

March or Die: road-killed herpetofauna along BR-040 highway, an ancient road on the Atlantic Forest from Southeastern Brazil

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Abstract: The construction of highways is responsible for access to previously protected areas, resulting in changes in landscape and dynamics of the animal populations that live in these areas. These enterprises are the major responsible for the mortality of wild animals, surpassing hunting and even the trafficking of animals. The objective of this study was to make a list that reflects the diversity of amphibians and reptile's road-killed along the BR-040, a highway that crosses the threaten lowland Atlantic Forest in Southeastern region of Brazil, including the use of microhabitats, lifestyle, activity pattern, reproductive cycles, and possible rare or endangered species. The study area consists of 180,4 km of highways. Monitoring began in 2006 and continues to the present day. A total of 1,410 individuals from 60 species were recorded in this study. The reptiles were more frequent in number of individuals and species. The commonest species recorded were *Crotalus durissus* and *Dipsas mikanii*. We have registered a single endangered species: *Ranacephala hogei*. The highest rates of road-kill were recorded during the wet season. Road-kills of fauna is a major threat to species, studies are of great importance to define plans that seek to mitigate the effects generated by these enterprises.

Keywords: Animal-vehicle collision; Roads; Road-kill mitigation; Road ecology; Reptiles; Amphibians.

Marche ou Morra: herpetofauna atropelada ao longo da rodovia BR-040, uma antiga estrada na Mata Atlântica do Sudeste do Brasil

Resumo: A construção de rodovias é responsável pelo acesso a áreas anteriormente protegidas, resultando em alterações na paisagem e na dinâmica das populações animais que vivem nessas áreas. Esses empreendimentos são os maiores responsáveis pela mortalidade de animais silvestres, superando a caça e até mesmo o tráfico de animais. O objetivo deste estudo foi realizar uma lista que reflita a diversidade de anfíbios e répteis atropelados ao longo da BR-040, uma rodovia que atravessa a ameaçada Mata Atlântica na região Sudeste do Brasil, incluindo o uso de microhabitats, estilo de vida, padrão de atividade, ciclos reprodutivos, e possíveis espécies raras ou ameaçadas. A área de estudo é constituída por 180,4 km de rodovias. O monitoramento começou em 2006 e segue até os dias atuais. Ao todo 1.410 indivíduos de 60 espécies foram registrados nesse estudo. Os répteis foram mais frequentes, em número de indivíduos e espécies. As espécies mais comumente registradas foram *Crotalus durissus* e *Dipsas mikanii*. Registramos uma espécie ameaçada de extinção: *Ranacephala hogei*. A maior taxa de atropelamento foi registrada durante a estação chuvosa. O atropelamento de fauna é uma grande ameaça as espécies, sendo de grande importância estudos para definição de planos que busquem mitigar os efeitos gerados por esses empreendimentos.

Palavras-chave: Colisão animal-veículo; Rodovias; Mitigação de atropelamentos; Ecologia de estradas; Répteis; Anfíbios.

Introduction

As a rule, roads make a major contribution to the high levels of biodiversity loss around the world (Coffin, 2007; Jochimsen et al., 2014; Van der Ree et al., 2015), being road-kills one of the main causes

of direct death of wild vertebrate species, overcoming the impacts generated by hunting and mortality rates from natural causes (Seiler & Helldin, 2006; Valadão et al., 2018; Hill et al., 2019). Every second 15 wild animals die on Brazilian roads, and these numbers can reach 1.3 million per day and exceed 475 million per year with extrapolated data

(CBEEE, 2022). However, the actual number may be even higher, since several deaths are not recorded and road impacts go beyond collisions between wild animals and vehicles (Casella et al., 2006; Van der Ree et al., 2015; Boyle et al., 2019).

Due to the great urgency and growth of the road network around the world, road-kills and other direct and indirect road impacts have received attention in many studies (Bager & Rosa, 2010; Almeida, 2013; Vélez, 2014). Among the various threats to wildlife are, in addition to direct collisions with vehicles, the increase in the level of air and noise pollution, the rise in temperature and the emergence of urban agglomerations on the roadside (Gomes et al., 2007; Bager & Rosa, 2010; Vélez, 2014; Shannon et al., 2016). To complicate matters, roads potentially reduce the size of natural populations, affecting their long-term persistence (Fahrig et al., 2003; Bueno et al., 2013; Gonçalves et al., 2018), by separating habitats by reducing their size, configuration and quality (McKinney, 2002; Fahrig, 2003; McKinney, 2006; Maynard et al., 2016), acting as barriers to dispersion (Parris & Schneider, 2008, Ware et al., 2015), limiting gene flow (Ascensão et al., 2017). Additionally, roads affect individual survival, and provide humans with easy access to previously difficult-to-reach areas, thereby increasing the negative pressure on wildlife (Laurance et al., 2009).

Nonetheless, wildlife road-kill's do not occur randomly (Sosa & Schalk, 2016; Filius et al., 2020). Several factors favor certain species to be road-killed more than others, such as biological characteristics (body size and diet; Barthelmess & Brooks, 2010), the characteristics of the landscape and the road itself (Bueno et al., 2013, 2015), as well as seasonal variations in temperature and rainfall (Bueno & Almeida, 2010; Santana, 2012; Santos & Carvalho, 2012). The vehicular traffic, as well as the vehicles' speed are also important determining characteristics that lead to the collision of vehicles with animals (Cunha et al., 2010; Lester, 2015). Various taxonomic groups are affected distinctly by vehicle collisions around the world, including mammals (Grilo et al., 2020; Hill et al., 2021; Navas-Suárez et al., 2022), birds (Bujoczek et al., 2011; Rosa & Bager, 2015 Grilo et al., 2020; Medrano-Vizcaíno et al., 2022), reptiles (Aresco, 2005; Shepard et al., 2008a; Hallisey et al., 2022), amphibians (Fahrig et al., 2003; Hels & Buchwald, 2001; Glista et al., 2008; Hallisey et al., 2022) and invertebrates (Seibert & Conover, 1991; McKenna et al., 2001). Ectotherms (amphibians and reptiles), despite being underrepresented in the literature on road ecology (Guns et al., 2011; Popp & Boyle, 2017), had a higher probability to be road-killed (D'Amico et al., 2015). This is probably because their metabolism causes slowness in amphibians (Hels & Buchwald, 2001; Puky, 2005), the behavioral freezing responses to threats (Andrews et al., 2005; Lima et al. 2015) and, mainly, due to characteristic basking behavior for reptile's thermoregulation (Ashley & Robinson, 1996; Tanner & Perry, 2007; Jochimsen et al. 2014; Andrews et al. 2015; D'Amico et al. 2015; Schalk & Saenz, 2016). In addition, there are other more intricate reasons, as such the cultural aversion to reptilian Bauplan in the Western civilization, mostly in the case of snakes and other limbless Squamata (Davey, 1994; Fernandes-Ferreira et al., 2011; Ceríaco, 2012; Castilla et al., 2020; Silva et al., 2021). Amphibians and reptiles are vulnerable to road-kills when they travel on roads that cross their area of origin, or when they are attracted by the resources available in the area surrounding road edges, often because they are not seen by drivers (Laurance et al., 2009; Bueno & Almeida, 2010; Carvalho et al., 2015). Sometimes, however, when stigmatized animals are in

sight of drivers on the road, some swerve the vehicle ever so slightly to run over them or simply do not try to swerve the vehicle to avoid hitting them (Ashley et al., 2007; Beckmann & Shine, 2012; Mesquita et al., 2014; Secco et al., 2014; Assis et al., 2020).

There is global concern about the road-kill threats in animal conservation (Freitas, 2015; Adárraga-Caballero & Gutiérrez-Moreno, 2019; Jarvis et al., 2019, Grilo et al., 2021). Although road ecology is a recent topic of interest in temperate and tropical regions (Rosa & Bager, 2013; Pereira et al., 2017), especially in the New World, the visibility of this theme has increased rapidly with the public becoming aware of its relevance to the protections of wild animal populations (Attademo et al., 2011). Nevertheless, there is still a paucity of accurate information on the spatial and temporal distribution of road-kill's. Understanding the dynamics of wildlife-vehicle collisions allows us to find alternative solutions to increase safety on the roads, reduce the impacts on humans and wildlife, reduce costs, and invest in mitigation measures aimed at conservation of biodiversity (Forman, 1998; Czech et al., 2000; Rytwinski et al., 2016; Abra et al., 2019; Ascensão et al., 2021; Silva et al., 2021). The objective of this study was to make a list of the diversity of amphibians and reptiles' road-killed along the BR-040, a highway that crosses the threaten Atlantic Forest in the Southeastern region of Brazil, including information on the use of microhabitats, lifestyle, activity pattern, reproductive cycles, and possibly rare or endangered species.

Material and Methods

1. Study area and source of data

The database used in the study come from the monitoring of the fauna road-killed along a 180 km stretch on the BR-040 (from km 125.2 in the municipality of Duque de Caxias, state of Rio de Janeiro to km 773.5 in the municipality of Juiz de Fora, state of Minas Gerais) (Figure 1). The project "Caminhos da Fauna" (free translation, Wildlife Pathways) started in 2006, is still in progress, and comprises the pioneering study in the monitoring of road-killed animals in the state



Figure 1. Land cover map for the selected area of surroundings from the BR-040 highway stretches where road-kill were recorded, from km 125.2 in the municipality of Duque de Caxias (red star), state of Rio de Janeiro, to the Km 773.5 in the municipality of Juiz de Fora (red triangle), state of Minas Gerais.

of Rio de Janeiro. In the present study, we analyzed the records from April 2006 to June 2022, comprising both the specimens discarded after identification at the lowest taxonomic level possible and those preserved for scientific purposes and deposited in the Amphibians and Reptiles collections of the Museu Nacional, Universidade Federal do Rio de Janeiro (MNRJ). These specimens comprise an important source of data in the amphibians and reptiles collections. The list of deposited specimens is in the appendix and the institutional abbreviation followed is as detailed in Sabaj (2020).

The area of study crosses the Biodiversity Corridor of the Serra do Mar National Park, whose main native vegetation cover is composed of tropical rain forest (Veloso et al., 1991). The topography varies from the lowlands in the municipality of Duque de Caxias (22°90'46"S, 43°18'43"W; 19 m above sea level, hereafter asl), through the mountain range (about 1,000 m asl) near the municipality of Petrópolis (22°30'18"S, 43°10'44"W; 838 m asl), up to the municipality of Juiz de Fora (21°41'20"S, 43°20'40"W; 715 m asl) (Figure 2). Since 1996, the BR-040 stretch from Rio de Janeiro to Juiz de Fora has been under the authority of a private company, CONCER. The mean traffic volume on this road is 37,000 vehicles/day (CONCER, 2020). Within this entire range, the road has 2 paved lanes in each direction, and for the stretch crossing the mountain range, the 2-lanes going up and the 2-lanes going down run separately.

2. Sampling design

The collection protocol is based on standard forms and techniques developed for the project Caminhos da Fauna, which includes taking pictures, removing carcasses, storing them in freezers, and recording their location, date and time of collection. The project has promoted the installation of three freezers located at the 104 km, 45 km and 816 km marks of BR-040 highway to provide a better preservation of the collected carcasses. Twice a month, the carcasses accumulated in the freezers were taken to the laboratory, at Veiga de Almeida University, in the municipality of Rio de Janeiro. After that, the specimens were donated to MNRJ, where they were defrosted, weighted and measured



Figure 2. Elevation map for the selected area of surroundings from the BR-040 highway stretches where road-kill were recorded, from km 125.2 in the municipality of Duque de Caxias (red star), state of Rio de Janeiro, to the Km 773.5 in the municipality of Juiz de Fora (red triangle), state of Minas Gerais.

(in the case of slightly damaged animals) and sampled for genetic material (muscle tissue was taken from most of reptiles specimens and for selected amphibians specimens). Carcass collections are carried out in partnership with the CONCER concessionaire throughout the week for 24 hours. The monitoring speed is 50 km/h which allows a best visualization of the road-killed animals (small reptiles and amphibians) along the entire highway. The data were converted to road-kill rate (number of individuals/km/day). For each record, a field form is filled out with: mileage, direction, location on the road, sex, taxonomic group of the road-killed animal, local speed limit, weather for the day of collection, presence of water nearby, surrounding vegetation, in addition all occurrences were georeferenced and made available in decimal degrees decimals. Unidentified species at least at gender level were not considered for further analyses.

Carcass collections are included in the SISBIO License Number: 30727-9. The animal carcasses used in this study meet and are in accordance with operation license No. 1187/2013 and authorization for capture, collection and transport of biological material - Abio (1st Renewal and 3rd Rectifier) 514/2014.

3. Species identification

The collected carcasses were identified by experts at the lowest taxonomic level possible using the relevant taxonomic literature, as well as by means of direct comparison with the specimens from the MNRJ collections of Amphibians and Reptiles. After identification, they are fixed in formalin solution and preserved in ethanol 70°GL and incorporated to the respective collection. The photographs aided in the taxonomic identification, but they were not considered alone for the species identification. Data regarding activity patterns, foraging, microhabitat selection and reproductive cycles were based on the available literature for each taxonomic group (e.g., Haddad et al., 2013; Marques et al., 2019) and are summarized in Tables 1–2, indicating the specific source of each natural history information.

Results

We recorded 1,411 road-killed individuals, being 934 reptiles (66.19%) of 45 species and 478 amphibians (33.81%) of 15 species (Figures 3–10). However, due to the poor morphological condition of some specimens, it was not possible to identify them to the level of species. In these cases, specimens were identified up to the generic level (records for 41 reptiles and 46 amphibians) or remained without identification (17 amphibians and 12 reptiles) (Tables 1–2).

Considering only road-killed reptiles, Serpentes was the most recorded group, corresponding to 72.91% (N = 681) of the entire sampling, followed by lizards with 24.19% (N = 226), Amphisbaenia 0.96% (N = 9), Testudines 0.42% (N = 4), and Crocodylia 0.32% (N = 3); without identification 1.20% (N = 12).

The most representative of the snakes (N = 681) were the Rattlesnake, *Crotalus durissus* Linnaeus, 1758 corresponding to 19.23% (N = 130) followed by the Neuwied's Tree Snake, *Dipsas neuwiedi* (Ihering, 1911) (11.45%; N = 78), and the Lancehead, *Bothrops jararaca* (Wied-Neuwied, 1824) (7.48%; N = 51). These three species together correspond to 38.16% of the sample for the snakes group.

The most commonly found lizards were the White Tegu, Salvator merianae Duméril & Bibron, 1839 (45.13%; N = 102) and the

Table 1. Complete list of road-killed amphibians on highway BR-040. **Abbreviations:** N = Sample number; No = Nocturnal; Di = Diurnal; Arb = Arboreal; Cry = Cryptozoic; Ter = Terrestrial; Art = Arthropods; Mol = Mollusks; Anu = Anura; S = Small; M = Medium; L = Large; REP. MODE = Reproductive Mode; LC = Least Concern; DD = Data Deficient;**REP. MODE:**(1) Direct development of terrestrial eggs; (2) Eggs and exotrophic tadpoles in still or running water; (3) Eggs on wet rocks, rock crevices, exotrophic semi-terrestrial tadpoles; (4) Eggs and exotrophic tadpoles in still water or eggs and early larval stages in natural or constructed basin; (5) Eggs and exotrophic tadpoles in still water or eggs and early larval stages in underground constructed chamber; (8) Foam nest floating on still water;**HABITS:**(1) Forest floor; (2) Swamp or pond; (3) Rock wall; (4) River or stream backwaters.

Таха	Ν	Activity	Habit	Diet	REP. MODE	Calling site	Size	ICMBio	IUCN
CLASS AMPHIBIA									
ORDER ANURA									
without identification BRACHYCEPHALIDAE	20								
<i>Ischnocnema guentheri</i> (Steindachner, 1864) BUEONIDAE	3	No.	Arb./Cry.	Art./Mol.	1	1	S	LC	LC
Rhinella dintycha (Cope 1862)	1	No	Ter	Δrt	2	2 or 4	T	IC	ממ
Rhinella icterica (Spix 1824)	157	No.	Ter	Art	2	2 or 4	L	LC	LC
Rhinella ornata (Spix, 1824)	10	No.	Ter.	Art.	2	2 or 4	M	LC	LC
Rhinella sp. CYCLORAMPHIDAE	35	1.01			_	2 01 1		20	20
<i>Thoropa miliaris</i> (Spix, 1824) CRAUGASTORIDAE	21	No.	Ter.	Art./Mol.	3	3	S	LC	LC
Haddadus binotatus (Spix, 1824) HYLIDAE	2	No.	Cry.	Art.	1	1	S	LC	LC
Boana faber (Wied-Neuwied, 1821)	20	No.	Arb.	Art.	4	2	М	LC	LC
Boana semilineata (Spix, 1824)	1	No.	Arb.	Art./Mol./ Anu.	2	2 or 4	М	LC	LC
<i>Bokermannohyla circumdata</i> (Cope, 1871)	4	No.	Arb.	Art./Mol.	5	2	S	LC	LC
Dendropsophus elegans (Wied-Neuwied, 1824)	2	No.	Arb.	Art./Mol.	6	2	S	LC	LC
Scinax eurydice (Bokermann, 1968)	4	No.	Arb.	Art./Mol.	6	2	Μ	LC	LC
Trachycephalus mesophaeus (Hensel, 1867) LEPTODACTYLIDAE	1	No./Di.	Arb.	Art./Mol.	6	2	М	LC	LC
Leptodactylus fuscus (Schneider, 1799)	2	No.	Ter.	Art./Mol.	7	2	М	LC	LC
Leptodactylus gr. latrans (Steffen, 1815)	4	No.	Ter.	Art./Mol.	8	2	М	LC	LC
Leptodactylus labyrinthicus (Spix, 1824)	15	No.	Ter.	Art./Mol.	8	2	L	LC	LC
Leptodactylus latrans (Steffen, 1815) Leptodactylus sp.	168 11	No.	Ter.	Art./Mol.	8	2	М	LC	LC

Amazon Lava Lizard, *Tropidurus torquatus* (Wied-Neuwied, 1820) (32.30%; N = 73). Together, these two species correspond to 77.43% of the sample for lizards.

The most frequently road-killed species of amphibians' was the Butter Frog, *Leptodactylus latrans* (Steffen, 1815) (N = 168; 35.14%), followed by the Yellow Cururu Toad, *Rhinella icterica* (Spix, 1824) (N = 157; 32.84%), and Military River Frog, *Thoropa miliaris* (Spix, 1824) (N = 21; 4.39%). These three species together correspond to 72.37% of the amphibian sample.

During the more than 15 years of sampling (2006 to 2022), the year with the highest number of records was 2014, with 462 road-kills of wildlife (32.77%), followed by 2015 (N = 301; 21.35%) and 2016 (N = 195; 13.83%). In 2014, the highest rate of accidents occurred in the rainy season, in the months of October (N = 49; 10.60%), November (N = 48; 10.39%), December (N = 45; 9.74%), January (N = 76; 16.45%), February (N = 65; 14.07%) and March (N = 59; 12.77%). These six months together corresponded to 74.02% of the trampling of wildlife of the year sampled.

Table 2. Complete list of road-killed reptiles on highway BR-040. **Abbreviations:** N = Sample number; No = Nocturnal; D = Diurnal; A = Arboreal; C = Cryptozoic; T = Terrestrial; Fo = Fossorial; Aq = Aquatic; Sa = Saxicola; M = Mammals; B = Birds; Ar = Arthropods; Mol = Mollusks; An = Anura; F = Fish; L = Lizard; E = Earthworm; Sn = Snake; G = Generalist; PMa = Plant material; Sm = Small; M = Medium; L = Large; REP. MODE = Reproductive Mode; S = Seasonal; C = Continuous; Bo = Both; V = Viviparous; O = Oviparous; D = Dry; R = Rainy LC = Least Concern; DD = Data Deficient; CR = Critically Endangered.

Taxa	Ν	Activity	Habit	Diet	REP.	Reproduction	Season	Size	ICMBio	IUCN
					MODE	p		~		
CLASS REPTILIA										
WITHOUT IDENTIFICATION	58									
ORDER SQUAMATA										
SERPENTES										
BOIDAE										
Boa constrictor Linnaeus, 1758	1	No/D	T/A	M/B	V	S	D	L	LC	LC
Corallus hortulana (Linnaeus, 1758) COLUBRIDAE	4	No	А	M/B	V	S	R	L	LC	LC
<i>Chironius bicarinatus</i> (WIED-NEUWIED, 1820)	26	D	T/A	An	Ο	S	R	L	LC	LC
Chironius exoletus (LINNAEUS, 1758)	1	D	T/A	An	0	S	R	М	LC	LC
Chironius fuscus (LINNAEUS, 1758)	12	D	T/A	An	0	С	Bo	М	LC	LC
<i>Chironius laevicollis</i> (WIED-NEUWIED, 1824)	8	D	T/A	An	0	S	R	L	LC	LC
Chironius sp.	7									
Leptophis ahaetulla (LINNAEUS, 1758)	5	D	T/A	An/L	0	S	D	М	LC	LC
Spilotes sulphureus (WAGLER, 1824)	21	D	T/A	M/B	0	S	R	L	LC	LC
Spilotes pullatus (LINNAEUS, 1758) DIPSADIDAE	22	D	T/A	M/B	Ο	S	R	L	LC	LC
Atractus zebrinus (JAN, 1862)	21	No	C/Fo	Е	0	S	R	Sm	LC	LC
Dipsas sp.	1									
Dipsas mikanii SCHLEGEL, 1837	2	No	Т	Mo	0	S	R	Sm	LC	LC
Dipsas neuwiedi (IHERING, 1911)	78	No	T/A	Mo	0	S	R	Sm	LC	LC
<i>Elapomorphus quinquelineatus</i> (RADDI, 1820)	14	D	С	Sn	0	S	Dry	М	LC	LC
Erythrolamprus aesculapii (LINNAEUS, 1758)	8	D	Т	Sn	0	С	Во	М	LC	LC
<i>Erythrolamprus miliaris</i> (LINNAEUS, 1758)	15	No/D	T/Aq	F/An	0	S	R	М	LC	LC
Erythrolamprus poecilogyrus (WIED- NEUWIED, 1824)	7	No/D	Т	An	0	С	Во	М	LC	LC
<i>Erythrolamprus reginae</i> (LINNAEUS, 1758)	1	D	T/Aq	F/ An/L	0	С	Во	Sm	LC	LC
<i>Helicops carinicaudus</i> (WIED- NEUWIED, 1824)	2	No/D	T/Aq	F/An	V	S	R	М	LC	LC
Hydrodynastes sp.	2									
Leptodeira annulata (LINNAEUS, 1758)	4	No	T/A	An	0	S	D	Sm	LC	LC
Oxyrhopus clathratus DUMÉRIL, BIBRON & DUMÉRIL, 1854	47	No	Т	Sn/L	0	S	R	Sm	LC	LC
Oxyrhopus petolarius (LINNAEUS, 1758)	42	No	Т	M/L	0	S	R	М	LC	LC
<i>Philodryas olfersii</i> (LICHTENSTEIN, 1823)	14	D	T/A	M/An	0	S	R	М	LC	LC
Pseudablades patagoniensis (GIRARD, 1858)	44	D	Т	An/L	0	S	R	М	LC	LC
<i>Pseudoboa nigra</i> (DUMÉRIL, BIBRON & DUMÉRIL, 1854)	2	No	Т	L	0	С	Во	L	LC	LC
Siphlophis compressus (DAUDIN, 1803)	20	No	T/A	L	Ο	S	R	М	LC	LC
Dibernardia affinis (GÜNTHER, 1858)	2	D	T/C	An/L	0	S	R	Sm	LC	LC

Continue...

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Таха	N	Activity	Habit	Diet	REP. MODE	Reproduction	Season	Size	ICMBio	IUCN
Thamnodynastes sp.	1									
Tropidodryas sp.	4									
Xenodon neuwiedi GÜNTHER, 1863	5	D	Т	An	0	С	Bo	М	LC	LC
ELAPIDAE										
<i>Micrurus corallinus</i> (MERREM, 1820) VIPERIDAE	3	D	С	Sn	0	S	R	М	LC	LC
Bothrops jararacussu LACERDA, 1884	8	No/D	Т	М	V	S	R	L	LC	LC
Bothrops jararaca (WIED-NEUWIED, 1824)	51	No	T/A	М	V	S	R	М	LC	LC
Crotalus durissus LINNAEUS, 1758 SAURIA	128	No	Т	М	V	S	D	М	LC	LC
DIPLOGLOSSIDAE										
Ophiodes cf. fragilis (RADDI, 1820)	5	Di.	С	Ar/Mo	V	S	R	М	LC	DD
Ophiodes fragilis (RADDI, 1820)	7	D	С	Ar/Mo	V	S	R	М	LC	DD
Ophiodes sp.	25							М	LC	DD
<i>Ophiodes striatus</i> (SPIX, 1824) SCINCIDAE	12	D	С	Ar/Mo	V	S	R	М	LC	DD
<i>Mabuya dorsivittata</i> (COPE, 1862)	2	D	Sa	Ar	V	S	R	Sm	LC	LC
Salvator marianae DUMÉRIL & BIBRON, 1839	102	D	Т	G	0	S	R	L	LC	LC
TROPIDURIDAE										
<i>Tropidurus torquatus</i> (WIED-NEUWIED, 1820)	73	D	Т	Ar/Mo	0	S	D	S	LC	LC
AMPHISBAENA										
AMPHISBAENIDAE										
Amphisbaena alba LINNAEUS, 1758	4	D	Fo	Ar	0	S	R	L	LC	LC
Leposternon microcephalum WAGLER, 1824	5	D	Fo	Ar	0	S	R	Sm	LC	LC
CROCODYLIA ALLIGATORIDAE										
Caiman latirostris (DAUDIN, 1801) TESTUDINE CHELIDAE	3	D	T/Aq	G	0	S	R	М	LC	LC
Mesoclemmys hogei (MERTENS, 1967)	1	D	T/Aq	Ar/ Mo/F/	0	S	R	S	CR	CR
				An						
Phrynops geoffroanus (SCHWEIGGER, 1812)	1	D	T/Aq	Ar/ Mo/F/ An	Ο	S	D	М	LC	NE
TESTUDINIDAE										
Chelonoidis carbonarius (SPIX, 1824)	2	D	Т	PMa	0	S	R	М	LC	NE

The road-kill rate for the stretch of highway studied was 0.04 road-kill's per kilometer per month. It was possible to observe a higher number of road-kills in the stretches where the speed limit is higher. The three stretches with the highest incidence of road-kills have speed limits of 110 km/h (N = 698; 49.50), 70 km/h (N = 533; 37.80%), and 90 km/h (N = 145; 10.28%). The remaining road-kills (2.42%) occurred on stretches with speed limit between 30 and 60 km/h. The higher

number of road-kills occurred on two-lane and one-lane stretches of the highway. Together, these stretches corresponded to 96.74% of all road-kills. The sections with three and four lanes had respectively 36 (2.55%) and 10 (0.71%) records of road-kills. The number of tracks showed that the stretches with 2 (N = 508; 36.03%) and 4 (N = 812; 57.59%) lanes were the most susceptible to trampling, together corresponding to 93.62% of the road-kill events.



Figure 3. Road-kill species registered in the monitored stretch. A – *Boana faber*, municipality of Bandeira, state of Minas Gerais, Brazil; B – *Dendropsophus elegans*, municipality of Bandeira, state of Minas Gerais, Brazil; and C – *Haddadus binotatus*, municipality of Bandeira, state of Minas Gerais, Brazil. Photos by Teles, A.

Among the 60 species identified throughout the study, 52 species were classified as LC (Least Concern) for both red lists consulted. Five species were classified as LC for ICMBio (Instituto Chico Mendes de Conservação da Biodiversidade) and DD (Data Deficient) for IUCN (International Union for Conservation of Nature). Only one species was listed as CR (Critically Endangered, in both lists) and two species had no information (NE, Not Evaluated) for the IUCN list (LC in ICMBio list) (see Tables 1 and 2).

No difference in the impact of road-kills was observed between the patterns of activity reported for the group of reptiles: diurnal (N = 439; 47%), nocturnal (N = 403; 43.14%) and those active in both periods (N = 33; 3.53%). For snakes, we observed a higher number of road-kill on species that presented predominantly nocturnal activity (N = 403; 59.17%), species with diurnal activity recorded 28.92% (N = 197) while species active in both periods 4.84% (N = 33). For



Figure 4. Road-kill species registered in the monitored stretch. A – *Bothrops jararaca,* municipality of Bandeira, state of Minas Gerais, Brazil; B – *Dipsas mikanii,* Serra do Caraça, state of Minas Gerais; and C – Brazil *Chironius bicarinatus,* Serra do Caraça, state of Minas Gerais, Brazil. Photos by Soares, M.

reptiles considered as a whole, the three lifestyles attributed to the most recorded animals were terrestrial (N = 495; 52.99%) followed by semi-arboreal (N = 276; 29.55%) and cryptozoic (N = 64; 6.85%), these three totaling 89.39% of the records. The other lifestyles: semi-aquatic (N = 25; 2.67%), saxicolous (N = 2; 0.21%), fossorial (N = 9; 0.96%), and arboreal (N = 4; 0.42%), together totaled 4.26% of the sample. For snakes we noticed the same pattern observed above, species with terrestrial (N = 293; 43.02%), semi-arboreal (N = 276; 40.52%) and cryptozoic (N = 40, 5.87%) lifestyles were the most affected by road-kill, totaling 89.41% of the records. Snakes that



Figure 5. Road-kill species registered in the monitored stretch. A – *Philodryas* olfersii, Serra do Mendanha, state of Rio de Janeiro, Brazil; B – *Xenodon neuwiedii*, municipality of Rio de Janeiro, state of Rio de Janeiro, Brazil; and C – *Elapomorphus quinquelineatus*, municipality of Simão Pereira, state of Minas Gerais, Brazil. Photos by Soares, M (A – B) and Silva, F (C).



Figure 6. Road-kill species registered in the monitored stretch. A–*Erythrolamprus miliaris*, Serra do Caraça, state of Minas Gerais, Brazil; B–*Atractus zebrinus*, Serra do Caraça, state of Minas Gerais, Brazil; and C – *Pseudablades patagoniensis*, state of Rio de Janeiro, Brazil. Photos by Silva, F (A) and Ferreira-Cunha, L (B – C).

feed exclusively on mammals or that combine mammals and other taxonomic groups (i.e., generalist), were the most sampled (N = 282) corresponding to 41.40% of road-kill's. Followed by snakes that feed exclusively on anurans or that combine anurans and other taxonomic groups (i.e., generalist; N = 155), corresponding to 22.76% of road-kill's. Reptiles with seasonal reproduction (N = 777; 95.67%) were more road-killed than those with continuous reproduction throughout the year (N = 35; 4.33%). Animals with reproduction in the wet season had 545 records (67.45%), while in the dry season 228 records (28.22%). For reptiles, medium-sized individuals (500 < CRC < 1000 mm) were more commonly found on the highway (N = 403; 48.49%), followed by small animals (CRC < 500 mm) (N = 231; 27.79%) and finally large animals (CRC > 1000 mm) (N = 197; 23.70%). In a more directed view, for the snake's group, we found the same pattern, the

species most hit were the medium-sized ones (500 < CRC < 1000 mm) (N = 373; 60.85%), followed by the small ones (CRC < 500 mm) (N = 150; 24.47%) and large ones (CRC > 1000 mm) (N = 90; 14.68%).

For amphibians, the lifestyle attributed to the animals most hit was the terrestrial (N = 378; 92.42%), followed by arboreal (N = 26; 6.36%) and cryptozoic (N = 5; 1.22%) individuals. Regarding habitat, the two most frequently found groups were animals with habits strictly associated with swamp or pond (N = 216; 52.55%) or swamp or pond and river or stream backwaters (N = 169; 41.12%), together amounting to 93.7%. The other two habits, Forest floor (N = 5) and Rock wall (N = 21), together corresponded to 6.3% of the road-kills. Large individuals (CRC > 100 mm) were the most found (N = 193; 46.96%), followed by medium-sized (50 < CRC < 100 mm) (N = 186; 45.26%) and small-sized (CRC < 50 mm) (N = 32; 7.79%).



Figure 7. Road-kill species registered in the monitored stretch. A–*Bokermannohyla circumdata*, Serra do Caraça, state of Minas Gerais, Brazil; B–*Boana semilineata*, Simão Pereira, state of Minas Gerais, Brazil; and C–*Ischnocnema guentheri*, Parque Nacional da Serra dos Órgãos, state of Rio de Janeiro, Brazil. Photos by Soares, M (A) and Silva, F (B–C).



Figure 8. Road-kill species registered in the monitored stretch. A – *Rhinella ornata*, Serra do Caraça, state of Minas Gerais, Brazil; B – *Trachycephalus mesophaeus*, Serra do Caraça, state of Minas Gerais, Brazil; and C – *Thoropa miliaris*, Rebio do Tinguá, state of Rio de Janeiro, Brazil. Photos by Silva, F (A) and Ferreira-Cunha, L (B – C).

Discussion

Road ecology is a recent topic of interest in evolutionary biology, initiated in Brazil in the late 1980s (see Novelli et al., 1988) with the objective of understanding the patterns and processes related to the interactions between the road network and the ecosystems, establishing effective mitigation measures for the negative effects of roads on wildlife (Huijser et al., 2009; Rosa & Bager, 2013). However, these recent studies still show aggregations of trampling in Brazil and the World (Cáceres et al. 2012; Teixeira et al., 2013; Carvalho-Roel et al., 2019; Miranda et al., 2020; Spanowicz et al., 2020; Ascensão et al., 2021). In the present study we observed an increase in the number of road-killed during the rainy season, which indicates a seasonal pattern of trampling, as observed in other studies (Bencke & Bencke, 1999; Seibert & Conover, 1991; Machado et al., 2015; Garriga et al., 2017). This period is usually associated with the reproductive season of many groups (e.g., amphibians and reptiles) (Toledo et al., 2003; Jochimsen, 2005; Zina et al., 2007; Shepard et al., 2008a) and the increased availability of food at foraging sites. These factors stimulate the greater movement of animals, thus increasing the chance of trampling of the fauna (Forman & Alexander, 1998; Smith & Dodd, 2003; Jochimsen, 2005; Pinowski, 2005).

For instance, some studies point to ectotherms (amphibians and reptiles) as the largest victims of road-kills in wet areas (Ashley & Robinson, 1996; Glista et al., 2008; Shepard et al., 2008b) because they are strongly influenced by environmental conditions in terms of humidity and temperature (Zug et al., 2001). This pattern can be observed here, for both groups, where the trampling peak occurred during the rainy season (hot moments), coinciding with the time of greatest activity for foraging and reproduction. Due to aspects intrinsic



Figure 9. Road-kill species registered in the monitored stretch. A – *Aspronema dorsivittata*, Serra do Caraça, state of Minas Gerais, Brazil; B – *Salvator merianae*, municipality of Rio de Janeiro, state of Rio de Janeiro, Brazil; and C – *Tropidurus torquatus*, Serra do Caraça, state of Minas Gerais, Brazil. Photos by Andrade-Jr, A (A), Ferreira-Cunha, L (B) and Carvalho, B (C).

to each species (e.g., biological cycle, population density, speed of movement and use of surrounding areas close to highways) (Steen & Gibbs, 2004; Aresco, 2005) monitoring protocols should be established targeting the study area and taxonomic group of interest (Glista et al., 2008; Attademo et al., 2011).

As a rule, road-kills are concentrated in a few species of the faunal elements in a given region, usually species presenting generalist habits (non-specialized diet and microhabitats), relatively abundant population density, with high mobility and that use the highways (primarily or secondarily) as a source of resources (e.g., food intake and/or thermoregulation opportunity) (Forman et al., 2003, Secco et al., 2014). Not surprising, the group most affected by trampling in our study were reptiles, especially snakes. We raised four, not mutually exclusive, possibilities that could contribute to this high rate, as such: (i) use of the road to maximize thermoregulatory behavior at night and



Figure 10. Road-kill species registered in the monitored stretch. A – *Phrynops geoffroanus*, municipality of Paracatu, state of Minas Gerais, Brazil; and B – *Mesoclemmys hogei*, municipality of Faria Lemos, state of Minas Gerais, Brazil. Photos by Silva, F (A) and Carrara, R (B).

in cold days (Sullivan, 1981; Mccardle & Fontenot, 2016; Gonçalves et al., 2018); (ii) motionlessness as a defensive tactic used by some species (Andrews & Gibbons, 2005); (iii) intentional road-killing predominantly of snakes by cultural motivation (Secco et al., 2014; Assis et al., 2020); and (iv) the scavenging behavior of some species that are attracted to carcasses on highways (Schwartz et al., 2018; Muszynska et al. 2022).

The high number of road-killed Crotalus durissus species (N = 128) and Salvator merianae (N = 102) can be explained by the fact that they are species commonly found and adapted to open and anthropized areas, such as residential and commercial regions along roadsides. On the other hand, as in several other studies carried out in Brazil (Coelho et al., 2008; Kunz & Ghizoni-Jr, 2009; Turci & Bernarde, 2009; Abra et al., 2019; Ascensão et al., 2021) larger animals were more represented in our records of road-kills and this effect can be explained by the monitoring speed (50 km/h) and the means of transport used for monitoring (car assistance), a general standard used in our collection methodology and in several other studies (Enge & Wood, 2002; Taylor & Goldingay, 2004; Coleman et al., 2008; Delgado et al., 2019). These choices likely result in lower detection of small animals as reported in other studies that used bicycles and/or lower speed during monitoring (Pracucci et al., 2012; Rosa et al., 2012; Pinheiro & Turci, 2013; Santos et al., 2016; Wang et al., 2022), indicating that monitoring carried out with the help of cars can generate biased results for large animals.

Considering the presence of amphibians (mostly nocturnal animals) in the sample, it is believed that most of the road-kills occurred between 18:00 p.m. and 07:00 a.m. (Silva et al., 2007). This period is off-peak road traffic activity, which usually occurs in the beginning of the day and in the end of the afternoon, so that, even with this asynchronism with the peak moment on the highways, amphibians are greatly affected by trampling of wildlife, although they are still poorly sampled in studies on this topic (Glista et al., 2008). Previous studies indicated that frogs of the genus Rhinella are among the amphibians most road-killed (Dornas et al., 2017), result also observed in the present study: *Rhinella* spp. were the amphibian most road-killed with 203 register (42.47%). One possible explanation for the high rate of road-kills of individuals of this genus is that they are commonly found foraging around light poles (Coelho et al., 2012; Bastos et al., 2019). In addition to that, Rhinella species are explosive breeders, that dislocate to breeding areas during the reproductive season (the rainy season) (e.g., Rhinella ornata, Dixo et al., 2009). Leptodactylus latrans is another very abundant species in records of road-kills, as it is a species frequently recorded in areas modified by humans (Bastos et al., 2019).

Another point to be discussed is that small vertebrates, such as frogs, are usually less visualized on highways than large animals, such as some representatives of the mammal group (Santos et al., 2016; Filius et al., 2020), with this more than half of these small animals that road-killed easily go unnoticed in monitoring (Delgado et al., 2019). Due to this fact, slower research methods employing bike or walking and with more than one agent are encouraged for better visualization of smaller animals as they can result in detectability up to 8.4 times greater than using a car (Medrano-Vizcaíno et al, 2022; Wang et al., 2022). This may explain in parts the low number of road-kills recorded for the amphibian group in the present study when compared to the work performed by Filius et al. (2020), in which monitoring with bicycle and walking was carried out. Another two points that can help explain this low number of records for the amphibians are (i) smaller animals can more easily be thrown off the road and even get stuck in tires and (ii) the shorter persistence time of reptile and amphibian carcasses on highways, especially for smaller representatives (Santos et al., 2016; Filius et al., 2020).

As proposed by Sosa & Schalk (2016), our results suggest that roads can act as a barrier to the dispersion of amphibians and reptiles, especially for fossorial and arboreal species (snakes) and small species (some amphibians and lizards), since the members of this guild are more unfeasible, either by the style of movement and the crossing time, or by the lack of connectivity between the forest areas on each side of the road. Despite the high number of species found in our study, we believe that this number may be underestimated. Due to two factors: (i) species that were not yet recorded in our dataset of road-killed animals, but are expected for the region (e.g., Dactyloa punctata; Hemidactylus mabouia; Gymnodactylus darwinii; Echinanthera cephalostriata), (ii) some species may have been thrown out of the track, or even, if they took refuge in the forest and later died outside the area of collection, in addition to the possibility that they served as a food source for carnivorous and scavenger animals (Rodrigues et al., 2002; Bagatini, 2006; Silva et al., 2007; Pracucci et al., 2012; Ratton et al., 2014; Machado et al., 2015). In fact, the accumulation of carrion along the highways attracts animals, which consequently may be also road-killed (Muszynska et al. 2022). The behavior of scavenging is quite reported for the group of mammals (Gonzáles-Suarez et al.,

2018). Although the scavenging behavior is not commonly recorded among snakes, as theoretically they have the preference for live prey (Sazima & Strussmann, 1990; Greene, 1997), there are some records in the literature reinforcing such behavior in the group (see Sazima & Strussmann, 1990; Lillywhite et al., 2002; Gomes et al., 2017; Marques et al., 2017). In this way, some snakes can lead two stages in what we call "the cycle of road-kills", constituting of two main steps: (i) as a source of food for scavengers animals (attracting other carnivorous animals like birds and mammals) and/or (ii) as carrion consumers of amphibians (e.g., Chironius spp.) or other vertebrates (e.g., Philodryas spp.) along the roads. Another very important point when we talk about road ecology is the rate of decomposition of carcasses along the roads and the difficulties generated by this factor. The estimate for the disappearance of carcasses in the snake group is that approximately 50% disappear within the first 8 to 24 hours (de Gregorio et al., 2011; Santos et al., 2016; Cabrera-Casas et al., 2020), depending on traffic and time of year. We extrapolate that for most amphibians and other small reptiles (several lizards and small snakes), this rate should be even higher due to the smaller body size, directly affecting the number of recorded individuals. However, this is not the only problem caused by the decomposition of carcasses, another known issue is the difficulty for the identification of very damage specimens (Bastos et al., 2019). For this reason, 20 amphibians and 58 reptiles could not be identified to the specific level. In addition, some specimens have been identified only to the generic level, such as Tropidodryas sp. due to the fact that more than one species occurs sympatrically in the region in combination with lacking preservation of key characters for diagnosing between congeners.

About road-kill rates, most information available were also estimated for the entire vertebrate clade, not for specific taxonomic groups, or as the number or record per kilometer, which is affected by the duration of the study. In Brazil, vertebrate road-kill rates varied from 0.18 road-kills/km/month in Pantanal wetlands (Fischer, 1997), 0.19 road-kills/km/month in stretches of Cerrado (Prada, 2004), and 0.21 and 0.46 road-kills/km/month in two roads in the sandy and wet restinga (Coelho et al., 2008), important remnants of Atlantic Forest in the south of Brazil. The rate found in the section analyzed by us (0.04 road-kill's/km/month) can be considered high, since it is relative only to the herpetofauna and for a stretch of highway. Several concepts about the ecology of roads were not and still are not taken into account in the environmental licensing process (Machado et al., 2015), causing the various ecological problems presented and discussed throughout the present study. Despite a myriad of mitigating measures to road-killssuch as the construction of ecological corridors, bridges, fences, and catwalks-are constantly encouraged to prevent animals from being road-killed when crossing the roads, some of these are criticized for their efficiency (e.g., isolated use of signs) and sometimes related to an increase in the rate of predation, hunting and trafficking of animals with economic interest (e.g., tunnels and underpasses) (Smith & Dodd, 2003).

Future Directions

The use of continuous fences and tunnel system for fauna (ecopassages or wildlife culverts) are currently the most recommended strategies for mitigate the impact of road-kills of amphibians and reptiles (Schmidt & Zumbach 2008; Lesbarreres & Fahrig, 2012; Beebee, 2013; Yue et al., 2019) and can be used by other taxonomic groups, including invertebrates and small mammals.

Mitigation strategies focused on one taxonomic species or group need to be beneficial, or at least not bring negative effects, to other animals present in the study region (Jarvis et al., 2019). With this, to reduce the road-kills of amphibians and reptiles in the stretch analyzed by us we recommend, in addition to speed reducers, faunasignaling plates and environmental education campaigns, the addition of continuous fences (no spaces for the animal to pass through it) and tunnels for fauna prioritizing the highest and well-preserved areas in order to mitigate damage to populations of more vulnerable and fragile species to automotive enterprises. We also recommend the preparation of further studies along the stretch aimed at detecting hotspots and the proposal of new strategies that help in conservation of local species.

Supplementary Material

The following online material is available for this article:

Appendix – List of specimens deposited in the Amphibians and Reptiles collections of the Museu Nacional, Universidade Federal do Rio de Janeiro (MNRJ).

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Author Contributions

Daniel Faustino Gomes: contribution to conceptualization; methodology; writing – original draft.

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Pedro H. Pinna: contribution to resources; methodology; resources writing – review & editing.

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Paulo Passos: contribution to conceptualization; methodology; resources writing – review & editing.

Conflicts of Interest

The authors declares that they have no conflict of interest related to the publication of this manuscript.

Ethics

This study did not involve human beings and/or clinical trials that should be approved by one Institutional Committee.

Data Availability

The data used in our analysis is available at Zenodo Dataverse https://doi.org/10.5281/zenodo.7459911>.

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