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Spatial and temporal distribution of bat mortality on a highway in southeast Brazil

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Roads and highways can work as barriers to the movement of many species, thereby preventing the individuals from accessing feeding and reproduction sites and the immediate risk of colliding wild species with vehicles. Identifying the impacts of roads on wildlife can contribute to the establishment of actions that promote conservation. In Brazil, data on collisions between bats and vehicles are scarce and underestimated in the literature. We described bat roadkill from 2008 to 2019 on a stretch of the BR-040 highway, which crosses an area of Atlantic Forest. Roadkill species were identified and the sites with high collision frequencies were characterized. A total of 923 individuals of 57 species and five families of chiropterans were identified. Frugivore bats showed the largest number of affected individuals, with *Artibeus lituratus*, a common species in the study region, with the highest number of roadkills. The diet and foraging behaviour were the most likely factors explaining most of the bats killed on the highway. The highest roadkill rate was documented in the fall, and the critical points located nearby the APA Petrópolis and REBIO of Tinguá, environmental protection areas. We reinforce the need to mitigate these roadkills, ensuring that road systems, which constitute municipal, state and federal highways, are built to prevent major disturbance of habitat and displacement routes of these species. We believe in the need for mitigations, and considering the various species involved, we suggest speed bumps construction reducing the speed limit, installing bridges, and signaling the presence of wildlife, before the stretches identified as hotspots.

Los caminos y carreteras pueden funcionar como barreras para el movimiento de muchas especies, impidiendo así el acceso de los individuos a los sitios de alimentación y reproducción y el riesgo inmediato de colisión de especies silvestres con vehículos. Identificar los impactos de los caminos sobre la vida silvestre puede contribuir al establecimiento de acciones que promuevan la conservación. En Brasil, los datos sobre colisiones entre murciélagos y vehículos son escasos y subestimados en la literatura. Describimos murciélagos atropellados entre 2008 y 2019 en un tramo de la carretera BR-040, que atraviesa un área de Mata Atlántica. Se identificaron las especies de animales atropellados y se caracterizaron los sitios con alta frecuencia de colisión. Se identificaron un total de 923 individuos de 57 especies y cinco familias de quirópteros. Los murciélagos frugívoros mostraron el mayor número de individuos afectados, con *Artibeus lituratus*, una especie común en la región de estudio, con el mayor número de atropellamientos. La dieta y el comportamiento de búsqueda de alimento fueron los factores más probables que explicaron la mayoría de los murciélagos muertos en la carretera. La mayor tasa de atropellamientos se registró en otoño, y los puntos críticos se ubicaron en las cercanías de APA Petrópolis y REBIO de Tinguá, áreas de protección ambiental. Reforzamos la necesidad de mitigar estos atropellamientos, asegurando que los sistemas viales, que constituyen las carreteras municipales, estatales y federales, se construyan para evitar que se alteren el hábitat y las rutas de desplazamiento de estas especies. Creemos en la necesidad de mitigaciones y considerando las diversas especies involucradas. Sugerimos la construcción de topes para reducir el límite de velocidad, instalar puentes y señalizando la presencia de fauna, antes de los tramos identificados como hotspots.

Keywords: Chiroptera; collisions; mitigation; road ecology; southeast Brazil.

Introduction

The human population in recent decades has caused several changes in the environment, including road and highway construction that is currently a significant concern for biodiversity conservation (do Prado *et al.* 2006; Gumier-Costa and Sperber 2009; Bueno *et al.* 2015). Road impacts on wildlife include deaths from vehicle collisions, habitat destruction and fragmentation, barrier effect, edge effects and disturbances caused by light, noise and chemical pollution (Forman and Alexander 1998; Gibbs and Shriver 2002; Row *et al.* 2007; Kerth and Melber 2009; Stone *et al.* 2009; Zurcher *et al.* 2010; Berthinussen and Altringham 2012; Bueno *et al.* 2013; Esperandio *et al.* 2019). For several vertebrate species, some of the ecological consequences include a reduction in the gene flow between populations and a decrease in both migration rates and genetic diversity (Corlatti *et al.* 2009; Esperandio *et al.* 2019).

The order Chiroptera is the second largest among mammals, with more than 1,400 living species (Simmons and Cirranello 2020). In Brazil, this order is represented by 68 genera and 181 species subdivided into nine families (Garbino *et al.* 2020). The chiropterans play a fundamental role in maintaining the ecological processes of different vegetation domains, being excellent objects of study for characterizing habitats and their quality since they are also sensitive to changes (Fenton *et al.* 1992; Medellín *et al.* 2000;

Gorresen *et al.* 2005). However, most data on bat deaths on the roads comes from Europe, where several studies have been developed (Bafaluy 2000; Lesiński 2007; Gaisler *et al.* 2009; Kerth and Melber 2009; Berthinussen and Altringham 2012; Medinas *et al.* 2013; Ramalho and Aguiar 2020), while in Brazil, data on bat collisions are scarce (Pracucci *et al.* 2012; Pinheiro and Turci 2013; Almeida and Cardoso Junior 2014; Ceron *et al.* 2017; Valadão *et al.* 2018; Damásio *et al.* 2021; de Figueiredo Ramalho *et al.* 2021). The bat's low body mass, flight speed and nocturnal behavior make it difficult to find bat carcasses, resulting in the subsampling of the group in studies of road ecology (Bafaluy 2000; Lesiński *et al.* 2011; Berthinussen and Altringham 2012).

The bats forage using echolocation (Kalko *et al.* 1996; Siemers and Schnitzler 2000; Schaub *et al.* 2008) and hearing the sound of prey such as amphibians and insects (Marimuthu and Neuweiler 1987; Faure and Barclay 1992; Altringham and Kerth 2016). According to Schaub *et al.* (2008), the 'passive listening' strategy is adopted by bat species specialized in hunting arthropods in vegetation or on the ground, where other sounds of the environment mask the noises emitted by prey; thus, the sounds produced by cars can compromise the echolocation of bats nearby, reducing their feeding effectiveness. Therefore, roads act as barriers, reducing the bats' ability to access suitable places to forage or shelter, thus being a concerning factor, especially regarding migratory species (Zurcher *et al.* 2010; Lesiński *et al.* 2011; Ceron *et al.* 2017; Claireau *et al.* 2019).

Although some studies show that bats recognize vehicles as a threat (Baxter *et al.* 2006; Zurcher *et al.* 2010; Altringham and Kerth 2016), many others show that bats are killed on the roads by vehicle collisions (Bafaluy 2000; Gaisler *et al.* 2009; Ceron *et al.* 2017). Moreover, some studies recent provide only a list of affected species (Ceron *et al.* 2017; Novaes *et al.* 2018). Important variables that affect the collision rates between bats and vehicles, such as the diversity and abundance of specific plants in the landscape, the presence of flight corridors, habitat fragmentation and foraging routes (Gaisler *et al.* 2009; Medinas *et al.* 2013), are poorly documented in Brazil. Despite these factors, bat species moving on the road may vary from region to region, indicating other differences in the structure of the local landscape (Lesiński 2007; Kerth and Melber 2009).

Due to the lack of studies on collisions between vehicles and bats on the roads, the need to obtain data on mortality from being run over in different regions and landscapes is evident since the impact on natural populations can be severe and may cause a decline due to the cumulative effect of roadkills (Lesiński *et al.* 2011; Berthinussen and Altringham 2012; de Figueiredo Ramalho *et al.* 2021). In that regard, monitoring of the occurrence of collisions between vehicles and bats was carried out on a long stretch on the BR-040 highway in the period from 2008 to 2019, identifying the affected species and the points with the highest collision frequencies.

Materials and Methods

Study area. Data was collected on the BR-040 highway, which covers nine municipalities, from Rio de Janeiro to Juiz de Fora (Figure 1), accounting for 180.4 km. In the State of Rio de Janeiro, it goes from 0 km to 125.2 km, and in the State of Minas Gerais from 773.5 km to 828.7 km. Six municipalities are located in the state of Rio de Janeiro: Rio de Janeiro, Duque de Caxias, Petrópolis, Areal, Três Rios, Comendador Levy Gasparian, and three in the state of Minas Gerais: Simão Pereira, Matias Barbosa and Juiz de Fora. Along the studied stretch, the BR-040 highway crosses three Conservation Units of Atlantic Forest: Petrópolis Environmental Protection Area (APA Petrópolis), the Tinguá Biological Reserve (REBIO for Tinguá), and the Serra da Estrela Wildlife Refuge. The climate along the monitored highway is humid subtropical with a hot temperate summer and a dry winter, according to the Köppen classification, with precipitation concentrated in the summer, in an annual total that varies in the region between 1,300 and 2,200 mm (Alvares *et al.* 2013). We plot a final map with the critical areas of fatalities identified from analysis in Siriema software in the ArcGIS Desktop (Ormsby *et al.* 2010).

Monitoring and data collection. The data were compiled in the period from 2008 to 2019, and the stretches were travelled weekly at 40 km/h by two observers, in

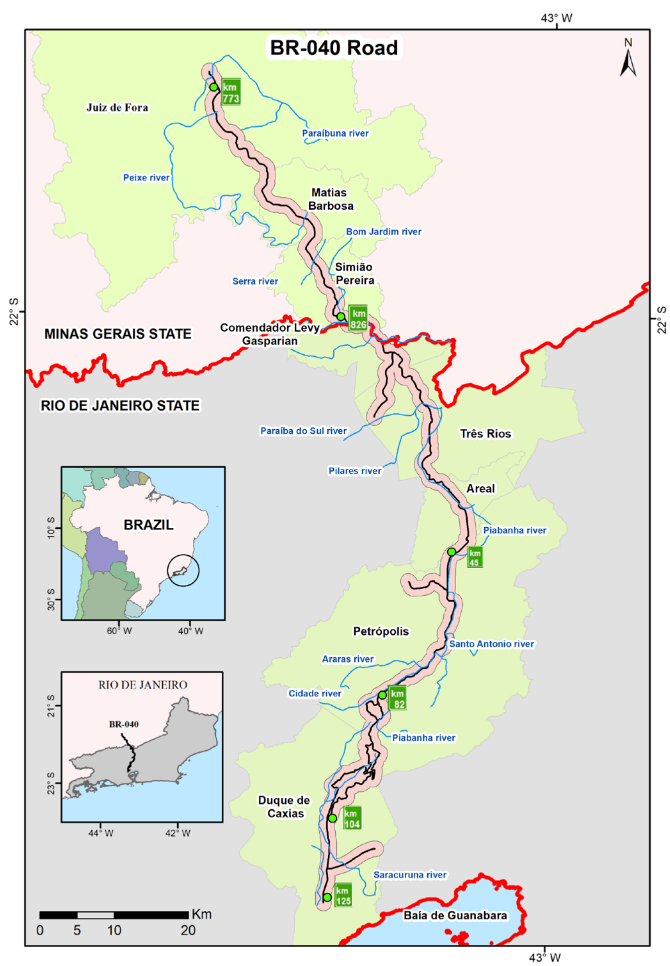

Figure 1. BR-040, stretch from the municipality of Rio de Janeiro to Juiz de Fora.

addition to highway traffic inspectors monitoring 24 hours a day, seven days a week. The bats found dead were collected; when possible, they were identified at the lowest taxonomic level, stored in individual plastic bags, and frozen at the four User Service Centers. Subsequently, the specimens were sent to the Wild Animal Studies Center located at the Veiga de Almeida University, where they were prepared for deposition in the mammalogy section of the National Museum of Rio de Janeiro, a reference collection. Roadkill locations were georeferenced, and bats were photographed, sexed, measured, and identified more accurately in the laboratory. In this study, we included all individuals that could be identified within the order Chiroptera. Bats were identified with the help of identification keys provided by Gardner (2007), and Reis *et al.* (2017), and whenever necessary, identification was checked in the laboratory with available literature (Gregorin and Taddei 2002; Dias and Peracchi 2008; Velazco *et al.* 2010; Moratelli *et al.* 2013; Cirranello *et al.* 2016).

The collections of roadkill specimens were made under SISBIO License, Number: 30727-12. The use of these animals in this study complies with and is under the Operating License, Number: 1187/2013 and Authorization for Capture, Collection, and Transport of Biological Material - Abio (Renewal) No. 514/2014.

Data analysis. Siriema 2.0 software was used (Coelho *et al.* 2014) to identify the main stretches of significant groupings within a highway, the hotspots, and the analyses were 2D HotSpots and Ripley 2D K statistics. Ripley's K statistic describes the dispersion of data across a range of spatial scales (Ripley 1981; Levine 2004). To define the different scales evaluated, we used an initial radius of 100 m and an increment of 400 m for each step. To assess the meaning of possible aggregations, we subtracted the observed K values from the average obtained in 1,000 simulations of random roadkill distributions for each stop. The values above the 95% confidence limits obtained in the simulations indicate scales with significant aggregations (Levine 2004; Teixeira *et al.* 2013; Coelho *et al.* 2014). To find the critical hit-and-run points, we performed a two-dimensional analysis of the identification of the hotspots. Using an average of 1,000 simulations with a 95% confidence interval, we divided the highway into segments of 200 meters each (Teixeira *et al.* 2013; Coelho *et al.* 2014).

Data on the number of individuals run over in each of the four years and comparing the seasons were tested for normality of residues, using the Shapiro-Wilk test and homoscedasticity of residues, using the Bartlett test. Having the data sets in which the assumptions of normality and homoscedasticity of the residuals were accepted, ANOVA (one way) was performed, and a BoxPlot was plotted to verify differences between the results obtained in the present study. The significance level used in the tests was 0.05%, and the R software was used to perform all the statistical tests (R Core Team 2021).

Results

A total of 923 roadkilled individuals of 57 species of bats were recorded. Five families (Table 1) were observed; amongst them, the Phyllostomidae (n = 652; 71%) and Vespertilionidae (n = 204; 22 %) were the most common roadkilled. The species with the highest collision frequency were *Artibeus lituratus* (n = 216; 23.4 %), *Glossophaga soricina* (n = 61; 6.61 %), *Carollia perspicillata* (n = 55; 5.96 %), *Platyrrhinus recifinus* (n = 46; 4.98 %), *Sturnira lilium* (n = 41; 4.44 %), *Platyrrhinus lineatus* (n = 40; 4.33 %), *Phyllostomus hastatus* (n = 38; 4.12 %), *Nyctinomops laticaudatus* (n = 33; 3.58 %), *Artibeus fimbriatus* and *Anoura caudifer* (n = 31; 3.36 %); other species had less than 20 records each, and 151 individuals (16.36 %) could only be identified to the order level.

Frugivores represented the most roadkills, represented by 19 species and 52.87 % of the total number of individuals; however, the greatest recorded richness was that of insectivores, represented by 24 species and 14 % of individuals roadkills. The nectarivores were represented by five species and 10.83 % of the total roadkill. The other trophic guilds were hematophagous, with two species, and carnivore, omnivore and piscivore, represented by one species each. These represent a total of 5.9 % of roadkill recorded in the study. In most of the specimens, it was not possible to identify sex and age; however, in those where it was possible, the male/female rate was similar, and most of them were adults (Table 1). Differences were observed in the frequency of bat collisions between the seasons. The highest mortality was documented in the fall with 330 (35.75 %) individuals killed in collisions, followed by 251 (27.19 %) in the summer, 217 (23.51 %) in the winter and 125 (13.54 %) in the spring. Considering the number of individuals run over in relation to the seasons and each monitoring year (2014, 2015, 2016, 2017, and 2018). According to the Shapiro-Wilk test, the data are normally distributed ($W = 0.98471$, $\alpha = 2$, $P = 0.8891$) and are homoscedastic according to the Bartlett test (2.1022, $\alpha = 3$, $P = 0.5515$). Therefore, the data were submitted to the parametric ANOVA (One Way) test, where significant differences were observed between the data set of individuals hit by cars in relation to the seasons of the year in each monitored year ($F = 1.337$, $\alpha = 3$, $P = 0.297$). The BoxPlot showed a difference, in terms of the number of individuals run over, between the spring season and the other seasons of the year (Figure 2).

In the analyses to determine the scales in which road deaths were significantly aggregated in space, we found different aggregation points for bats. The stretch in the city of Rio de Janeiro, that cover less than 1 km, was the only one that had no record of bat's roadkills. The spatial distribution of roadkill along the BR-040 highway was not random, indicating that the records concentrated on some specific points along the highway. Many stretches had significant aggregations, with the most probable and most significant extension being located between the Duque de Caxias and Petrópolis kilometers. This most critical stretch begins at the ascent of the sierra of Petrópolis. Other peaks of signifi-

Table 1. Species of bats roadkill during the study and the number of collisions according to their age and sexual classification. Undefined sex = SexInd; Undefined age category = AgeInd. Conservation status for species at global level follows IUCN (2020). Data Deficiente (DD); Least Concern (LC); Near Threatened (NT); No identification (S/ID).

Family / Species	Female	Male	SexInd	Adult	Young	AgeInd	IUCN
Emballonuridae							
Saccopteryx leptura	0	0	1	1	0	0	LC
Phyllostomidae							
Anoura caudifer	6	6	19	16	0	15	LC
Anoura geoffroyi	1	1	1	1	0	2	LC
Anoura sp.	0	0	1	0	0	1	
Artibeus cinereus	1	0	2	1	0	2	LC
Artibeus fimbriatus	5	9	17	16	0	15	LC
Artibeus lituratus	43	50	123	105	4	107	LC
Artibeus obscurus	0	0	1	0	0	1	LC
Artibeus planirostris	0	1	0	1	0	0	LC
Artibeus sp.	0	0	17	8	0	9	
Carollia perspicillata	17	11	27	24	2	29	LC
Chiroderma doriae	3	1	2	3	0	3	LC
Chiroderma sp.	0	0	1	1	0	0	
Chiroderma villosum	1	2	1	2	0	2	LC
Choeroniscus minor	1	0	0	1	0	0	LC
Chrotopterus auritus	0	0	4	0	0	4	LC
Desmodus rotundus	2	2	5	6	0	3	LC
Diphylla ecaudata	0	1	1	1	0	1	LC
Glossophaga soricina	18	13	30	35	1	25	LC
Lonchophylla peracchii	0	1	2	1	0	2	LC
Macrophyllum macrophyllum	0	0	1	0	0	1	LC
Micronycteris hirsuta	0	0	1	1	0	0	LC
Micronycteris megalotis	1	1	0	2	0	0	LC
Micronycteris microtis	0	0	1	0	0	1	LC
Micronycteris minuta	1	0	0	1	0	0	LC
Mimon bennettii	2	2	1	4	0	1	LC
Phyllostomus hastatus	5	7	26	24	1	13	LC
Platyrrhinus sp.	0	0	1	0	0	1	
Platyrrhinus lineatus	12	4	24	17	0	23	LC
Platyrrhinus recifinus	10	8	28	20	1	25	LC
Pygoderma bilabiatum	1	2	10	3	0	10	LC
Sturnira lilium	9	8	24	16	3	22	LC
Sturnira tildae	0	0	3	0	0	3	LC
Uroderma sp.	0	0	1	0	0	1	
Vampyressa pusilla	5	1	1	5	0	2	DD
Vampyrodes caraccioli	1	0	0	1	0	0	LC
Noctilionidae							
Noctilio leporinus	1	0	0	1	0	0	LC
Molossidae							
Eumops sp.	0	0	1	0	0	1	
Molossops neglectus	1	0	0	0	0	1	DD
Molossus molossus	1	3	11	5	0	10	LC
Molossus rufus	1	0	5	1	0	5	LC
Nyctinomops aurispinosus	0	0	1	0	0	1	LC
Nyctinomops laticaudatus	1	4	28	18	0	15	LC
Nyctinomops sp.	0	0	5	2	0	3	
Tadarida brasiliensis	0	0	1	0	0	1	LC
Molossidae (unidentified)	0	0	2	2	0	0	

Table 1. Continuation...

Family / Species	Female	Male	SexInd	Adult	Young	AgeInd	IUCN
Vespertilionidae							
Eptesicus brasiliensis	1	1	3	3	0	2	LC
Eptesicus diminutus	1	0	0	1	0	0	LC
Eptesicus sp.	1	0	2	2	0	1	
Histiotus velatus	1	0	0	1	0	0	DD
Dasypterus ega	1	1	4	2	0	4	LC
Myotis albescens	0	0	1	1	0	0	LC
Myotis izecksohni	1	1	1	2	0	1	DD
Myotis nigricans	1	3	5	3	0	6	LC
Myotis riparius	2	0	2	1	0	3	LC
Myotis ruber	0	1	0	1	0	0	NT
Myotis sp.	0	0	16	4	0	12	
Vespertilionidae (unidentified)	0	0	3	0	0	3	
S/ID	0	1	150	27	1	123	
Total	159	146	618	394	13	516	

cant aggregations were observed between the kilometers of Três Rios and Comendador Levy Gasparian (Figure 3). The most critical points on the road, according to the analyses, were in the Atlantic Forest Biodiversity Corridor, which includes the Tinguá Biological Reserve, the Serra da Estrela State Wildlife Refuge and the APA Petrópolis (Figure 4).

Discussion

Bats are valuable indicators of biodiversity and ecosystem health, as a result of his sensitivity to fragmentation, that can decrease the richness and abundance of the order in altered areas (Reis *et al.* 2003; Alurralde and Díaz 2021). Thus, habitat fragmentation could be an aggravating factor in the seed dispersal process carried out by Chiroptera species. Despite the relative richness and abundance observed during the monitoring of the current study, these values can be related to the time and size of the sample. However, still can be underestimated for the region due to the limitation of the study that only recorded species found dead by collisions. Species richness during this monitoring was similar to other studies in southeastern Brazil (Dias and Peracchi 2008; Esbérard and Bergallo 2008; Tavares *et al.* 2010; Perini *et al.* 2014), yet, we observed a high variation between the richness and diversity of these studies, which is probably due to differences in capture techniques, such as the use of mist nets or active search for shelters in areas with landscape mosaic varying from conserved to degraded environments (Dias and Peracchi 2008; Esbérard and Bergallo 2008; Tavares *et al.* 2010; Perini *et al.* 2014; Alurralde and Díaz 2021), and differences in collision probabilities by bat species in the present study.

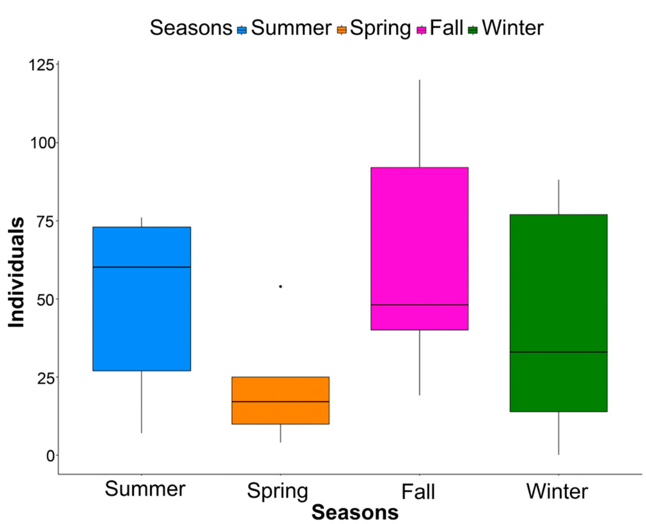

Figure 2. Boxplot (median with upper and lower quartiles) of the number of individuals of bats killed in collisions by season of the year on highway BR-040.

Frugivore bats are usually the most frequent group in collisions with vehicles on the roads, according to our data and other studies on South America (Pinheiro and Turci 2013; Ceron *et al.* 2017; Valadão *et al.* 2018; de Figueiredo Ramalho *et al.* 2021), where frugivores are more representative (Fenton *et al.* 1992; Martins *et al.* 2014). Species as *Artibeus lituratus*, *Carollia perspicillata,* and *Sturnira lilium* are not inhibited by fragmentation and are more abundant in disturbed areas than other bat species (Medellín *et al.* 2000; Reis *et al.* 2003; Alurralde and Díaz 2021). However, these species have been most affected by collisions with vehicles on the roads (Pinheiro and Turci 2013; Ceron *et al.* 2017; Valadão *et al.* 2018; de Figueiredo Ramalho *et al.* 2021). These species have been considered abundant in the study region (Dias and Peracchi 2008; Tavares *et al.* 2010; Luz *et al.* 2011), and have characteristics that make them good indicators of damaged areas, providing advantages for plants whose fruits are consumed by them, is a key factor for dispersion and genetic flow of plants in regions affected by fragmentation (Muller and dos Reis 1992; Reis *et al.* 2003; Bianconi *et al.* 2006).

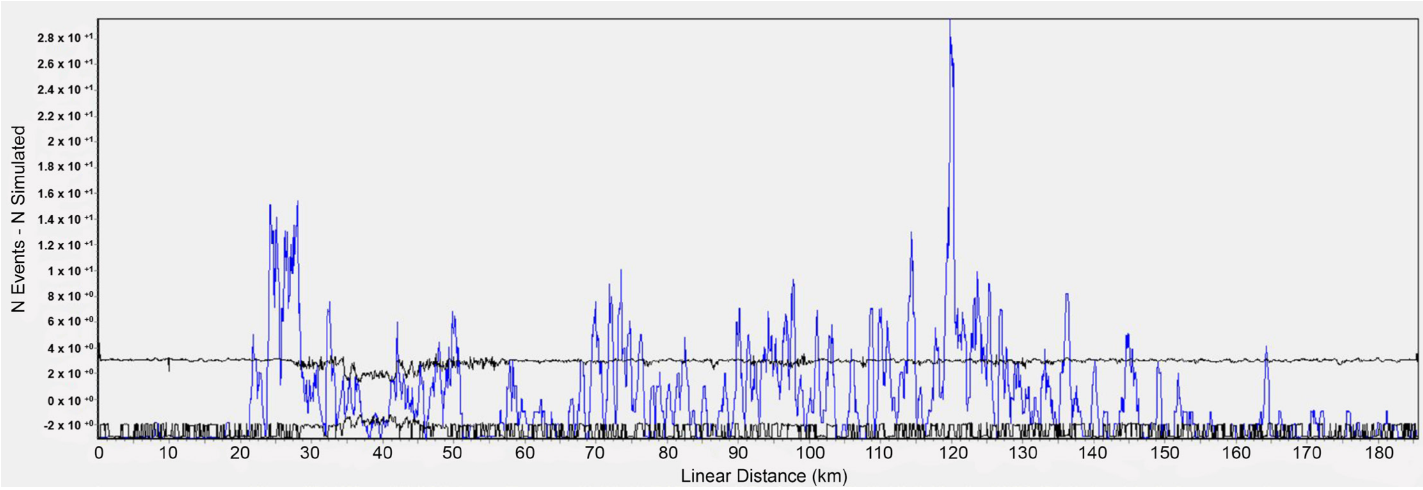

Figure 3. Bat roadkill aggregation intensity (blue line) and 95% confidence limits (black line) along 180.4 km of BR-040.

This higher rate of collisions with frugivores bats is probably due to some associated factors, such as flight height, displacement patterns in foraging, the temporal and spatial distribution of the fruits and the plants, as well as the landscape structure (Bernard and Fenton 2003; Clevenger *et al.* 2003; Bianconi *et al.* 2006; Gumier-Costa and Sperber 2009; Pracucci *et al.* 2012; Gomes *et al.* 2020). An essential factor is that many of these frugivorous species are consumers of pioneer plants, which are usually found in large densities in degraded areas, such as the vicinity and edges of roads (Muller and dos Reis 1992; Reis *et al.* 2003). The species most impacted by the collisions on BR-040, *Artibeus lituratus*, was also the most abundant in studies in the southeastern region (Esbérard and Bergallo 2008; Tavares *et al.* 2010; Luz *et al.* 2011; Perini *et al.* 2014). Although roadkills did not show marked seasonal trends, fewer bat collisions were documented during the spring. Moreover, there is evidence of the difference in the number of collisions by bats in the seasons (Lesiński *et al.* 2011), but little is known about the factors that can influence the number of roadkills by these species, needing further investigations.

Different species of bats can be impacted differently by the fragmentation of habitats resulting from a road network. Some factors may be contributing to this difference in the amount of roadkill by bat species, such as foraging strategies, diet, or resilience of species in degraded areas (Kalko *et al.* 1996; Stone *et al.* 2009; Bhardwaj *et al.* 2017; Claireau *et al.* 2019), and need be investigated. Rare species can potentially be affected even by small mortality rates (Fensome and Mathews 2016; Damásio *et al.* 2021). In the present study, many insectivores considered rare were found roadkilled. The study by Bhardwaj *et al.* (2021) pointed out that the activity of seven of ten insectivorous bats species decreased significantly within the proximity of a highway due to changes created in the surrounding environment by traffic, such as pollution, light, noise, and vibrations. The authors called the surrounding environment a "road effect zone," and in this area, the habitat is degraded or entirely unsuitable for wildlife, which leads to an indirect loss of habitat (Bhardwaj *et al.* 2021).

One of the stretches identified in this study as a highly significant hotspot is in an area of Atlantic Forest in the municipality of Petrópolis, a region inserted in the APA Petrópolis, the Serra da Estrela State Wildlife Refuge, and close to the Biological Reserve (REBIO) of Tinguá. Twenty-eight species of bats have been registered in REBIO so far, the most abundant being *C. perspicillata*, *S. lilium,* and *A. lituratus* (Dias and Peracchi 2008), also the most frequently recorded in the present study. We highlight the species *Myotis ruber*, which had a run-over record close to the REBIO area, and which is listed as almost threatened with extinction in the IUCN Red List (IUCN 2020). The most critical points were on the stretch that crosses Tinguá Biological Reserve, highlighting the need for more urgent conservation measures in this region. However, the simple counting of carcasses found on the roads is an underestimation of the actual rate of bats killed by being run over. Even in regular monitoring, some factors may influence this count. Bat carcasses can be completely destroyed by continuous vehicle traffic, some can be thrown off the road and lost in the vegetation, and some individuals could be removed by other animals for consumption (Pracucci *et al.* 2012; Cunha *et al.* 2015; Ramalho and Aguiar 2020).

The roads that cross parks and forest reserves can have an extremely negative impact, a barrier to the natural movement of the species, and establishing a population decline, as well as decreasing the genetic flow for the wildlife of these regions (Samson *et al.* 2016). Critically, bat collisions with vehicles have been observed mainly in these areas where endangered and endemic bat species occur (Lesiński 2007). Moreover, these regions also harbor other wild species, thus making roadkills more critical for the conservation of species (IUCN 2020). Efforts to mitigate these accidents in the region are carried out with wildlife warning signs, yet these had not shown been proved effective. Roads are already proven to be harmful to many wild animals (Trombulak and Frissell 2000; Fahrig and Rytwinski 2009; Gumier-Costa and Sperber 2009), and there are several approaches that can be used to reduce the effects of

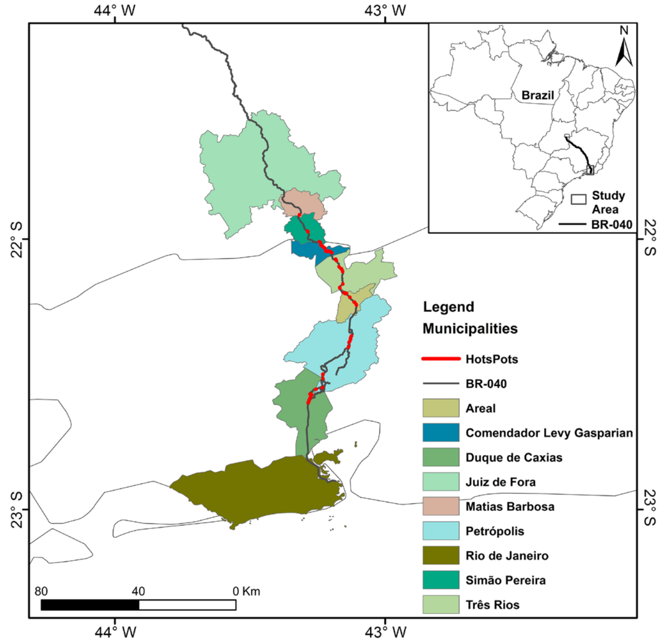

Figure 4. The sections marked in red indicate the critical areas of fatalities (HotSpots) identified from monitoring of run over bat fauna, analysis in Siriema software.

roads and mortality in wild animal populations (Jackson 2000; Glista *et al.* 2009). The use of underpasses and ecological bridges for wild fauna probably varies from species to species, depending on their behavior in terms of movement (Berthinussen and Altringham 2012; Bhardwaj *et al.* 2017). However, a combination of monitoring and ecological bridges has shown great effectiveness in decreasing roadkill rates (Bhardwaj *et al.* 2017).

Our results suggest that bats are particularly vulnerable to the impact of roads. Consequently, they may take a long time to recover from disturbances due to their low fertility, longevity, and foraging activity in large areas of the landscape (Findley 1993). Roads can affect the amount of area available to wildlife habitat, particularly through the fragmentation, and also affect the quality of this area by traffic of vehicles that cause collisions and deaths of wild animals (Bafaluy 2000; Bueno *et al.* 2015; de Figueiredo Ramalho *et al.* 2021). Other factors that influence this mortality are the lack of proper signs and awareness of drivers who travel on these highways. Hybrid mitigation is applied to reduce the adverse effects of roads on wildlife and comply with environmental legislation. These mitigations should account for several species, considering the broad diversification of habits showed by bats (Bernard and Fenton 2003; Bianconi *et al.* 2006; Gomes *et al.* 2020), and their sensitivity to increased lighting, which can change their activity pattern (Stone *et al.* 2009; Appel *et al.* 2017; Gomes *et al.* 2020). We suggest speed bumps construction to reduce the speed limit, install bridges, and signaling the presence of wildlife on the stretches identified as hotspots in our analyses, that is, with a higher risk of collision for these species of bats.

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