carvalho, 2015; Orlandin *et al.*, 2015). This is an extraordinary type of food chain which leads to a roadkill cascade as animals seeking for food on the road are particularly prone to roadkill events.

Roadkills had a significant non-random distribution over the course of the year. The rainy season was the period with the highest number of roadkilled mammals (61%). The seasonal pattern of roadkills may be a consequence of the increased movement rates in the rainy season (Loretto & Vieira, 2006; Cáceres *et al.*, 2012). Mammals search for food since there is greater availability of resources in this season, as well as an intense proliferation of insects (Gumier-Costa & Sperber, 2009). Another possibility is the increased search for mates, since there is an overlap with the breeding season of some species (Loretto & Vieira, 2006). Our result contradicts what was reported by Bueno & Almeida (2010) and Faria *et al.* (2022), who pointed out the dry season as the season with high roadkill numbers. In this way, it is evident that roadkills do not occur homogeneously, but so far no pattern was identified.

The two identified hotspots in the RJ-122 (Km 23 and 24) were in areas with dense natural vegetation. Despite the high degree of forest degradation, many species persist in these forest fragments which are structurally isolated by a matrix of pastures, plantations, and urban areas (Vieira *et al.*, 2009). These areas can function as forest corridors, since some animals tend to avoid crossing open areas, as it increases the risk of exposure to potential predators and their preys (H-Saranholi *et al.*, 2016; Costa *et al.*, 2022). Indeed, the areas with the lowest number of roadkills in the RJ-122 (Km 7, 9, 10 and 11) were open areas (presence only of undergrowth or small bushes) with fenced rural properties in the vicinity. A low roadkill rate was also recorded near two local rivers: Guapiaçu and Orindiaçu rivers. Several studies have associated the presence of rivers close to roads as a potentiator of roadkill events (Bueno *et al.*, 2013; Freitas *et al.*, 2015; Costa *et al.*, 2022). We suggest that the low mortality rate near the two local rivers can be explained by the use of rivers as an alternative route for animals that move through the landscape, avoiding crossing the road, thus resulting in a lower mortality rate near riparian forests. However, more refined research is necessary to answer this question with proper characterization of the landscape.

The identification of two hotspots along the RJ-122 highway provides subsidies for the implementation of mitigation measures for the prevention of new wildlife-vehicle collisions. We suggest three possible mitigation measures: (1) modifying driver's behavior (*e.g.*, appropriate road signs and speed bumps should be part of the road where animals are frequently recorded), (2) educational and awareness-raising campaigns for drivers (*e.g*., installation of signs about the incidence of roadkills), and (3) preventing animal access to the highway (*e.g.*, construction of physical barriers or facilitators of safe movement through wildlife passages and ecological corridors (LESTER, 2015; RYTWINSKI *et al.*, 2016). Although awareness-raising measures are widely used, their implementation must be associated with other mitigation measures, as indicated by RYTWINSKI *et al.* (2016). The use of fences to prevent animal access to roads has been constantly implemented, and their effectiveness has been proven (COLLINSON *et al.*, 2019; WILLIAMS *et al.*, 2019; PARK *et al.*, 2021). RYTWINSKI *et al.* (2016) verified a 54% reduction in accidents on roads that had fences. We believe that the presence of fences along stretches of the RJ-122 highway, for example, around rural homes, may be one of the reasons that lead to a decrease in the number of roadkills. Given this scenario, the construction of crossing structures, together with fences, appears to be the best solution to connect forest fragments, directing animals to safe routes where there is no passage of vehicles, thus reducing the number of roadkills and allowing animal dispersal (RYTWINSKI *et al.*, 2016).

Here we have shown that the overall number of roadkilled mammals at RJ-122 was high, despite the short time period of monitoring. These numbers can be even higher since many animals, at the moment of impact, can be thrown off the road or can move away from it where they end up dying. Roadkills were concentrated mainly in two areas with dense natural vegetation. The most roadkilled species were in fact from forested areas, which reinforces the need to apply mitigation measures in areas which facilitate the movement of species and connect forest fragments over the landscape. Importantly, we recorded two threatened species during the study period, the jaguarundi (*Herpailurus yagouaroundi*) and the tapiti (*Sylvilagus tapetillus*), which are classified as Vulnerable, at the national list, and Endangered, at the global list, respectively. The results discussed here can contribute to a better understanding of roadkill dynamics, in addition to providing parameters that allow assessing the sustainability of highways, as well as improving and suggesting mitigation studies in several other Brazilian highways.

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REFERENCES

ABRA, F. D.; GRANZIERA, B. M.; HUIJSER, M. P.; FERRAZ, K. M. P. M. B.; HADDAD, C. M. & PAOLINO, R. M. 2019. Pay or prevent? Human safety, costs to society and legal perspectives on animal-vehicle collisions in São Paulo state, Brazil. **PLoS One 14**(4):e0215152. https://doi.org/10.1371/journal.pone.0215152

ABRA, F. D.; HUIJSER, M. P.; MAGIOLI, M.; BOVO, A. A. A. & FERRAZ, K. M. P. M. B. 2021. An estimate of wild mammal roadkill in São Paulo state, Brazil. **Heliyon 7**(1):e06015. https://doi.org/10.1016/j.heliyon.2021.e06015

ALMEIDA, R. L. F.; FILHO, J. G .B.; BRAGA, J. U.; MAGALHÃES, F. B.; MACEDO, M. C. & SILVA, K. A. 2013. Man, road and vehicle: risk factors associated with the severity of traffic accidents. **Revista de Saúde Pública 47**(4):718-731. https://doi.org/10.1590/S0034-8910.2013047003657

ASCENSÃO, F.; LUCAS, P. S.; COSTA, A. & BAGER, A. 2017. The effect of roads on edge permeability and movement patterns for small mammals: a case study with Montane Akodont. **Landscape Ecology 32**(4):781-790. https://doi.org/10.1007/s10980-017-0485-z

BAGER, A. & ROSA, C. A. 2010. Priority ranking of roads sites for mitigating wildlife roadkill. **Biota Neotropica 10**(4):149-153. https://doi.org/10.1590/S1676-06032010000400020

BARTHELMESS, E. & BROOKS, M. S. 2010. The influence of body-size and diet on roadkill trends in mammals. **Biodiversity and Conservation 19**(6):1611-1629. https://doi.org/10.1007/s10531-010-9791-3

BÍL, M.; ANDRÁ, R. & JANOSKA, Z. 2013. Identification of hazardous road locations of traffic accidents by means of kernel density estimation and cluster significance evaluation. **Accident Analysis & Prevention 55**:265-273. https://doi.org/10.1016/j.aap.2013.03.003

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

BUENO, C.; FAUSTINO, M. T. & FREITAS, S. R. 2013. Influence of landscape characteristics on capybara road-kill on highway. **Oecologia Australis 17**:130-137. https://doi.org/10.4257/oeco.2013.1702.11

BUENO, C.; SOUSA, C. O. M. & FREITAS, S. R. 2015. Habitat or matrix: which is more relevant to predict road-kill of vertebrates? **Brazilian Journal of Biology 75**:228-238. https://doi.org/10.1590/1519-6984.12614

CÁCERES, N. C. & LESSA, L. G. 2012. O papel de marsupiais na dispersão de sementes. *In:* CÁCERES, N. C. ed. **Os marsupiais do Brasil: biologia, ecologia e conservação**. Campo Grande, Editora UFMS, p. 407-426.

CÁCERES, N. C.; CASELLA, J. & GOULART, C. S. 2012. Variação espacial e sazonal de atropelamentos de mamíferos no bioma cerrado, rodovia BR 262, sudoeste do Brasil. **Mastozoología Neotropical 19**(1):21-33.

CARVALHO, C. F.; CUSTÓDIO, A. E. I. & JÚNIOR, O. M. 2015. Wild vertebrates' roadkill aggregations on the BR-050 Highway, State of Minas Gerais, Brazil. **Bioscience Journal 31**(3):951-959.

CBEEE - CENTRO BRASILEIRO DE ESTUDOS EM ECOLOGIA DE ESTRADAS. 2022. **Mais de 2 milhões de animais morrem atropelados em rodovias todo ano.** Available at <https://ecoestradas.com.br/2milhoes/>. Accessed on 10 August 2022.

CHEN, H. L. & KOPROWSKI, J. L. 2019. Can we use body size and road characteristics to anticipate barrier effects of roads in mammals? A meta-analysis. **Hystrix, The Italian Journal of Mammalogy 30**(1):1-7. https://doi.org/10.4404/hystrix-00185-2019

Cirino, D. W.; Lupinetti-Cunha, A.; Freitas, C. H. & Freitas, S. R. 2022. Do the roadkills of different mammal species respond the same way to habitat and matrix? **Nature Conservation 47**:65-85. https://doi.org/10.3897/natureconservation.47.73010

Coelho, I. P.; Kindel, A. & Coelho, A. V. P. 2008. Roadkills of vertebrate species on two highways through the Atlantic Forest Biosphere Reserve, Southern Brazil. **European Journal of Wildlife Research 54**:689-699.

Collinson, W. J.; Marneweck, C. & Davies-Mostert, H. T. 2019. Protecting the protected: reducing wildlife roadkill in protected areas. **Animal Conservation 22**(4):396-403. https://doi.org/10.1111/acv.12481Costa, I. M. D. C., Ferreira, M. S., Mourão, C. L. B., & Bueno, C. 2022. Spatial patterns of carnivore roadkill in a high-traffic-volume highway in the endangered Brazilian Atlantic Forest. **Mammalian Biology 102**(2):477-487. https://doi.org/10.1007/s42991-022-00247-1

Cunha, H. F.; Moreira, F. G. A. & Silva, S. S. 2010. Roadkill of wild vertebrates along the GO-060 road between Goiânia and Iporá, Goiás State, Brazil. **Acta Scientiarum Biological Sciences 32**(3):257-263. https://doi.org/10.4025/actascibiolsci.v32i3.4752

Cunha, A. & Vieira, M. 2002. Support diameter, incline, and vertical movements of four didelphid marsupials in the Atlantic Forest of Brazil. **Journal of Zoology 258**(4):419-426. https://doi.org/10.1017/S0952836902001565

Eisenberg, J. F. & Redford, K. H. 1999. **Mammals of the Neotropics (Volume 3). The Northern Neotropics. The Central Neotropics: Ecuador, Peru, Bolivia, Brazil**. Chicago, University of Chicago Press. 624p.

Fahrig, L. 2003. Effects of Habitat fragmantation on Biodiversity. **Annual Review of Ecology, Evolution and Systematics 34**:487-515.

Faria, H. H.; Pires, A. S. & Abra, F. D. 2022. Monitoramento dos impactos de rodovia sobre a fauna como componente de gestão de uma área protegida nos domínios da Mata Atlântica brasileira. **Periódico Eletrônico Fórum Ambiental da Alta Paulista 18**(1):1-17.

Ferraz, K. B; Siqueira, M.; Martin, P.; Esteves, C. & Couto, H. 2010. Assessment of *Cerdocyon thous* distribution in an agricultural mosaic, southeastern Brazil. **Mammalia 74**(3):275-280. https://doi.org/10.1515/mamm.2010.036

Ferreira, M. S.; Vieira, M. V.; Cerqueira, R. & Dickman, C. R. 2016. Seasonal dynamics with compensatory effects regulate populations of tropical forest marsupials: a 16-year study. **Oecologia 182**:1095-1106. https://doi.org/10.1007/s00442-016-3735-x

Filius, J.; Hoek, Y. V. D.; Jarrín-V, P. & Hooft, P. V. 2020. Wildlife roadkill patterns in a fragmented landscape of the Western Amazon. **Ecology and Evolution 10**(13):6623-6635. https://doi.org/10.1002/ece3.6394

Freitas, S. R.; Oliveira, A. N.; Ciocheti, G.; Viera, M. V. & Matos, D. M. S. 2015. How landscape features influence road-kill of three species of mammals in the brazilian savanna? **Oecologia Australis 18**:35-45.

Gumier-Costa, F. & Sperber, C. F. 2009. Atropelamentos de vertebrados na Floresta Nacional de Carajás, Pará, Brasil. **Acta Amazonica 39**(2):450-466. https://doi.org/10.1590/S0044-59672009000200027

H-Saranholi, B.; Bergel, M. M.; Ruffino, P. H. P.; Rodríguez-C., K. G.; Ramazzotto, L. A.; De Freitas, P. D. & Galetti, P. M. 2016. Zonas de alto impacto de atropellamientos en un area protegida de Cerrado (Brasil): planeando acciones para la conservacion. **Revista Medicina Veterinaria y Zootecnia 21**(2):5441-5448. https://doi.org/10.21897/rmvz.609

ICMBio - Instituto Chico Mendes de Conservação da Biodiversidade. 2018. Livro Vermelho da Fauna Brasileira Ameaçada de Extinção: Volume II - Mamíferos. *In:* Instituto Chico Mendes de Conservação da Biodiversidade. org. **Livro Vermelho da Fauna Brasileira Ameaçada de Extinção**. Brasília, ICMBio. 622p.

INMET - Instituto Nacional de Meteorologia do Brasil. 2021. **Normais climatológicas (1991/2020)**. Brasilia, INMET. 27p.

IUCN - International Union of Conservation of Nature. 2021. **The IUCN Red list of threatened species**. Available at <https://www.iucnredlist.org/>. Accessed on 11 August 2022.

Laurance, W. F.; Goosem, M. W. & Laurance, S. G. W. 2009. Impacts of roads and linear clearings on tropical forest. **Trends in Ecology & Evolution 24**(12):659-669. https://doi.org/10.1016/j.tree.2009.06.009

Lester, D. 2015. Effective Wildlife Roadkill Mitigation. **Journal of Traffic and Transportation Engineering 3**:42-51. https://doi.org/10.17265/2328-2142/2015.01.005

Magioli, M.; Ferraz, K. & Rodrigues, M. 2014. Medium and large-sized mammals of an isolated Atlantic Forest remnant, southeast São Paulo State, Brazil. **Check List 10**:850-856. https://doi.org/10.15560/10.4.850

Monteiro-Alves, P. S.; Helmer, D. M.; Ferreguetti, A. C.; Pereira-Ribeiro, J.; Rocha, C. F. D. & Bergallo, H. G. 2019. Occupancy, detectability, and density of crab-eating fox (*Cerdocyon thous*) in two protected areas of restinga habitats in Brazil. **Canadian Journal of Zoology 97**(10):952-959. https://doi.org/10.1139/cjz-2018-0322

Orlandin, E.; Pìovesan, M.; Favretto, M. A. & D'Agostini, F. M. 2015. Mamíferos de médio e grande porte atropelados no Oeste de Santa Catarina, Brasil. **Biota Amazônica 5**(4):125-130. http://dx.doi.org/10.18561/2179-5746/biotaamazonia.v5n4p125-130

Paglia, A. P.; Da Fonseca, G. A.; Rylands, A. B.; Herrmann, G.; Aguiar, L. M.; Chiarello, A. G.; Leite, Y. L. R.; Costa, L. P.; Siciliano, S.; Kierulff, M. C. M.; Mendes, S. L.; Tavares, V. C.; Mittermeier, R. A. & Patton, J. L. 2012. Annotated checklist of Brazilian mammals. **Occasional Papers in Conservation Biology 6**:1-82.

Park, H.; Woo, D.; Choi, T. Y.; Hong, S. 2021. Assessment of the behavioural response of Korean water deer (*Hydropotes inermis argyropus*) to different fence heights. **Animals 11**(4):938. https://doi.org/10.3390/ani11040938

Parris, K. M. & Schneider, A. 2009. Impacts of traffic noise and traffic volume on birds of Roadside habitats. **Ecology and Society 14**(1):29.

Pinowski, J. 2005. Roadkills of Vertebrates in Venezuela. **Revista Brasileira de Zoologia 22**(1):191-196.

Prevedello, J. A. & Vieira, M. V. 2009. Plantation rows as dispersal routes: A test with didelphid marsupials in the Atlantic Forest, Brazil. **Biological Conservation 143**(1):131-135. https://doi.org/10.1016/j.biocon.2009.09.016

Qgis Development Team. 2019. **QGIS geographic information system**. Open-Source Geospatial Foundation Project

R Core Team. 2019. **R: A language and environment for statistical computing**. R Foundation for Statistical Computing, Vienna.

Rytwinski, T.; Soanes, K.; Jaeger, J. A. G.; Fahrig, L.; Findlay, C. S.; Houlahan, J.; Van Der Ree, R. & Grift, E. A. V. D. 2016. How effective is road mitigation at reducing road-kill? A Meta-Analysis. **Plos One 11**(11):e0166941. https://doi.org/10.1371/journal.pone.0166941

Santana, G. S. 2012. Fatores influentes sobre atropelamentos de vertebrados na região central do Rio Grande do Sul, Brasil. **Neotropical Biology and Conservation 7**(1):26-40.

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.

Smith, L. L. & Dodd Jr, C. K. 2003. Wildlife mortality on U.S. highway m441 across paynes prairie, Alachua County, Florida. **Florida Scientist 66**(2):128-140.

Van Der Ree, R.; Grilo, C. & Smith, D. J. 2015. **Handbook of road ecology.** Chichester, Wiley. 560p.

Vélez, C. A. D. 2014. Adiciones al atropellamiento vehicular de mamíferos en la vía de el escobero, envigado (Antioquia), Colombia. **Revista de Ingeniería de Antioquia 11**(22):147-153.

Vieira, M. V.; Olifiers, N.; Delciellos, A. C.; Antunes, V. Z.; Bernardo, L. R.; Grelle, C. E. V. & Cerqueira, R. 2009. Land use vs. fragment size and isolation as determinants of small mammal composition and richness in Atlantic Forest remnants. **Biological Conservation 142**:1191-1200. https://doi.org/10.1016/j.biocon.2009.02.006

Ware, H. E.; Mcclure, C. J. W.; Carlisle, J. D. & Barber, J. R. 2015. A phantom road experimente reveals traffic noise is an invisible source of habitat degradation. **Proceedings of the National Academy of Sciences 112**(39):12105-12109. https://doi.org/10.1073/pnas.1504710112

Williams, S. T.; Collinson, W.; Patterson-Abrolat, C.; Marneweck, D. G. & Swanepoel, L. H. 2019. Using road patrol data to identify factors associated with carnivore roadkill counts. **Peer Journals 7**:e6650. https://doi.org/10.7717/peerj.6650

Zanzini, A. C. S.; Machado, F. S.; Oliveira, J. E. & Oliveira, E. C. M. 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. https://doi.org/10.4257/oeco.2018.2203.04