

DON'T SPEED UP, SPEED KILLS: MAMMAL ROADKILLS ON HIGHWAY SECTIONS OF PR-445 IN THE SOUTH OF BRAZIL

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Abstract: Animal-vehicle collisions are the main negative impact of roads on wildlife, where they cause population declines, shifts in community structure, and potential changes in species behavior. Here, we determined mammal roadkill rates and the hotspots with higher rates for medium- and largebodied mammals on the Highway PR-445 in the state of Paraná, Brazil. We have also evaluated possible differences in the frequency of roadkills concerning species activity patterns and their feeding habits. In doing so, we monitored the PR-445 twice a week between the kilometers 1 and 76, from March 2018 to March 2019, totaling 7296 km after 96 trips. We have recorded 93 mammal roadkills belonging to 17 species, representing a rate of 0.013 individual/km/day. The Carnivora, Didelphimorphia, and Cingulata orders showed to be the most common representatives among the roadkills. Omnivores were more prone to get hit by vehicles than herbivores and carnivores. The type of mammal activity pattern was not determinant in explaining the differences in roadkill rates. Highways in Paraná are among the roads that register the highest number of vehicle collisions with vertebrates in the country. This issue, together with extensive habitat loss and fragmentation, increases the threats to the relictual fauna. Our results indicate that the regions with the highest incidence of mammal roadkills on PR-445 are those close to stretches over rivers and with remnants of native vegetation. Thus, we emphasize that more comprehensive measures (e.g., wildlife passages and speed reducers) are essential to mitigate the impact of roads on wildlife.

Keywords: activity period; animal-vehicle collisions; diversity loss; conservation; species diet.

INTRODUCTION

In the 21st century, roads account for some of the most important anthropogenic changes on the environment, resulting in a high impact on natural

landscapes (Laurance *et al.* 2014). These impacts include an increase in noise and light pollution, erosion, loss of the natural environment, and land use change, aggravating the edge effect of forest fragments associated with the often-abrupt verges

of roads and linear clearings (Forman & Alexander 2002, Laurance *et al.* 2009). The consequences of roads on wildlife reach different ecological scales, including changes in the structure of communities (Laurance *et al.* 2009) and behavioral dynamics (Ascensão *et al.* 2014, Poessel *et al.* 2014). It also influences species presence and persistence in surrounding forest fragments (Borda-de-Água *et al.* 2011), and causes considerable economic losses (Abra *et al.* 2019).

Besides the abovementioned road-related threats to animals, roadkill is the main negative impact of roads on wildlife, with direct effects on local populations, influencing species abundance and distribution (Eigenbrod *et al.* 2008). Population declines caused by road impacts have effects on species genetic variability and viability (Jackson & Fahrig 2011). Roadkill is the primary death cause for some species, including threatened taxa (Forman *et al.* 2003), proving to be a direct death cause among terrestrial vertebrates, which is considered to be as important as hunting (Seiler & Heldin 2006).

Brazil has one of the most extensive network of roads in the world, covering more than 200,000 km of surfaced roads (ANTT 2019). According to the estimates provided by the Brazilian Center for Road Ecology Research (CBEE), more than 15 animals die on Brazilian roads every second. The vertebrate roadkill rate in Brazil is 8.65 (26.37) per kilometer per year (Dormas et al. 2012). This can potentially represent close to 14.7 (44.8) million animals running over per year throughout the Brazilian territory (Dormas et al. 2012). Among these, it is estimated that five million mediumand large-bodied animals, such as giant anteaters, peccaries, deer, jaguars, monkeys, and maned wolves, are killed annually on the roads and highways in Brazil (CBEE 2020). Therefore, road monitoring and the implementation of mitigation strategies to prevent road impacts on the native biota are essential (Costa et al. 2015, Abra et al. 2019). This action is even more crucial in areas of great value for biodiversity conservation, such as the Atlantic Forest biome, which is considered one of the 34 biodiversity hotspots worldwide (Mittermeier et al., 2004).

In the northern part of the Paraná state, the predominant vegetation is seasonal semideciduous forest (SSF), which is one of the most disturbed ecosystems in the Atlantic Forest, occurring in refuge areas of the South and Southeast Brazil (SOS Mata Atlântica & INPE 2018). Recent estimates have shown that only 7 % of the area initially covered by SSF still remains, which are represented by small fragments (typically less than 50 ha), immersed in matrices of intensive farming and urban areas crossed by extensive road networks with intense traffic (SOS Mata Atlântica & INPE 2018). Within the Paraná state, studies involving mammal roadkills have been conducted in the surroundings of Iguaçu National Park (Brocardo & Cândido-Junior 2012), in the coastal region of the state (i.e. Serra do Mar) (Leite et al. 2012, Belão et al. 2014), and in the region of Campos Gerais (i.e. central plateaus) (Zaleski et al. 2009, Weiss & Vianna 2013). However, no studies have extensively explored the impact of roads on the mammalian fauna of the northern region. This meta-region is of great importance for biodiversity conservation due to its location in a transition zone between the tropical and subtropical regions of the Atlantic Forest.

Evaluating the importance of animal-vehicle collisions is a complex challenge in conservation ecology, involving ecological, economic, social and technical perspectives, and considering both large and small spatial scales (Seiler & Heldin 2006). The information collected from road data surveys is often used to choose the best strategies for reducing collisions with animals and to evaluate their effectiveness (Cureton & Deaton 2012, Lesbarreres & Fahrig 2012, Costa *et al.* 2015).

Thus, this study determined roadkill rates and pointed out the hotspots with higher incidences of roadkills for medium- and large-bodied mammals along the Highway PR-445, located in the subtropical portion of the Atlantic Forest in South Brazil. We have also evaluated the possible variation in the frequency of roadkills according to the activity pattern and food habits of the recorded mammalian species. Therefore, two hypotheses were tested. (I) Assuming the mostly nocturnal habit of mammals in the region, and that their detection during the night by drivers would be difficult when compared with daylight (Caceres 2011), there will be a difference in roadkill rates in relation to the activity pattern, with the higher frequency of roadkills of mammals with nocturnal habit. (II) Since mammals with herbivorous and carnivorous food habits tend to cover larger areas to find food than omnivorous and insectivorous animals, they are expected to have higher roadkill rates than species from other feeding guilds.

MATERIAL AND METHODS

Study area

Highway PR-445 (also called Celso Garcia Cid) is a 95 km long highway located between the localities of Mauá da Serra (23°54'26.41" S; 51°11'32.93 W") and Warta (23°16'50.71" S; 51°8'55.83" W) in northern Paraná. This highway is located in the transition zone between the tropical and subtropical Atlantic Rainforest (Tropic of Capricorn). It is a state-administered two-lane highway with a short four-lane section in the city of Londrina. PR-445 maximum speed is 80 km/h, which is controlled by electronic radars and mobile surveillance. Londrina metropolitan region is the fourth largest metropolis in southern Brazil, with an estimated population of more than one million inhabitants, which creates a heavy traffic. Traffic with heavy vehicles ($e.g. \ge 3,500 \text{ kg}$) is constant, mainly due to the transport of crops during the harvest time.

The predominant vegetation in the region is seasonal semideciduous forest. The climate of the region is classified as humid subtropical mesothermal (Cfa), with an annual average temperature around 21 °C, and an annual rainfall of 1,450 mm (Peel *et al.* 2007). Highway PR-445 is mainly surrounded by agricultural matrices (soybean, wheat, and corn crops), eucalyptus monocultures, pastures, urban areas, and small native remnants of SSF (SOS Mata Atlântica & INPE 2018).

Sampling

The data on mammal roadkills was obtained between kilometers 1 and 76 of PR-445 (80 % of the whole road), which includes the counties of Londrina, Mauá da Serra, and Tamarana (Figure 1). We adopted a monitoring protocol following

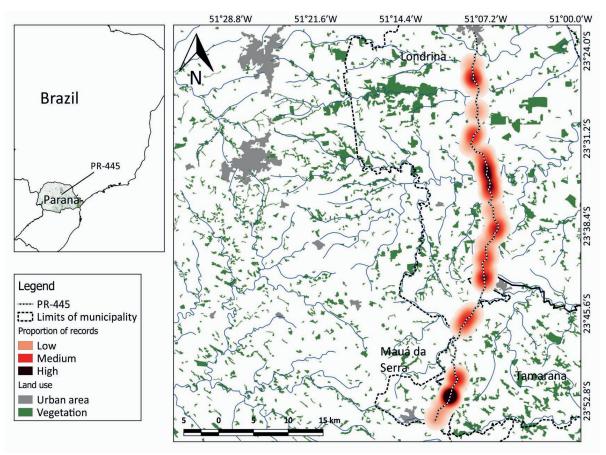


Figure 1. Location of the Highway PR-445 in the Paraná state. Brazil indicating the sector measured in this study from 2018 to 2019. The hotspots highlighted in red indicate places with high rates of mammal roadkills.

Costa *et al.* (2015), which have used mixed ways of sampling. This methodology is based on monthly and weekly samplings to get species records with no marked mortality peak (*i.e.* species with higher activity in warmer periods [southern hemisphere summer]) (Costa *et al.* 2015).

We monitored PR-445 twice a week between kilometers 1 and 76, from March 2018 to March 2019, totaling 7,296 km across 96 trips. The search for medium- to large-bodied mammal carcasses was done during the mornings (from 7:00 h to 12:00 h) with at least two observers present in a car with an average speed of 80 km/h.

The roadkilled mammals that were found, were photographed and identified in situ whenever possible. Accordingly, we have used specific field guides for each taxonomic group (Reis et al. 2014). For the carcasses in advanced decomposition stage or showing any damage that hindered accurate species identification, we have manually collected hair samples on slides for species identification based on microstructural hair characteristics, namely cuticular and medullary patterns. We extracted small tufts of guard hairs from the back at the intersection of the median line and scapular waistline, following the identification protocol described by Quadros & Monteiro-Filho (2006). Species identification was confirmed using the identification keys of Quadros & Monteiro-Filho (2010), and Miranda et al. (2014).

The alpha taxonomic nomenclature followed Paglia et al. (2012) after considering recent taxonomic changes (e.g. Leopardus guttulus (Hensel, 1872) based on Trigo et al. (2013) and in the genus Sapajus according to Lynch-Alfaro et al. (2012). For the Pilosa and Rodentia orders, we have also consulted Patton et al. (2015) for an accurate species identification. All species with an adult body mass ≥ 1 kg were considered to be medium-to large-bodied mammals (Chiarello 2000). Mammalian activity pattern and food habits followed Reis et al. (2011) and Paglia et al. (2012), respectively. Moreover, for each species, we obtained their conservation status according to the Brazilian Wildlife Red Book (MMA 2018), the List of Threatened Fauna in the Paraná state (Paraná 2010), and IUCN Red List of Threatened Species (IUCN 2019).

Data analysis

The location of each animal was recorded using a Global Positioning System (GPS). In order to analyze the highway regions with the highest concentration of roadkills (*i.e.* hotspots), we created a density map of records using the kernel density estimator (Wand & Jones 1995). The kernel approach allows estimating the number of events per unit area in each cell of a regular grid covering the study area, in addition to filtering dataset variability (Wand & Jones 1995). The analysis was performed in R environment (R Core Team 2019) using the MASS package (version 2.23-15, Wand 2015) and "KernSmooth" function.

The frequency (F) of roadkills for each recorded species was calculated by the formula:

$$Fi = \left(\frac{Ni}{N}\right)100$$

where: F_i = frequency of occurrence for the ith species; N_i = number of records of the *i*th species; N = total number of records.

We have used the Kruskal-Wallis nonparametric test, a non-parametric equivalent of an ANOVA, due to a non-normal data distribution determined *a priori* by frequency histogram and Shapiro-Wilk test (Shapiro & Wilk 1965), to evaluate possible differences in the number of records regarding activity patterns and feeding habits. For the cases in which we have observed statistically significant differences, we posteriorly performed pairwise comparisons using the Tukey and Kramer (Nemenyi) tests with Tukey-Dist approximation for independent samples (Nordstokke & Stelnicki 2014). All analyses were performed in R environment using the Vegan package (version 2.4-1, Oksanen *et al.* 2019).

RESULTS

We have recorded 93 mammal roadkilled specimens on PR-445, resulting in 17 species distributed in 12 families and seven orders (Table 1), corresponding to a roadkill rate of 0.013 individual/km/day. The kernel density estimator indicated three points along the highway with the highest concentration of roadkilled animal records, *i.e.* hotspots. One hotspot occurred between Mauá da Serra and Tamarana (km 4; 23°52'56.79"S, 51°10'29.01"W to 23°51'47.49"S,

38 | Mammal roadkills on highway sections of PR-445

Table 1. List of roadkill medium- to large-bodied mammalian species on Highway PR-445. Acronyms are as follows. N: Total number of occurrences. F: Frequency of occurrence by species. Diet: carnivore (Ca); frugivore and folivore (Fr/Fo); frugivore and omnivore (Fr/On); herbivore or grazer (Hb); insectivore and omnivore (In/On) and myrmecophage (Myr). **Activity pattern:** crepuscular/nocturnal (CrN); diurnal (Dn); nocturnal (Nt). **Threat category:** data deficient (DD); endangered (EN); least concern (LC); not evaluated (NE); near threatened (NT) and vulnerable (VU). Exotic species (*). Small-bodied species (**).

Taxon	Number of records		Diet	Activity	Threat category		
	Ν	F%		pattern	IUCN	BR	PR
Didelphimorphia							
Didelphidae							
Didelphis albiventris Lund, 1840	13	14	Fr/On	CrN	LC	NE	LC
Didelphis aurita Wied-Neuwied, 1826	3	3.2	Fr/On	Nt	LC	NE	LC
Pilosa							
Myrmecophagidae							
Tamandua tetradactyla (Linnaeus, 1758)	3	3.2	Myr	Nt	LC	NE	NE
Cingulata							
Dasypodidae							
Dasypus novemcinctus Linnaeus, 1758	13	14	In/On	CrN	LC	NE	LC
Primates							
Cebidae							
Sapajus nigritus (Goldfuss, 1809)	3	3.2	Fr/On	Dn	NT	NE	DD
Carnivora							
Canidae							
Cerdocyon thous (Linnaeus, 1766)	19	20.4	In/On	CrN	LC	NE	LC
Felidae							
Leopardus guttulus (Hensel, 1872)	3	3.2	Ca	Nt	VU	VU	VU
Leopardus wiedii (Schinz, 1821)	2	2.2	Ca	Nt	NT	VU	VU
Puma yagouaroundi (E. Geoffroy, 1803)	1	1.1	Ca	Dn	LC	VU	DD
Mustelidae							
<i>Eira barbara</i> (Linnaeus, 1758)	1	1.1	Fr/On	Dn	LC	NE	LC
Galictis cuja (Molina, 1782)	1	1.1	Ca	CrN	LC	NE	LC
Procyonidae							
Nasua nasua (Linnaeus, 1766)	12	12.9	Fr/On	Dn	LC	NE	LC
Lagomorpha							
Leporidae							
Lepus europaeus Pallas, 1778*	5	5.4	Hb	CrN	LC	NE	NE
Sylvilagus minensis (Thomas, 1901)	3	3.2	Hb	CrN	LC	NE	VU
Rodentia							
Cuniculidae							
<i>Cavia aperea</i> Erxleben, 1777**	3	3.2	Hb	Nt	LC	NE	LC
Erethizontidae							
Coendou spinosus (F. Cuvier, 1823)	4	4.3	Fr/Fo	Nt	DD	NE	LC
Myocastoridae							
Myocastor coypus (Molina, 1782) *	4	4.3	Fr/On	Nt	LC	NE	LC

51°10'3.96" W). The second stretch near the bridge over the Taquaruna River (Km 40; 23°35'14.27"S, 51° 6'54.11"W to 23°33'16.56"S, 51° 7'10.04"W). The third hotspot was located eight kilometers perpendicular to the Parque Estadual Mata dos Godoy protected area, towards the urban area of Londrina (km 58; 23°27'50.11"S, 51° 8'0.89"W to 23°26'33.48"S, 51° 8'8.58"W) (Figure 1).

Higher numbers of roadkill events were found for the orders: Carnivora (41.9 % of roadkill records), Didelphimorphia (17.2 %), and Cingulata (14 %) in relation to the others. We have also recorded roadkills of the Lagomorpha (8.6 %), Erethizontidae (4.3 %), Myocastoridae (4.3 %), Primates (3.3 %), Rodentia (3.3 %), and Myrmecophagidae (3.3 %) orders. Among the 17 species recorded, four of them have some degree of threat, either according to state, national, or international level, and two are exotic species (*Lepus europaeus* and *Myocastor coypus*; Table 1).

Based on the analysis of the cuticular and medullary patterns of mammal hair microstructure, we were able to perform an accurate identification of feline species (Figure 2). We found three cuticular patterns, namely wide rhombus (*Puma yagouaroundi*), narrow rhombus (*Leopardus guttulus*), and narrow leaf shape (*Leopardus wiedii*), and also two medullary patterns, namely trabecular fimbriate with vacuoles in *P. yagouaroundi* and *L. guttulus*, and trabecular fimbriate without vacuoles for *L. wiedii* (Figure 2).

We did not find significant differences in the frequency of roadkills with regard to the activity patterns of mammals (H = 3.2919; df = 2; p > 0.05; Figure 3A). However, we found significant

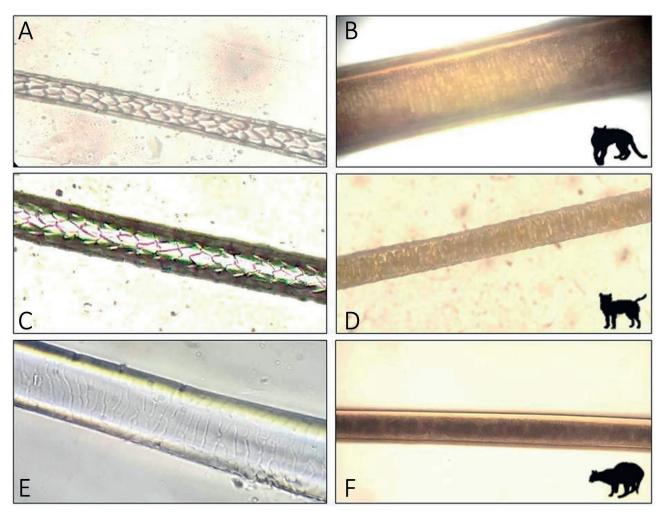


Figure 2. Microstructural hair patterns for species of the Felidae family: *Leopardus wiedii* (A) cuticular pattern and (B) medullar pattern; *Leopardus guttulus* (C) cuticular pattern and (D) medullar pattern; *and Puma yagouaroundi* (E) cuticular pattern and (F) medullar pattern.

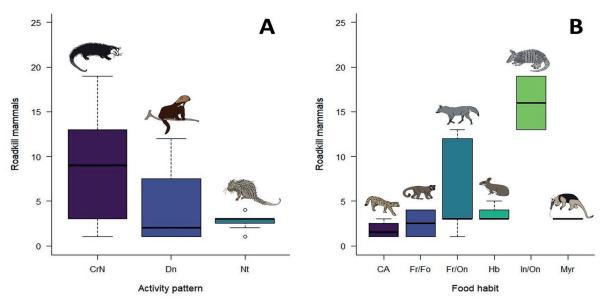


Figure 3. Boxplot illustrating the differences in roadkill rates for medium- to large-bodied mammals on the Highway PR-445 section between the municipalities of Londrina and Mauá da Serra, Paraná state. (A) Activity patterns: Cr/N - crepuscular/nocturnal; Dn – diurnal; Nt - nocturnal. (B) Food habits: CA - carnivore; Fr/Fo - frugivore and folivore; Fr/On - frugivore and omnivore; Hb - herbivore or grazer; In/On - insectivore and omnivore; Myr - myrmecophage.

differences in the frequency of roadkills with regard to their food habits (H = 6.869; df = 5; p < 0.05; Figure 3B).

DISCUSSION

Direct mortality is the most widespread and significant effect of roads on wildlife (Bissonette 2002), which has been evidenced in our study. However, the number of roadkills presented here may be underestimated, since larger animals may not die immediately after the collision, as they move to areas away from the road, making it difficult to include them in the count (Bruinderink & Hazebroek 1996).

We found a rate of 0.013 dead animals per day for each kilometer. Considering this crude rate > 4,745 mammal specimens were estimated to be succumbing during a year on the highway along PR-445. The current mammal roadkill rate is similar to the rate found for the PR-406 and PR-508 highways in the coastal region of the Paraná state (0.017 dead animals per day for each kilometer) (Leite *et al.* 2012). Zaleski *et al.* (2009) have also reported similar results to the ones observed in the present study when monitoring the PR-160, a highway with similar characteristics to PR-445, which is located in Telêmaco Borba municipality. In the region of Campos Gerais of Paraná, Weiss & Vianna (2012) reported an average of 10 mammal roadkills per kilometer during the four years monitoring the highways BR-376, BR-373, and BR-277. The high roadkill rates for many species is appalling and indicates that these accidents are commonplace and widespread (Seiler & Heldin 2006). Yet, the Brazilian Atlantic Forest has high defaunation rates of medium- to large-bodied mammals (Bogoni *et al.* 2018), especially in highly fragmented areas, such as the area of the present study, which makes the road impacts on wildlife a crucial factor to be considered in conservation strategies.

A series of empirical studies have showed that roads cause substantial impacts on species abundance and species composition in ecosystems (Polak *et al.* 2014, Magioli *et al.* 2019). Among the roadkilled species recorded in our study, 43 % belonged to the order Carnivora. Presumably, the high number of carnivoran (species of the Order Carnivora) roadkills may be related to their ecological and behavioral aspects, because carnivorans have large home ranges (a powerful descriptor of species or populations spatial requirements). Moreover, carnivoran species show the ability to explore human-disturbed environments neighboring native vegetation (Lyra-Jorge *et al.* 2010).

Studies indicate that roads affect carnivore movements, restricting their distribution, particularly those living in habitats near urbanized areas, including felids (Poessel et al. 2014). The population declines of carnivores due to roadkills may compromise forest stability and ecosystem functioning (Poessel et al. 2014, Červinka et al. 2015). Carnivores play an important role in providing ecosystem services (e.g. increase diversity), and are considered apex predators or mesopredators in food chains, contributing to the growth regulation of other populations (Terborgh et al. 1999). In the absence of carnivores in modified ecosystems where they originally occurred, the subsequent trophic levels would also be affected, influencing the entire commuity by a cascade effect (Poessel et al. 2014, Červinka et al. 2015).

In this study, the most frequently roadkilled species were *Cerdocyon thous,* Dasypus novemcinctus, Nasua nasua, and Didelphis albiventris. In the Atlantic Forest, these species are easily found in altered environments and a have preference for open areas (Reis et al. 2014). Although C. thous does not appear in the Brazilian Red Book of Threatened Species of Fauna (MMA 2018), the high roadkill rates for this species is of conservation concern. In many studies throughout Brazil, C. thous has been shown to be the most affected species by roadkills (Zaleski et al. 2009, Cunha et al. 2010, Belão et al. 2014, Brum et al. 2018, Zanzini et al. 2018). Other species did not have such high frequencies of vehicle collisions compared to C. thous, D. novemcinctus, N. nasua, and D. albiventris. In theory, the number of animal-vehicle collisions should depend on the density and activity of the animals, and on the number of vehicles passing on the highway (Seiler & Heldin 2006).

For some species, the low number of roadkill events is mainly related to low species abundances (*e.g.* large felids that in general have low population densities within an area) (Jaeger *et al.* 2005). In addition, roadkill records may reinforce the vulnerability of some species to landscape modifications. Among the 17 roadkilled species on PR-445, four of them (*Sapajus nigritus, Leopardus guttulus, Leopardus wiedii,* and *Herpailurus* *yagouaroundi*) are listed in the "Vulnerable" or "Nearly threatened" categories, both on national and international scales (MMA 2018, IUCN 2019). In addition, *Sylvilagus minensis* (Thomas, 1901), which has been recently recognized as a valid species (see Bonvicino *et al.* 2015 and Ruedas *et al.* 2017), needs a new evaluation.

Although nocturnal species are expected to be more susceptible to be run over on highways than those of diurnal habit (Bruinderink & Hazebroek 1996), this was not evident in our study. We did not find significant differences in roadkill rates between species with different activity patterns. Moreover, activity pattern peaks are strongly related to climatic conditions and are more evident in groups of amphibians, reptiles, and migratory birds (Costa *et al.* 2015).

Our initial hypothesis was that herbivorous and carnivorous mammals were more run over because they tend to walk and cover larger areas for food than omnivorous and insectivorous animals (Tucker *et al.* 2014). However, our results indicate that omnivorous species are more prone to being run over than herbivorous or carnivorous ones (Figure 2B). Omnivorous, generalist, and opportunistic animals are more tolerant to fragmentation and are consequently more abundant in these modified areas than other species. Thus, they are more subject to being roadkilled (Araújo *et al.* 2019).

Our results also indicate that the analysis of cuticular and medullary patterns of mammal hairs is a useful tool for identifying roadkilled felines. Hair microstructure is a useful tool to identify mammals and is applied in several disciplines, such as forensic sciences and ecology (Quadros & Monteiro-Filho 2010), but there are still few studies that use this tool for roadkill identification. This technique allows the confirmation of species identification considering non-intact individuals, particularly small wild felines, which is especially important because the main characters usually utilized for species identification can be destroyed by vehicle collisions (Quadros & Monteiro-Filho 2010).

In Brazil, there are some initiatives to reduce the impact of roads on biodiversity. The monitoring of vehicle collisions with wildlife carried out by the Brazilian Center for Road Ecology Research (CBEE), provides an app (Urubu System App) based on citizen science for monitoring animal accidents on Brazilian roads. Other important actions for mitigation measures on Brazilian roads have been developed by the environmental enterprise ViaFauna in São Paulo, called Passa-Bicho ("Animal Pass"). This initiative enables the detection of animals based on positioning a set of sensors on stretches of the roadway where animal crossing rates are high (Vasconcelos 2017, Abra *et al.* 2019).

The roads in the Paraná state are among those that have the highest records of roadkills in Brazil (CBEE 2020). Our results indicated that the sections with higher incidence of animals run over on PR-445 are associated with rivers and regions with higher concentration of native vegetation (see Figure 1). Previous studies like those of Bueno et al. (2015) and Freitas et al. (2015) indicated an association between river proximity and the roadkill of many mammal species, showing that rivers may be a preferential route for them, which suggests that these areas are a mitigation priority. We emphasize that along this entire highway, there are no basic systems to avoid collisions with wild animals. The installation of underpasses and external fences could be suitable measures for mitigating mammal roadkill (Rytwinski et al. 2016), especially near bridges crossing over rivers and streams, and in regions with higher concentration of native vegetation.

We also suggest placing speed reducers (warning signs, speed cameras, and speed bumps) and road fencing, which should be installed mainly in the places where this study indicated the highest densities of roadkills. However, we emphasize that the installation of these fences must be in strategic locations along the highway that lead to underground passages. Thus, such fences would force animals to use the underground passages to safely access the other side of the road, leading to a reduction in the risk of car collision. Finally, it is necessary to increase incisive advertising campaigns aimed at drivers in order to build a connection between the road transport authority and the public and reduce collisions between vehicles and animals.

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