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Analysis of scientific production and knowledge about wildlife roadkill in Brazilian protected areas

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ABSTRACT

Roads are responsible for great biodiversity loss, especially in protected areas (PAs). Thus, considering the great risk of roads to PAs and the lack of knowledge about these areas, we aimed to analyze the scientific production on wildlife roadkill in Brazil and compare the studies that surveyed roads with and without PAs. We searched for papers in five databases: SciELO, Google Scholar, Reet Brasil, Scopus and Plataforma Lattes. Studies considered to be near PAs (PPA) collected data within a radius of 1 km of PAs and the other studies were considered to have no PA (NPA). We found 126 studies that surveyed wildlife roadkill in Brazil, of which 57% are PPA. Publications on wildlife roadkill have increased in recent years, with a greater number of PPA studies than NPA studies (W = 618, p = 0.5992). Mammals are the most-studied group (n = 108), followed by reptiles (n = 79), birds (n = 73) and amphibians (n = 58). Most of the studies took place in the Cerrado (54) and the Atlantic Forest (45), where are the greatest number of surveyed PAs, greatest number of PAs and greatest number of PAs without studies. Only 18 papers suggest specific mitigation measures for the study site. The increase in PPA studies is positive, but researchers need to increase contact with PA managers to produce scientific knowledge and develop more efficient mitigation measures for these areas. We encourage increased surveying of roads near PAs, involvement of researchers with environmental agencies, and more studies with small animals.

Keywords: Local extinction; Biodiversity; Road Ecology; Wildlife Vehicle Collisions.

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SIGNIFICANCE STATEMENT

Brazil is a mega-biodiverse country and has a large number of protected areas with loss of wildlife diversity run over by motor vehicles. In this manuscript, we mainly seek to understand which protected areas are neglecting the problem of roadkill fauna. For this, we surveyed all works published up to the year 2021 as well as used geospatial tools to identify where these protected areas are located and which region deserves more attention from environmental managers. So, we believe that this work is fundamental to indicate the spatial gaps in the death of wild vertebrates particularly vulnerable to roadkill events in protected areas as well as the protected areas that need more attention from researchers, environmental managers, and government. With our review, it is also possible to understand how work on roadkill fauna is being prepared, which allows for drawing up plans for the future of research, especially research involving protected areas directly affected by roads. Thus, the work also indicates knowledge gaps that will serve as a guide for government environmental managers.

INTRODUCTION

Anthropogenic activities cause major problems for protected areas, including through the fragmentation of adjacent habitats, which reduces patch connectivity, and contamination of protected areas as a result of pollution in surrounding areas (Lovejoy 2006). Protected areas (PAs) are affected by a range of impacts, such as illegal harvesting of plants, poaching, invasive species, fires, deforestation and construction of roads (Schulze et al. 2017). Furthermore, PAs located near roads are more likely to be colonized by alien species and to be deforested, due to the ease of access (Mcdonald et al. 2009; Pfaff et al. 2015). Other effects of roads on PAs include habitat loss, alteration of the biotic community, changes to species' behavior, edge effects, and the division and isolation of populations (Spellerberg 1998).

Roads are one of the main factors that isolate PAs, by restricting the movement and dispersion of animals (Newmark 2008). This problem is even worse with more developed roads (i.e., 4-lane paved roads are more developed than single-lane paved roads, which are more developed than dirt roads), which increase the risk to PAs and reduce the efficiency of PAs closer to roads in maintaining biodiversity (Lupinetti-Cunha et al. 2022). Thus, the expansion of the road network puts these areas at risk, as it tends to favor deforestation and habitat loss (Aguirre et al. 2021), mainly by making the regions more accessible, which drives deforestation (Barber et al. 2014). Furthermore, construction of roads leads to an increase in the number of collisions between vehicles and wild animals (Oddone-Aquino and Nkomo 2021).

Roadkill is the most visible negative effect of roads on biodiversity (Forman and Alexander 1998; Ferreira et al. 2023; Oliveira et al. 2023), and one that can cause major environmental impacts, such as extinctions of local populations (Barrientos et al. 2021; Tres et al. 2024). Therefore, it is considered a major problem for PAs (Collison et al. 2019). Roadkill can increase the loss of biodiversity in PAs (Bager et al. 2016), as they tend to have a higher diversity than unprotected areas and, consequently, higher collision rates than other areas (Garriga et al. 2012; Kioko et al. 2015). Thus, due to the serious threat and likelihood of occurrence it is essential to monitor roadkill on roads near PAs (Saranholi et al. 2016), especially in a megadiverse country such as Brazil, which has great biodiversity, including two conservation hotspots (Rylands and Brandon 2005).

We have numerous examples of the occurrence of wildlife-vehicle collisions in PAs in Brazil. One study in the region of the Sooretama Biological Reserve showed that the BR-101 highway offers a major risk to the local population of Lowland Tapir (*Tapirus terrestris*, Mammalia, Perissodactyla), given that this site is considered one of the last refuges for viable populations of this species in the Atlantic Forest (Banhos et al. 2021). Diniz and Brito (2013) state that the death of Giant Anteater (*Myrmecophaga tridactyla*, Mammalia, Pilosa) by roadkill is the greatest threat to population viability in the Brasília National Park, located in the Cerrado. Smaller animals are also strongly affected by the roads that surround protected areas, such as the Sooretama Biological Reserve, which has one of the highest rates of roadkill bat biodiversity in the world (Damásio et al. 2021).

Considering the impact of roads on PAs (Garriga et al. 2012; Bager et al. 2016) and the scarcity of knowledge about biodiversity inside and outside of PAs in Brazil, new steps need to be taken to maintain the efficiency of PAs in maintaining biodiversity (Oliveira et al. 2017). Thus, we need to understand what is already known about roadkill inside and outside of PAs in order to suggest steps to take in both environments.

Given the above, the present study aimed to analyze the research on roadkill and to compare studies of areas near to and far away from PAs in Brazil. We believe that the majority of studies occur near to PAs, but they are limited to the more developed regions because these regions have the easiest access. We presumed that studies near to PAs would suggest mitigation measures, and we analyzed the presence of these measures. Finally, we pointed out gaps in areas without surveyed roads near to PAs.

MATERIAL AND METHODS

Bibliographical research

We created a database of scientific papers about wildlife roadkill in Brazil published up to 2021 and available in journal databases and on a curriculum platform (Table 1). We used broad keywords (Table 1) because we preferred to refine the data manually. We prioritized Google Scholar due to the efficiency of the platform (Prins et al. 2016; Martín et al. 2018), carrying out two searches, one in Portuguese and one in English. To increase the scope of papers published in English we also searched on Scopus (see Martín et al. 2018). We chose SciELO because it grouped papers from Brazilian journals, Plataforma Lattes because it grouped the Curriculum (CV) of researchers from Brazil, and the list of papers available on the Rede Brasileira de Especialistas em Ecologia de Transportes (REET Brasil - Brazilian Network of Specialists in Transport Ecology).

We only included scientific articles, excluding books, monographs, theses, dissertations, and abstracts. We only considered papers that focused on surveying or monitoring of wildlife roadkill, i.e., those papers that actively searched for animals that had been killed on a stretch of road, either with roadkill as the main focus or as a secondary method for sampling fauna in a region. As a result, we discarded papers related to the use of roadkill specimens for biological description, taxonomy, parasitology, and occurrence notes. Duplicate papers were excluded.

Table 1. Description of how the research was carried out, including databases, predictors, date, and results before selection of the papers. Databases are organized according to the research date.

Databases	Link	Search	Date	Results
REET Brasil	https://reetbrasil.wixsite.com/reetbrasil	The site provides a list of papers	07/04/2022	42 papers
SciELO	https://www.scielo.br	"roadkill"	07/04/2022	28 papers
Google Scholar	https://scholar.google.com.br	"roadkill" + "Brazil"	12/04/2022	2130 papers
		"fauna atropelada" + "Brasil"	03/05/2022	196 papers
Scopus	https://www.scopus.com	"Roadkill AND Brazil", in title, abstract and keywords	15/04/2022	99 papers
Plataforma Lattes	https://lattes.cnpq.br	"Roadkill"	16/04/2022	160 curriculum / 164 papers

Collection and analysis of literature data

Firstly, we defined which papers surveyed roads with direct influence on a PA. For this, we considered the road to have a 1 km radius of direct influence (see Ibisch et al. 2016; Lupinetti-Cunha et al. 2022), and all studies conducted with protected areas within this radius were considered as being near to protected areas (Presence of Protected Areas – PPA), while studies with no PA within a 1 km radius of the surveyed road were considered as being far from a PA (No Protected Areas – NPA). We selected the PPA studies by using Google Earth to draw the surveyed routes, which were exported in ".kmz" and opened in QGIS 3.16.10 (QGIS 2021). We then created a 1 km zone around the surveyed roads and recorded any PAs. We used the outlines of Brazil's Protected Areas provided by the Ministério do Meio Ambiente (MMA - Ministry of the Environment), which contains every PA in Brazil, updated with PAs created up until the beginning of 2022 (BRASIL, 2022). After selecting the papers, we collected the information described in Table 2.

Table 2. Description of literary variables collected and analyses performed. PA = Protected Area; PPA = Presence of Protected Areas; NPA = No protected Areas.

Variable	Description	Analysis
a. Presence of PAUsed to record the PPA and NPA papers.	The minimum distance between the surveyed road and PAs. We counted the PAs within a 1 km radius of the road (see Ibisch et al. 2016; Lupinetti-Cunha et al., 2022), and separated the papers into two categories:- With the presence of protected areas (PPA)- No protected area (NPA)	We used QGIS and Google Earth to measure distance and group papers into two categories: PPA and NPA
b. YearUsed to analyze the number of studies per year, and to test the difference between the NPA and PPA studies over the years.	Paper publication date.	- Correlation between year and total number of papers, PPA and NPA- Mann-Whitney to analysis the difference in production between PPA and NPA papers
c. ApproachesUsed to find out the topics studied in the articles, comparing the PPA and NPA studies to see which of those groups used more approaches, and to test whether the papers with more approaches were in the journals with highest IF.	What approaches the papers adopted, divided into:- Descriptive, when the paper only described roadkill species and the state of conservation;- Temporal, when analysis was performed to compare seasonality;- Spatial, when analysis was carried out to find points with higher rates of wildlife roadkill;- Landscape, when analyzing the landscape around the road or the carcasses;- Modeling, when using some model of occupation or situations (e.g. to predict roadkill or create landscapes to study animal movement);- Method proposal, when studying and proposing methods for future research;- Biological aspects, when considering aspects of the species, such as size, diet and behavior;- Mitigation, when studying the efficiency of mitigation measures;- Monetary, when studying the expenses and costs of roadkill animals;- Fauna in the surroundings, when the roadkill species and the species around the roads were analyzed;- DNA analysis, when DNA analysis was performed for populations or to identify species;- Carcasses, when the focus was on aspects of carcasses, such as length of time on the road.	- PERMANOVA to see the difference between PPA and NPA - Correlation between the number of approaches and IF, to discover whether more approaches are present in studies with higher IF (see "e")- Graph with the approaches per year
d. Biological groupUsed to compare the number of papers per group, and find the most studied group in PPA and NPA.	The groups studied in each study. We recorded the number of studies per group, therefore, a paper that studied all groups was counted once for each group. We considered four groups:- Amphibians;- Birds;- Mammals;- Reptiles.	- Mann-Whitney for group, to test the difference between PPA and NPA studies, using the number of studies per group- Maps of groups and biomes
e. Journal Impact Factor (IF)Used to find the possible correlation between IF and approaches	Journal's impact factor over the last two years, using the "Academic Accelerator" website in October 2022 (https://academic-accelerator.com/)	- Correlation between the number of approaches and impact factor (see "c")- Mann-Whitney between IF and PPA and NPA

f. SurveyingUsed to find out how the papers collected data, and verify differences in the PPA and NPA surveyed roads	We analyzed the type of surveying, separated into:- Systematic, when it followed a surveying routine (e.g., monthly or weekly);- Not defined, when the authors are not clear about the surveying- Occasional, when the authors reported surveying being occasional or sporadic;- Various methods, when the authors collected data over several periods in different ways;- Not systematized, even when collected periodically, if it was not systematized and changed several times over a week or month, depending on the authors;- Seasonal, involving data collection campaigns using the seasons as a parameter.	- We organized the data in a table, separating PPA and NPA
g. Surveying time Used to find out the surveying time for NPA and PPA	We counted the number of months when surveys were conducted to find the overall data collection time for the study, and analyzed the difference between NPA and PPA. If the study collected data once or twice in a month we considered it as a sampled month, regardless of the number of times the road was covered.	- Mann-Whitney between PPA and NPA- Boxplot graphic
h. Biomes	We separated the papers by biome (Amazonia; Caatinga; Cerrado; Atlantic Forest; Pantanal; Pampa) to find the number of works per biome	- Number of papers in each biome to create a map
i. Surveyed roadsUsed to locate the areas where the studies collected data	We traced the route covered by the authors to record where the research took place	- We used Google Earth to draw the routes, which were saved in ".kmz" and exported to QGIS.- Afterwards, we created a map displaying the surveyed road
j. Departure and arrival points	We marked the start and end points of each surveyed route to find the most-studied areas using spatial analysis	- Points used to create the heatmap (Kernel) with QGIS
k. First year of surveyingUsed to find the difference from the year of the PA's creation	The year of the first survey for each study	- We calculated the difference between the year of creation of PAs within a 1km radius and the initial year of data collection in the area (see "l")
l. Year of PA's creationUsed to see the difference from the first year of surveying	Year informed in the PA outlines provided by the Brazilian Ministry of Environment	- We calculated the difference between the year of the PA's creation and the first year of surveying in the area (see "k")
m. Authors' affiliationUsed to find the involvement of government agents in the research	Authors' institutions used to identify studies involving government agencies, e.g., Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA - Brazilian Institute of the Environment and Renewable Natural Resources) and Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio - Chico Mendes Institute for Biodiversity Conservation)	- Description of papers with author from government agency
n. Mitigation measures	Analysis of which measures were recommended in the conclusion and/or final considerations	- We organized the data in the form of a table

We performed all tests using R 4.1.1 (R Core Team, 2021), including PERMANOVA (Anderson 2001) with 9999 permutations, using the "vegan" package (Oksanen et al. 2022). We created a boxplot with the "Ggplot2" package (Wickham 2016). The other tests (correlation and Mann-Whitney) did not require a package. We considered the 5% confidence limit for all tests.

Spatial analysis

We summarized the papers by biome: Amazonia, Atlantic Forest, Caatinga, Cerrado, Pampa and Pantanal. For this, we used the outlines available on the website of the Instituto Brasileiro de Geografia e Estatística (IBGE - Brazilian Institute of Geography and Statistics) (IBGE 2019), overlaid the routes covered by each study, and manually recorded which regions the surveyed roads were located in. As such, some papers surveyed two or more biomes and were considered for all of the biomes that they surveyed. The data were organized in maps that indicate the number of studies per biome and per group (birds, mammals, amphibians and reptiles). This data enables us to find which regions contained the most PPA studies and which animal groups within PAs are most studied by biome. We used the spatial location of the start and end points of the routes covered by the studies to create a kernel density map with a radius of 130 km and pixel size of 300m, because the average route length surveyed per study was 130km. This indicated whether there were locations covered by more than one study. To find the longest length of road surveyed, we intersected the outline of roads in Brazil provided by the IBGE (2022) and the outline of roads surveyed in the country up until the year 2021. The outcome of the analysis is a map with points of intersection between the two outlines (Brazilian roads and surveyed roads), indicating where the lines cross. Thus, locations with a greater number of lines crossing each other had more points. We used the intersection outline to perform kernel density analysis, with a radius of 500 km and a pixel size of 300m. We conducted all the spatial analysis and created the maps in QGIS 3.16.10 (QGIS 2021).

Analysis of PAs in the area of influence of surveyed roads

We counted the number of PAs within the area of direct influence of the roads for each study and classified them by type (federal, state and municipal). We organized the data in a table containing the number of PAs in the area of influence in each biome. We also computed the existing PAs in each biome using the outlines mentioned above and added them to the table. We recorded the first year of the first paper carried out in each biome and compared it with the year of creation of the PAs within a 1 km radius of each study. This provided us with information as to when the surveying started and when the PAs were created, if there were any studies before the creation of the PAs, and how long after creation the roads closer to the PAs were surveyed. These data were organized in a table.

RESULTS AND DISCUSSION

RESULTS

We found 237 papers related to wildlife roadkill in Brazil, with the first record being from 1988. However, only 126 papers actually involved a survey of wildlife roadkill (Supplementary Table S1). The remaining papers related to wildlife roadkill are publications in several areas, including data reviews, analysis of the length of time the carcass remains on the road, parasite studies and use of roadkill animals to obtain physiological and morphological data.

Publications about Brazilian wildlife roadkill

Of the 126 studies that surveyed wildlife roadkill, 57% (n = 72) collected data on roads with at least one PA within a radius of 1 km. The number of studies with protected areas within a 1 km radius (PPA) and the number of studies without protected areas within a 1 km radius (NPA) have both increased. However, roads near to PAs were surveyed more than roads far away from PAs, although this difference is not statistically significant (W = 618; p = 0.5992). We noticed two moments when there was an increase in the number of studies: after 2014, with the creation of the "Urubu mobile app" and after 2019, with the creation of the Rede Brasileira de Especialistas em Ecologia de Transportes (REET Brasil - Brazilian Network of Specialists in Transport Ecology). Other important events that may have encouraged research in the area include the publication by Forman and Alexander (1998) that serves as a reference for road ecology, the enactment of the Law establishing the Sistema Nacional de Unidades de Conservação da Natureza (SNUC – Brazilian System of Protected Areas) and the creation of the Centro Brasileiro de Estudos em Ecologia de Estradas (CBEE - Brazilian Center for Studies on Road Ecology) (Figure 1).

We observed that in 2016 (n = 7), 2017 (n = 7), 2018 (n = 8) and 2021 (n = 10) studies were published with different approaches, in addition to the description and listing of wildlife roadkill and spatiotemporal analyses. However, publications with descriptive and temporal analyses appeared every year. Only in 2018 and 2021 were there publications that used data collection on wildlife roadkill to present methodological proposals, while DNA analyses also appear only in two years: 2016 and 2017 (Figure 2).

Mammals were the most studied group (n = 108), followed by reptiles (n = 79), birds (n = 73) and amphibians (n = 58). When comparing the PPA and NPA studies, it was observed that only mammals have more studies near to PAs, but the difference between the groups is not significant (Table 3).

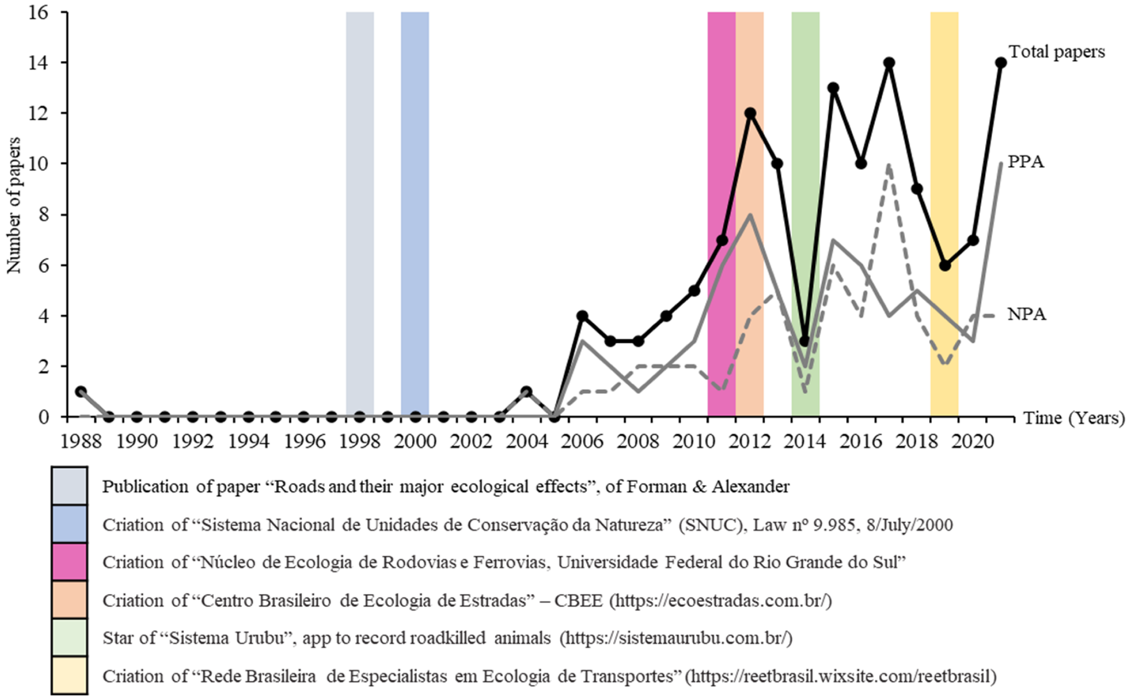

Figure 1: Number of studies that collected data on wildlife roadkill in Brazil published up until 2021 and some important events, with total number of studies (Total studies), only studies from near to protected areas (PPA) and only studies far from protected areas (NPA).

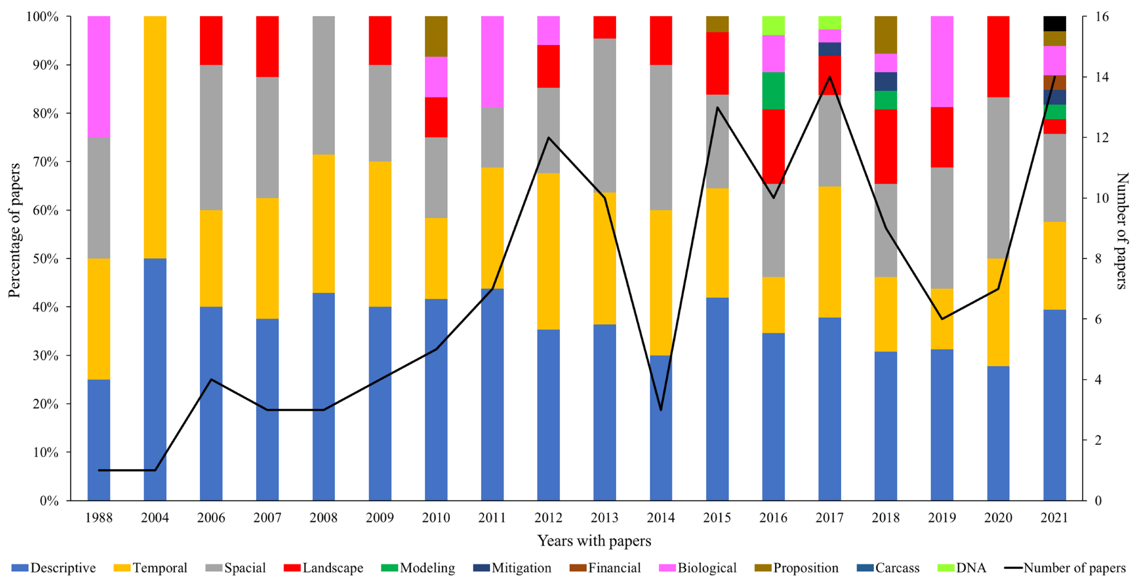

Figure 2: Demonstration of the approaches carried out by papers on wildlife roadkill in Brazil up until 2021, by year. Years missing from the graphic indicate that there were no publications.

Table 3. Description of the studies conducted near to (PPA) and far from (NPA) of protected areas according to the variables (biological group, approaches, journal impact factor, sampling, and data collection time) and the test value.

Variables		**PPA papers (n = 72)**	**NPA papers (n = 54)**	**All papers**	**p-value**	**Test value**	**Test**
Biological group (Frequency)*	Amphibian	25	33	58	882	588.5	Mann-Whitney
	Bird	35	38	73	582	617.5	
	Mammal	58	50	108	822	595.0	
	Reptile	38	41	79	626	543.0	
Approaches studied in the papers (%)	1 approach	13.9	14.8	14.3	0.0852	F(1, 124) = 2.4313	Permanova**
	2 approach	26.4	38.9	31.7			
	3 approach	40.3	35.2	38.1			
	4 approach	18.1	11.1	15.1			
	5 approach	1.3	0	0.8			
Journals Impact Factor (IF)***	Minimum	385	347	347	0.0002	1226.5	Mann-Whitney**
	Maximum	7963	4677	7963			
	Average	2290	1370	2020			
	Standard deviation	2190	1310	2000			
Sampling(%)	Systematic	68.06	74.07	70.63	-	-	-
	Not defined	9.72	7.41	8.73			
	Occasional	8.33	5.56	7143			
	Various methods	8.33	5.56	7143			
	Not systematized	4.17	1.85	3175			
	Seasonal	1.39	5.56	3175			
Time collected (months)	Minimum	3	2	2	0.00002	2798.5	Mann-Whitney**
	Maximum	120	108	120			
	Average	32.9	15.6	25.5			
	Standard deviation	28.1	18.1	25.7			

* We use frequency because a paper may have worked with more than one group;
** Test performed between PPA and NPA papers;
*** Papers without IF were disregarded.

Most of the studies used a descriptive approach, usually followed by a temporal and/or spatial approach, with the same pattern observed for studies inside and outside PAs. The studies described the species most often killed on roads and the conservation status of these species, in addition to temporal analyses to indicate in which season more carcasses were recorded and spatial analyses to indicate points of greater risk of collision with animals. This last factor was sometimes related to landscape analysis, as a predictor of roadkill (Table 3). Only one study addressed the financial costs of wildlife roadkill, with an emphasis on the expense for drivers (see Ascensão et al. 2021). There was no difference in the composition of analyses between the PPA and NPA studies (Table 3), and there was a low correlation between the number of approaches and the impact factor (IF) of the journals in which the papers were published ($r = 0.365$; $p < 0.000026$).

We observed that there was a significant difference between the impact factor (IF) of the journals in which the PPA and NPA studies were published (Table 3). The PPA studies were published in higher IF journals (= 2.29; sd= 2.19) than the NPA studies (= 1.37; sd = 1.31). While 32% (n = 23) of PPA studies did not have IF, 61% (n = 33) of the NPA studies did not have IF. Most PPA papers involved three or more approaches, while most NPA papers involved two approaches.

Most of the 126 studies found (n = 89) collected data systematically (e.g., monthly or weekly). Of the others, nine made only occasional records, generally taking advantage of trips for other purposes, nine varied the collection methods, four did not systematize the data collections, four made collections during campaigns that considered the seasonality of the region and 11 studies did not make it clear how data was collected (Table 3). The PPA studies showed the same result, with the majority (68.06%) performing systematic sampling.

In general, studies involving wildlife roadkill in Brazil collected data over a total period of between 2 and 120 months (Table 3). However, we observed that the difference between the total data collection time is greater for PPA than NPA studies ($W = 2798.5$; $p = 0.00002$). Thus, we noticed that there is a greater variation (Figure 3) in the collection time between PPA papers.

Most of the studies took place in the Cerrado (54 papers) and Atlantic Forest (45 papers), with a greater overlap in the Cerrado (indicated by the red points in Figure 4). On the other hand, the studies carried out in the Atlantic Forest are more spread out across the biome geographically. The Pampa has a great area covered by researchers with only 21 papers. Amazonia (09 papers), Caatinga (08 papers) and Pantanal (08 papers) have a smaller number of studies.

Monitoring wildlife roadkill in Brazilian protected areas

Although Amazonia has a high number of visible PAs on the map (Figure 5A), the Atlantic Forest has the greatest number of PAs (1183 PAs). The Cerrado is the region with the second most PAs (n = 358), followed by Amazonia (n = 301), Caatinga (n = 184), Pampa (n = 27), and Pantanal (n = 19). The Cerrado and Atlantic Forest also have a high density of roads (Figure 5B). Furthermore, the largest extension of surveyed roads is found in the Southeast of Brazil, between the Cerrado and Atlantic Forest, in the state of São Paulo (Figure 5C and 5D).

Amazonia, Caatinga and Pantanal make up a minority of studies, including a lower number of studies in PAs (Figure 6A). The Cerrado and Atlantic Forest hosted most of the studies, with 54 and 45 studies respectively (Figure 6A). These two biomes also have the most studies in Protected Areas (Figure 6B). When we separated the papers by biological groups we found the same result, with more studies in the Cerrado and Atlantic Forest in PAs for amphibians (Figure 6C), birds (Figure 6D), mammals (Figure 6E) and reptiles (Figure 6F). We observed that half of the papers about amphibians (Figure 6C) and birds (Figure 6D) are from the Cerrado.

We found 1054 PAs within a 1 km radius of Brazilian roads. Of these PAs, 126 had nearby roads surveyed (Supplementary Table S2), 26 of which are municipal, 66 are state and 34 are federal PAs. Most of the PPA studies are located in the Atlantic Forest (n = 33) and Cerrado (n = 30) regions, with the highest number of PAs within the direct influence area (1 km radius) of surveyed roads (Table 4). We found 992 PAs directly affected by roads that have not yet been surveyed, most of which are in the Atlantic Forest and Cerrado. As such, the Atlantic Forest has the greatest number of PAs near to roads that have not yet been surveyed (Supplementary Table S3), representing 57% of its PAs, followed by the Caatinga with 49% of its PAs near to roads that have not been surveyed, Pampa (48%), Amazonia (29%), Cerrado (18%) and Pantanal (15%).

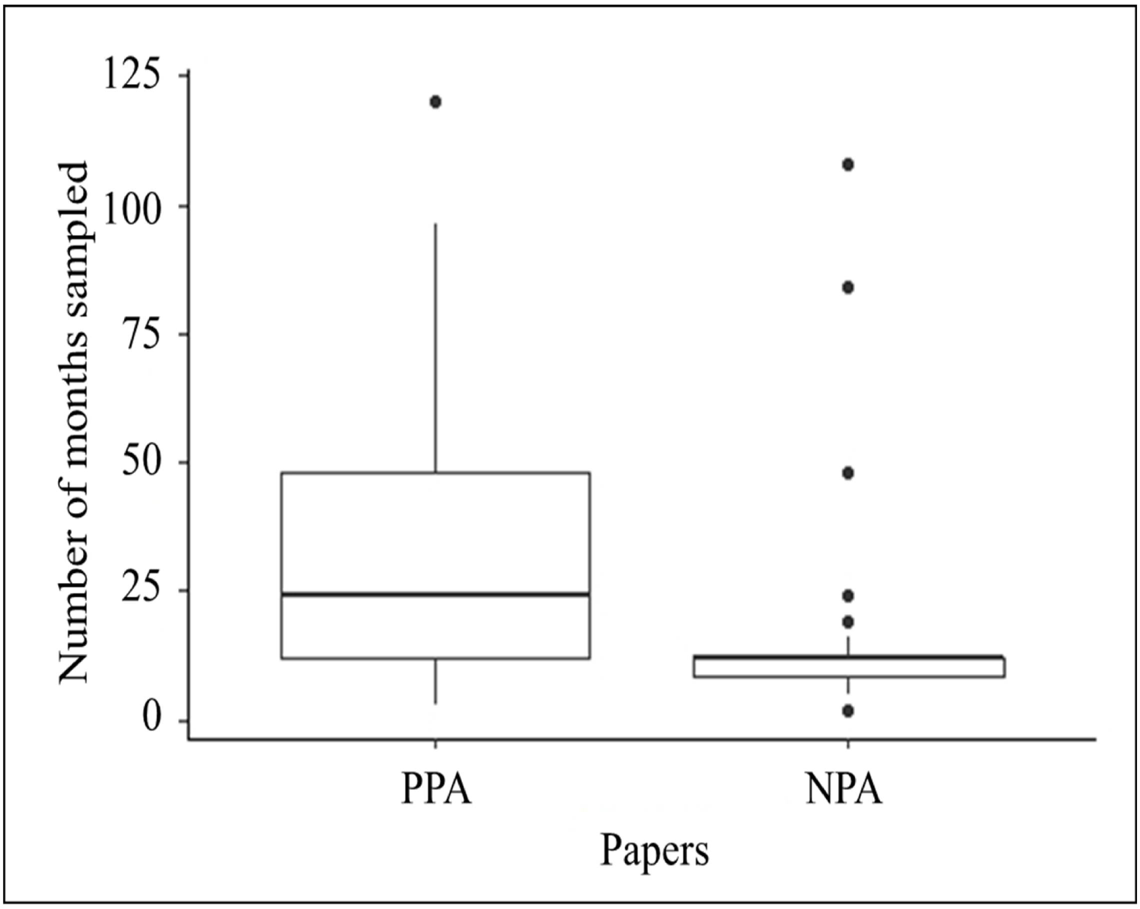

Figure 3: Number of months sampled for studies that surveyed wildlife roadkill in Brazil, near to (PPA) and far from (NPA) protected areas.

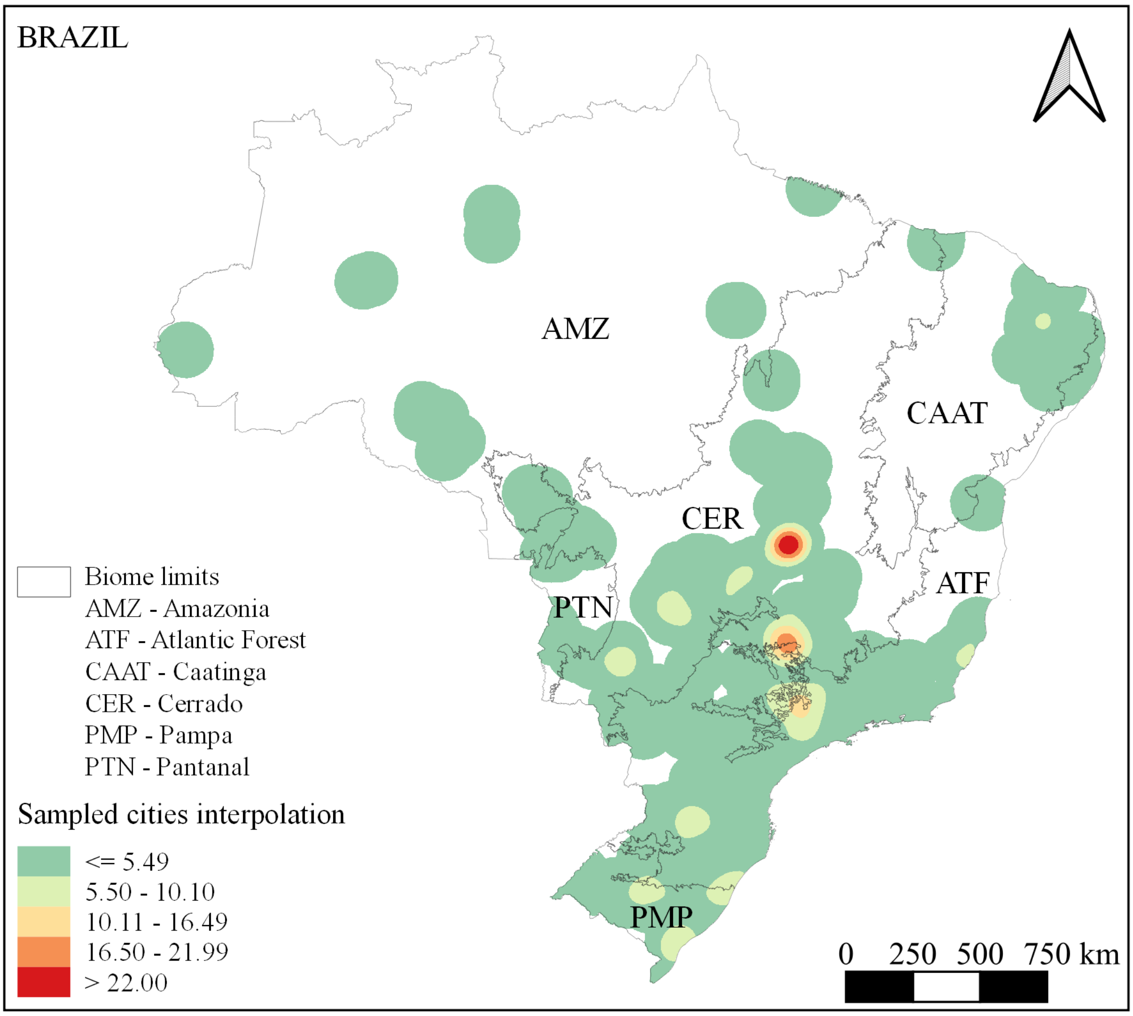

Figure 4: Interpolation of the starting and ending points of the routes where wildlife roadkill was surveyed up until 2021 in Brazil. Red regions indicate locations that have more published studies.

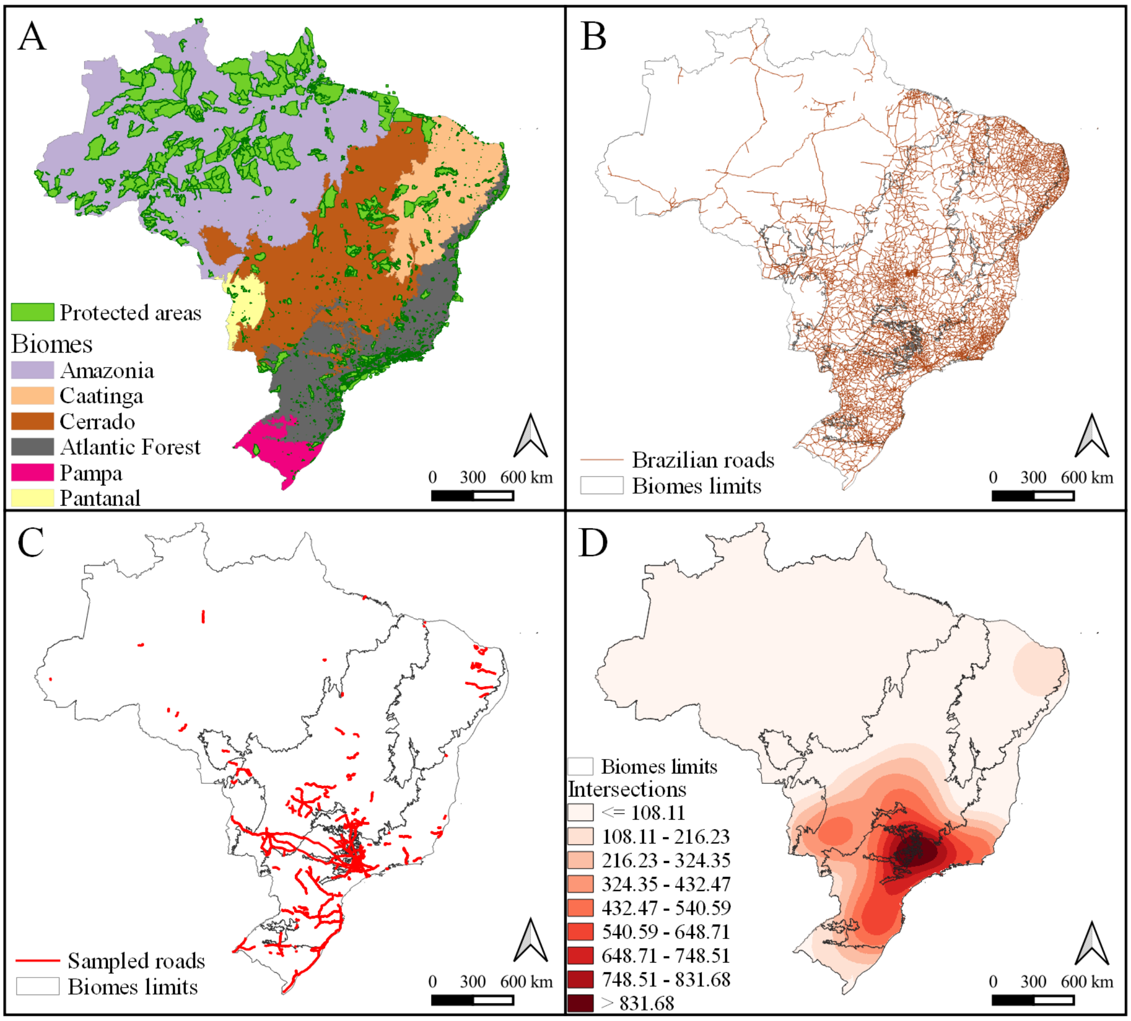

Figure 5: Roads surveyed in Brazil to 2021. A: Biomes and protected areas in Brazil. B: Roads in Brazil. C: Surveyed roads. D: Interpolation of the intersection between meeting points of roads in the country and roads surveyed to 2021. Biomes represented in colors that are easier to see for people with color vision deficiency (https://colorbrewer2.org).

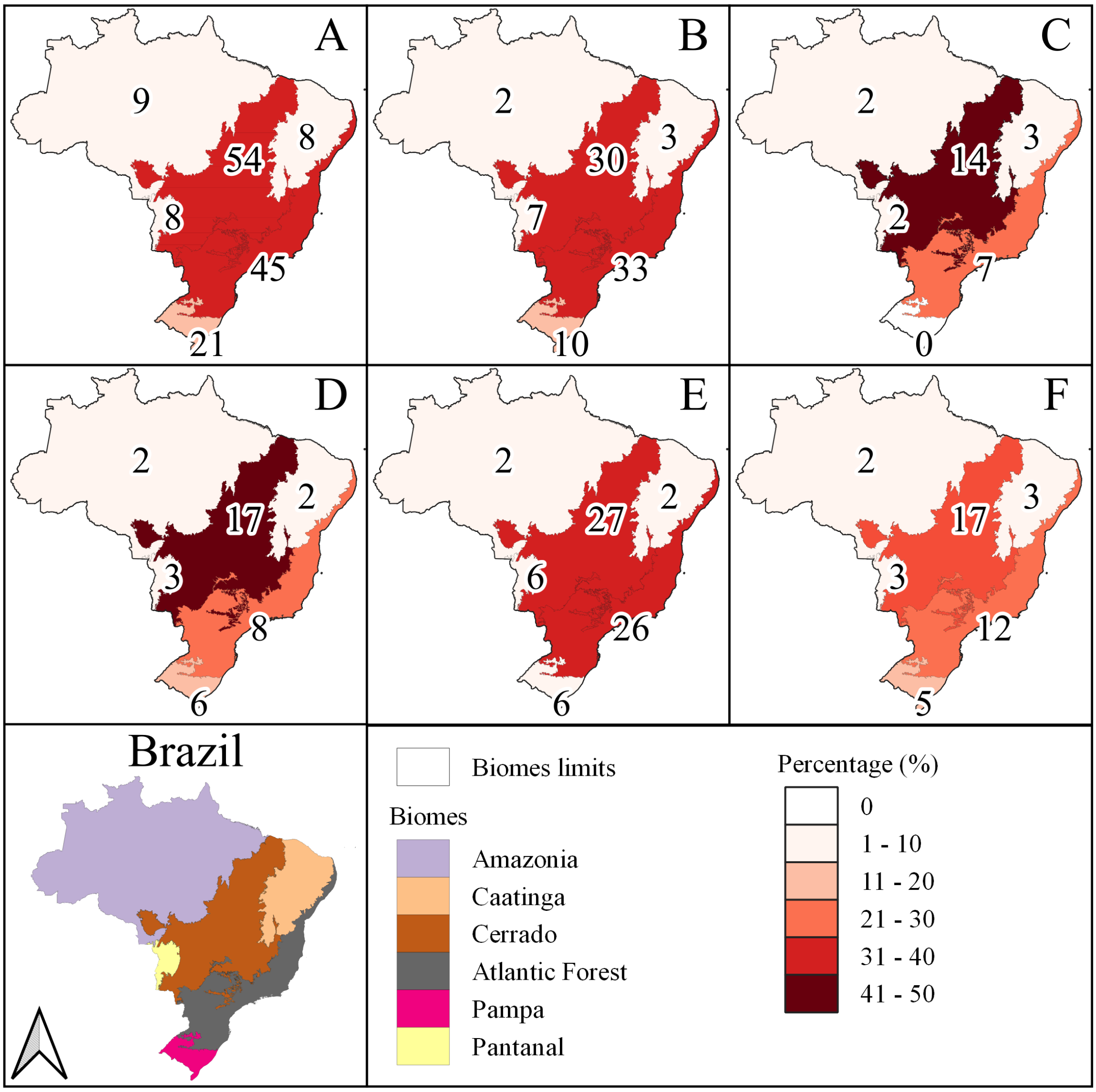

Figure 6: Number of studies (represented by the numbers on the maps) that collected wildlife roadkill data in Brazil and were published up until 2021, separated by biome, in which we have: total number of studies (A); studies that surveyed roads within a 1km radius of protected areas (B), with: amphibians (C), birds (D), mammals (E) and reptiles (F). Scales in percentage up to 50%, because this is the highest value. Biomes represented in colors that are easy to see for people with color vision deficiency (`https://colorbrewer2.org`).

Table 4. Number of studies about wildlife roadkill in Brazil, classified according to the Biome and the presence of protected areas. PAs = Protected areas; PPA = Presence of Protected Area; NPA = No Protected Area.

Biomes	**Number of papers**			**Number of PAs within a 1km radius of sampled roads***	**Number of PAs**	**Number of PAs without papers****
	PPA	NPA	All			
Amazonia	2	7	9	3	301	87
Atlantic Forest	33	12	45	78	1183	566
Caatinga	3	5	8	5	184	90
Cerrado	30	24	54	38	358	180
Pampa	10	11	21	6	27	13
Pantanal	7	1	8	1	19	3

Legend: *As there are PAs that are in more than one biome, the sum will not be the same as the total number of PAs studied in the country. **Number of PAs taken from the shape used for the previous analyses.

Most PAs had their surrounding roads surveyed after their creation (average = 18 years later; sd = 12.6; minimum = 1 year later; maximum = 64 years later), although some were surveyed before (average = 5 years before; minimum = 2 years before; maximum = 8 years before) and only two were surveyed in the same year of creation. We observed that most PAs were surveyed 13 years after their creation and only 14 areas that are currently protected areas had nearby roads surveyed before they were established (Figure 7).

Of the 126 published papers, only 16 were co-authored by a government agency, of which 13 were near to PAs. In other words, approximately 18% of the PPA papers involved the participation of an employee of a government agency. The institutions mentioned are Instituto Brasília Ambiental (IBRAM - Brasília Environmental Institute) (n = 5), ICMBio (n = 3), Municipal Departments (n = 2), State Departments (n = 2), IBAMA (n = 2), Polícia Rodoviária Federal (PRF - Federal Highway Police) (n = 1), and Instituto Nacional da Mata Atlântica (INMA – National Institute of the Atlantic Forest) (n = 1).

Only 18 (25.0%) of the PPA papers made specific suggestions, indicating the location or period and the mitigation measure that should be considered for the area (Table 5). However, 33 (45.8%) PPA papers made general suggestions, without discussing the specific problem, or only suggested further research in the area or topic. Unfortunately, 21 (29.2%) of PPA papers do not recommend or suggest mitigation measures.

DISCUSSION

The number of wildlife roadkill studies in Brazil has increased. The majority of these studies have surveyed roads near to PAs, performed descriptive and temporal analysis, collected data systematically, and focused on mammals as the main studied group. Although there was no significant difference between the approaches carried out by the PPA and NPA studies, the PPA studies addressed a greater diversity of topics. Even though we have found a wider range of approaches in publications in recent years, descriptive and temporal analyses have always been present. The number of approaches used in a paper does not seem to make a difference to the impact factor of the journal in which it was published, but we observed that PPA papers collected data over a longer period of time, on average, and are published in more reputable journals, given that more than half of the NPA studies are published in journals with no impact factor.

The increase in publications that surveyed wildlife roadkill reflects the increased interest in understanding and resolving the problem in Brazil. There are three reviews that point to the growing trend of studies on road ecology and wildlife roadkill in Brazil over the last two decades (Bager et al. 2007; Bager and Fontoura 2012; Oliveira et al. 2020). Currently Brazil has more publications on the subject than any other country in Latin America (Pinto et al. 2020) and ranks third in the world for productions on road ecology (Oliveira et al. 2020). The greater number of roads surveyed in the vicinity of PAs is a positive point for the country, coinciding with a need pointed out by Bager et al. (2016), i.e., to understand the impacts of road structures in areas that are essential for biodiversity conservation. Thus, more published papers implies not only that more areas are surveyed, but also that there is an increase in understanding of the real impact of road structures on Brazilian fauna, enabling us to establish recommendations for better procedures for mitigation of accidents.

Most papers only adopt a descriptive approach. These descriptive data can be of great importance for the area, especially in a country like Brazil that has a large land area. However, there is a need to understand the interactions between roads, animals and plants (Oliveira et al. 2020). Studies that use spatial analysis to identify roadkill hotspots, for example, despite being very important, should be seen as basic works to suggest where the parameters of influence of roadkill can be analyzed (Pagany 2020).

Other approaches, such as economics, are also important and have been little explored so far. As we found only one study that is related to economic losses, we believe that this is an area for future study. The lack of studies that assess economic losses is a gap that has been pointed out since 2009 (Dornas et al. 2012). Collisions with larger animals can cause major material damage (Abra et al. 2019) and thinking about the economic losses is fundamental to drawing the attention of the population and public authorities to the problem. Another necessary approach is to incorporate DNA analysis into research on wildlife roadkill. This encompasses the ecological discussion, especially if associated with landscape or functional attributes and used to support locally specific conservation measures (Rodríguez-Castro et al. 2017).

Research conducted near PAs has used a greater number of different approaches. In addition to the descriptive approach of describing which species were affected, these studies also collected data over a longer time period and are published in more reputable journals. As the practice of occasional wildlife roadkill data collection is recurrent (Dornas et al. 2012), it is possible that the PPA papers have more robust data, hence their published in better journals. Despite the problems of occasional data collection, we believe that these studies are still important to increase the knowledge base regarding wildlife roadkill, which until now has been concentrated in the Atlantic Forest and the Cerrado.

However, before entering into more complex analyses, we need to standardize the data collection. Although most of the papers present data collected systematically, there are still studies that collect data occasionally or do not even make it clear how they have performed the data collection. It is common for studies to monitor wildlife roadkill on roads that are routes already traveled by the authors, whether for work or leisure (Dornas et al. 2012). Just like Dornas et al. (2012), we believe that these studies may present a large sampling error and impair the comparison of data in future research, especially if the monitoring is performed at higher speeds. This is a common sampling design flaw; the design should be created primarily based on the species we wish to study, as a flawed sampling design can impact the spatiotemporal patterns

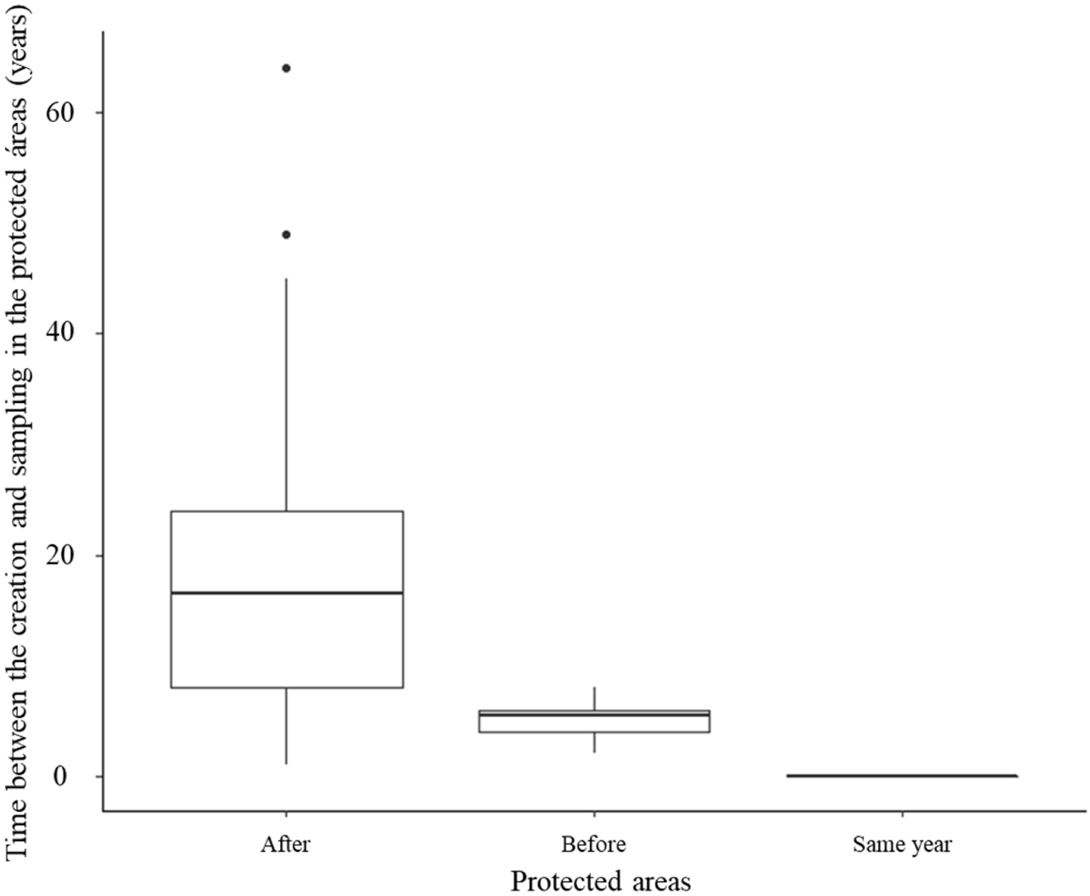

Figure 7: Difference between the first year of surveying for a study and the year of creation of the protected areas. Most roads were surveyed later (average = 18 years later; sd = 12.6; minimum = 1 year later; maximum = 64 years later), a few were surveyed before (average = 5 years before; sd = 1.8; minimum = 2 years before; maximum = 8 years before)..

observed (Silva et al. 2021b). Carcasses of larger animals persist for a long time on the road (Santos et al. 2011; Teixeira et al. 2013). Therefore detectability of carcasses is not the same for the researcher because large animals, e.g., some mammals, tend to be more easily spotted due to their greater body mass (Teixeira et al. 2013), therefore we need standardized data collection. In the case of amphibians, for example, monitoring on foot can be 25 times more efficient (Pereira et al. 2018). Driving at a higher speed can make it even more difficult to see smaller species (Dornas et al. 2012; Santos et al. 2016). So, perhaps it is because of the ease of spotting the carcasses that most studies have studied mammals, especially studies that involved travelling at the same time as data collection. In the other words, the large number of papers with mammals is a result of non-sampled works.

The majority of studies took place in the Atlantic Forest and Cerrado, both overall and by group (amphibians, birds, mammals and reptiles). The two regions not only have a greater extension of surveyed roads, but also more roads surveyed close to PAs (more than half of the surveyed PAs are in the Atlantic Forest). Spatially, the Cerrado-based studies are more sporadically distributed, with a greater number of studies in the same area. The Atlantic Forest-based studies are better distributed spatially, with not only more publications but also more surveyed areas. Pinto et al. (2020), demonstrated that in addition to most studies being in these two areas, the Cerrado regions stand out in relation to publications on biodiversity and mortality. In addition to these regions being responsible for the highest number of bird and mammal deaths (Medrano-Vizcaíno et al. 2022), they are also among the regions in which protected areas suffer most from lack of funding (Silva et al. 2021a). These aspect may contribute to reducing the chances of conducting studies and the possibility of implementing mitigation measures. As these two biomes have the largest number of PAs, descriptive studies may be helpful at first to increase knowledge about the diversity of roadkill animals. However, we emphasize the need to be careful with the sample design and to increase studies in PAs in other Brazilian biomes.

We also need to sample more roads in the Caatinga, for

Table 5. Suggestions for the conservation of species or for the mitigation of wildlife-vehicle collisions presented in the studies that surveyed roads close to PAs (PPA papers).

Suggestions in papers	Frequency in papers
Wildlife passage	25
Speed reduction	24
Road signs	19
Fences, like barriers to the animals	9
Environmental education	6
Environmental education programs for drivers in training	6
Improve the structures that exist on the highways (e.g., adaptation of culverts)	6
Monitor and supervise mitigating measures	5
Clear the road and prune the vegetation	3
Monitor the surrounding population	2
Reduce traffic on roads within the protected area	2
Create a program for continuous monitoring of roadkilled fauna	1
Study the habitats and land use in the surroundings	1
Install light bulbs that are less attractive to insects	1
Use occupancy detection models to install measures	1
Requiring the study of roadkills focused on bats in some projects	1
More research about the topic to indicate a point measure	8

three reasons. First, approximately half of the PAs near roads have not been surveyed, and we need more knowledge about the impact of roads on these PAs. Second, the Caatinga includes areas where roadkill has a major impact on birds and mammals (Medrano-Vizcaíno et al. 2022). Third, recently a portion of this biome was classified as a priority for research with birds (Medrano-Vizcaíno et al. 2023).

The roads in the vicinity of state-run PAs have been surveyed more. This may reflect the increase in state-run PAs and greater investment in these areas by the states, unlike what happens with federal and municipal PAs (Vieira et al. 2019). However, the participation of PA managers and other environmental agencies was low, indicating little involvement of scientists with the government's environmental area. It is likely that budget constraints in PA administration and growing political pressure, which are major disadvantages for managers (Marque and Peres 2014), contribute to the low participation of government officials in these research and conservation projects. Although one of the SNUC's objectives is "to provide means and incentives for scientific research activities, studies and environmental monitoring" (Brasil 2000), we note that there is not always integration between researchers from universities with managers of protected areas or that there is hardly any execution and publication of studies by managers of Brazilian PAs.

As most of the sampling took place after the creation of the PA, we infer that there was no analysis before the creation of these areas, which makes it impossible to compare the effects before and after the presence of the PA. The objective of PAs in Brazil is to intervene to prevent biodiversity loss, but currently they are not able to ensure protection for the various Brazilian habitats, and therefore legislation must be strengthened both for breeding (Vieira et al. 2019) and for encouraging research in these environments. In addition, long-term studies are important to identify trends in biodiversity and ecosystem functions that support Brazilian conservation (Marques et al. 2022).

Finally, most of the papers made only generalized recommendations for mitigating accidents with wildlife in their conclusions, such as installing signs and fauna passages, and did not make specific recommendations for the mitigation of collisions in the study area. Even so, at least there were a large number of papers that cited mitigation measures.

CONCLUSION

Through our results we conclude that it is necessary to increase surveying on roads surrounding protected areas in Brazil, so that more basic science can be carried out. Despite the importance of listing wildlife roadkill, it is important that the studies begin to adopt new approaches and analyses to complement the research already published. These other approaches include evaluation of the financial cost of collisions with wildlife, DNA analysis and biological aspects of the species, so that we can deepen our knowledge about wildlife roadkill, mainly in protected areas, which are so little surveyed.

We believe that it is essential for government agencies to have more involvement in research in Brazilian protected areas, with more interaction between managers and researchers. The interaction of managers and researchers is essential for the proposition, implementation and monitoring of mitigating measures on roads within and close to PAs.

Research that suggests mitigation measures around the world is focusing on underpasses for animals and fences for large mammals, reptiles and amphibians (Rytwinski et al. 2016). As this is somewhat different from what papers in Brazil are recommending for specific areas (speed reduction and road signs for drivers), we suggest that further research should focus on the efficiency of these measures here in Brazil and complement them with studies to indicate efficient and cost-effective technological alternatives, in view of the current budgetary reality of low incentives for research and conservation of the environment in the country.

Although we found numerous studies in the Atlantic Forest and in the Cerrado, we would encourage sampling of more areas of these biomes, as there are still many PAs without surveyed roads. We also highlight the need for more studies in the Caatinga, due to the priority in understanding the real impact of roads in this region, mainly in PAs.

Finally, we encourage projects aimed at smaller animals, such as frogs and small mammals, to be implemented. This will enable us to understand the real impact of the roads on these groups. Another suggestion is to create a systematic way of informing the government and agencies that work with roads in Brazil. This could facilitate the installation of mitigation measures.

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DATA AVAILABILITY

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

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

CONTRIBUTION STATEMENT

All authors contributed to the study. JESM had the idea for the article, performed the literature search and data analysis, and AS drafted and/or critically revised the work.

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