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SEASONAL AND SPATIAL VARIATION OF ROAD-KILLED VERTEBRATES ON BR-330, SOUTHWEST BAHIA, BRAZIL

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Abstract: In the present study, we aimed to characterize and quantify the vertebrates road-killed along 12.8 km of the BR-330 highway in southwestern Bahia. The road is surrounded by patches of Atlantic Forest and Caatinga and by human-modified areas. From May/2012 to August/2013, we performed 35 surveys, monitoring 448 km in total. During this period, we registered 146 road-killed individuals belonging to 60 species (35 birds, 15 reptiles, 7 mammals and 3 amphibians). The species most likely to be road-killed in the study area were the toad *Rhinella jimi* and the snakes *Oxyrhopus trigeminus* and *Sibynomorphus neuwiedi*. The Jaccard index showed higher values when compared to the observed richness of road-killed species, which is corroborated by the ten new species recorded during five additional surveys from August/2015 to July/2016. The number of road-killed vertebrates was significantly higher in the rainy season than in the dry season. We identified 26 road-kill hotspots along the studied stretch of road. The presence of water bodies associated with vegetational landscape surrounding these hotspots may be a reason for the higher concentration of road-kills. Besides being one of the few studies conducted in an ecotonal area in the northeast of Brazil, our data indicate some important patterns that should be considered for regional and national policies on impact mitigation.

Keywords: Caatinga; ecotone; road ecology; road impact; semi-arid environment.

INTRODUCTION

Conflicts between wildlife and human interests have increased over the past decades due mainly to demographic growth of the human population (Glista *et al.* 2008). This population growth results in high levels of anthropic interference in natural environments, such as the suppression and fragmentation of habitats, altering the landscape and interrupting horizontal ecological and gene flows (Forman 1998, Trombulak & Frissel 2000, Williams *et al.* 2003, Laurance *et al.* 2009, Tucker *et al.* 2018). Among the landscape modifications produced by human action in the last decades, the construction, maintenance and expansion of road networks have been some of the most widespread modifications in the world, especially in tropical countries (Laurance *et al.* 2009, 2014).

Roads are associated with negative effects on biotic integrity, in both terrestrial and aquatic ecosystems (Laurance *et al.* 2009). Trombulak & Frissel (2000) cataloged seven deleterious effects of roads on local fauna: (1) increase in mortality of species due to road construction; (2) increase in mortality due to collisions with vehicles; (3) behavior modification; (4) physical alteration of the environment; (5) chemical alteration of the environment; (6) increase in the geographic distribution of exotic species; and (7) increase in alteration and use of the environment by humans.

Road-killing is considered as the most significant impact of the roads (Rosa & Bager 2012) and to evaluate the dynamics of road-kill, some authors have proposed indexes that show the more important sites in terms of loss of individuals of a given population (Bager & Rosa 2010, Pracucci *et al.* 2012). These indexes are based on the abundance and composition of road-killed species and on the sites where endangered species are most likely to be road-killed. Thus, it is possible to determine wildlife road-kill incidence hotspots and then take appropriate mitigation measures (Coelho *et al.* 2008), such as the construction of wildlife passages and speed reduction measures (Van der Grift *et al.* 2013). Besides the identification of hotspots, a possible seasonal variation in road-kill events must also be examined, requiring that road impacts be accurately assessed for at least one year (one cycle) (Bager & Rosa 2011, Garriga *et al.* 2017). Commonly, road-kill occurrence presents a seasonal pattern (normally linked to species' more active periods, associated with foraging, reproduction and dispersion activity) and is often not homogeneous along the road, becoming more frequent closer to shelter and foraging areas (Coelho *et al.* 2008, Pracucci *et al.* 2012, Rosa & Bager 2012).

Although a vast road network spans Brazil's northeast region, the effects of roads on the mortality rate of wildlife in this region have not received attention (Dornas *et al.* 2017). This situation is expected, since even ecological studies of faunistic communities are rarer in the northeast than in the south and southeast of Brazil. In this sense, studies concerning road ecology are essential and should be conducted systematically throughout the region. Furthermore, the northeast encompasses one of the most interesting and yet poorly studied biomes of the country: the Caatinga (Fonseca 2017). Areas of equal biological interest include transition zones between the Caatinga and other biomes (Atlantic Forest and Cerrado). The scarcity of studies of both the Caatinga and its transition zones turns difficult the evaluation of impacts of human action on the local biological community, which in turn impedes the proposal and implementation of mitigation measures.

Therefore, in the present study we aimed to assess the vertebrate species road-killed along a stretch of the BR-330 highway, between the municipalities of Jequié and Jitaúna, in the state of Bahia, Brazil. We also analyzed the richness and abundance of road-killed species, the seasonality of these events and the road-kill hotspots.

MATERIAL AND METHODS

The BR-330 highway is 1,493.50 km long. The present study was conducted along a stretch of about 12.8 km (between km 728.1 and 740.9) in the municipality of Jequié, Bahia (Figure 1). This stretch is the fastest and shortest link between the municipalities of Jequié and Jitaúna and between the BR-116 and BR-101, two of the most important highways in the country. The BR-330 is a two-way paved road measuring approximately 8 m wide, with 3 m of shoulder. Most of the natural landscape along the road has been converted to cocoa crops, pasture and residential areas. However, some stretches are in a good state of conservation, comprising areas of Atlantic Forest, Caatinga and ecotone between these two biomes.

From May/2012 to August/2013, at approximately 15-day intervals (on random days of the week), we monitored a 12.8 km stretch of BR-330. Riding a bicycle at an average speed of 10 km/h, we covered a total of 448 km and collected 35 surveys. The monitoring started at 06:00 h and ended around 10:00 h. Five additional surveys were carried out from August/2015 to July/2016, following the same methodology (adding 64 km to our dataset, totaling 512 km sampled). We highlight that since we did not standardize the data collection of this second sample period, the data served only as additional information on the list of road-killed species and were not used for the spatial and temporal analyses. The rainy and the dry seasons were defined based on Moura *et al.* (2007), the rainy period being from December to April and the dry from May to November.

During the study period, we registered all road-

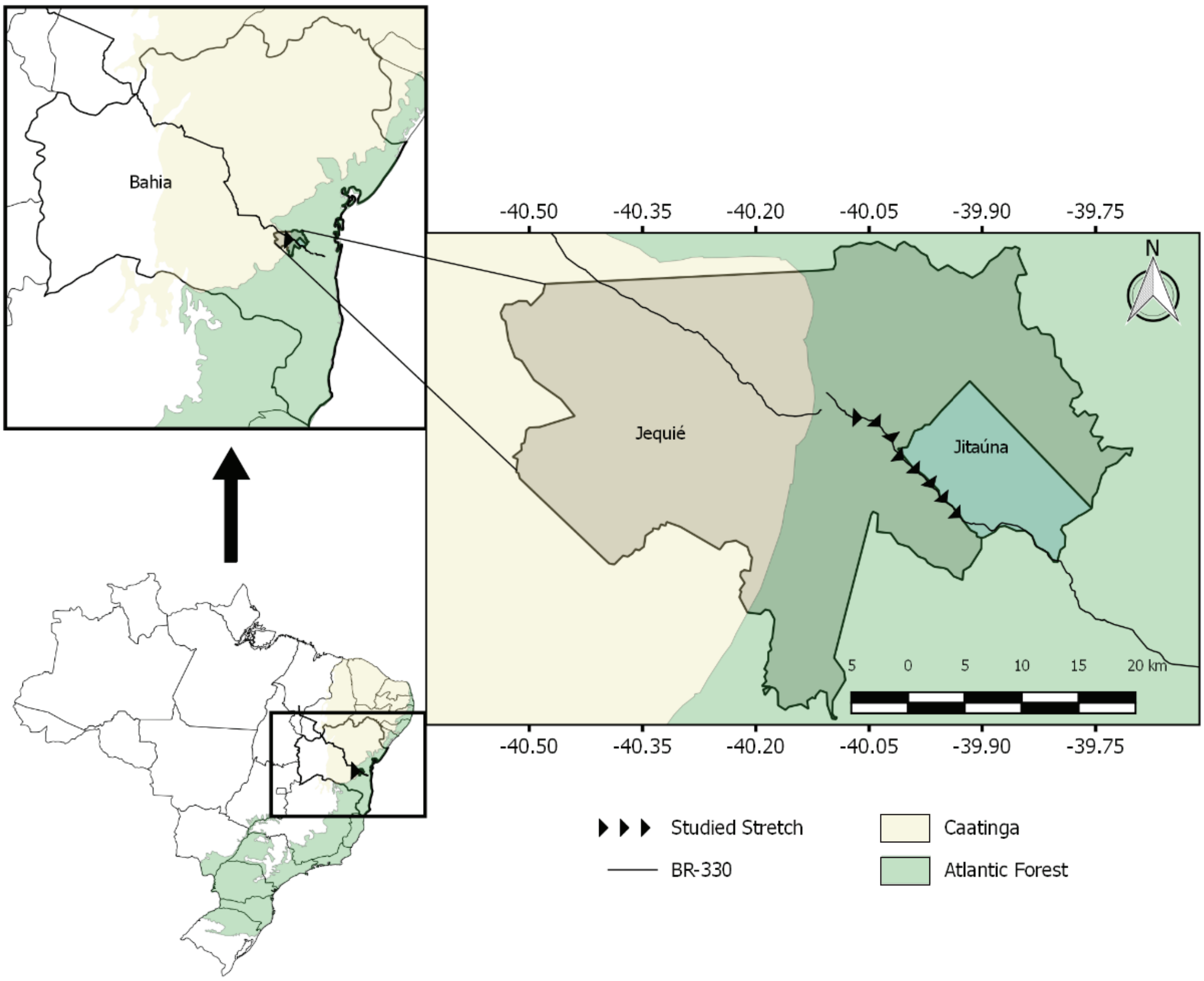

Figure 1. Map indicating study stretch in BR-330, municipality of Jequié, state of Bahia, Brazil.

killed vertebrates (amphibians, reptiles, birds and mammals). Domestic animals were not included in our data analysis. All road-kills were georeferenced to enable the analysis of road-kill concentration along the studied stretch. The road-killed animals were conditioned in plastic bags and transported in a styrofoam box on the bicycle to the Laboratório de Zoologia de Vertebrados of Universidade Estadual do Sudoeste da Bahia, campus of Jequié, where they were identified, fixed in 10% formalin and deposited (when relatively undamaged) in the zoological collection of this same institution.

All specimens were identified to the lowest possible taxonomic level, achieved through consultation with specialists on each taxonomic group (Paul François Colas Rosas for mammals, Joedison dos Santos Rocha for birds, Juliana Zina for reptiles and anurans) and through referral to field guides (Marques *et al.* 2005, Reis *et al.* 2007, Sigrist 2013, Marques *et al.* 2017). Information on the species' weight and size was obtained through field guides (Sick 2001, Reis *et al.* 2007, Sigrist 2013), a book (Sick 2001) and species description articles. The popular names of the species were obtained from the International Union for Conservation of Nature (IUCN 2018), Wikiaves (2018), Uetz (2018) and Frost (2018) platforms and from field guides (Marques *et al.* 2005, Sigrist 2013, Marques *et al.* 2017). Animals in advanced putrefaction were only photographed, registered, and removed from the road to avoid recounting. To measure the road-kill rate, we divided each species' total number of road-killed individuals by 12.8 (sampled length), divided by the total number of surveys.

To evaluate the sampling effort, we constructed a species accumulation curve (Colwell 2005) based on the number of road-killed individuals of each species, recorded between May/2012 and

August/2013. We considered each day (or each 12.8 km) of monitoring as our sample unit. The data were randomly rearranged by the Mao Tau method (1000 randomizations). The final value was compared with the mean and the standard deviation obtained by the Jackknife I estimator (Santos 2003). We extrapolated the species accumulation curve to verify the sample effort needed to reach the richness obtained by the estimators.

To test for the seasonality of road-kills, we used data (total number of road-killed vertebrates per month) collected during 24 surveys in the dry season and 11 surveys in the rainy season, and we performed a Mann-Whitney test because our data presented non-normal distribution ($p < 0.05$). We also performed a Mann Whitney test to check for seasonal differences in road-killed animals of each group (amphibians, reptiles, birds, and mammals) separately.

To determine the occurrence and scale of road-kill aggregations, we performed the 2D Ripley's K-Statistics test using an initial radius of 100 meters, a radius increase of 500 meters, confidence level of 95%, and one thousand simulations. The values of initial radius and radius increment corresponded to the scale at which mitigation measures can be effective (Teixeira *et al.* 2013). To evaluate the road-kill hotspots, we used a radius of 100 meters and 250 divisions of the road, considering that this was the smallest scale at which road-kill aggregation was significant according to the results of the 2D Ripley K-Statistics test. We performed all analyses using Siriema plots (Dornas 2018) in Siriema v2.0 software (Coelho *et al.* 2014). For the statistical analyses, we considered values of $p < 0.05$ as significant (Zar 2010).

RESULTS

Diversity

Through 35 surveys conducted in 2012 and 2013, we found 146 road-killed individuals from 60 species (35 birds, 15 reptiles, 7 mammals and 3 amphibians) (Table 1). The additional surveys from 2015 to 2016 found 70 road-killed individuals, of which we identified 31 species (15 birds, 7 reptiles, 6 mammals and 3 amphibians), adding ten newly recorded species to the road-kill list. In total (data collected in both periods), we registered 216 road-killed individuals belonging to 70 species. Considering the entire sampling effort, around 78% of road-killed individuals belongs to species that weighed less than 400 g.

Four snake individuals could not be identified due to their state of putrefaction. However, it was possible to verify, by counting scaling, that all represented different taxonomic units. Three other species of reptiles, including two snakes and a lizard, could be identified only to the genus level (Table 1). Two species of birds were identified to the

Table 1. Species of road-killed vertebrates on the BR-330 highway from May/2012 to August/2013, municipality of Jequié, state of Bahia. *Species with potential to reach more than 400 g. ** species recorded in the additional monitoring conducted between August/2015 and July/2016.

Taxon	Common name/Local name	N	Road-kill ratio (ind/km/day)
AMPHIBIA			
Order Anura			
Family Bufonidae			
Rhinella jimi (Stevaux 2002)*	Cururu Toad/sapo-cururu	18	0.040
Family Hylidae			
Boana crepitans (Wied-Neuwied 1824)	Rattle-voiced Treefrog/perereca gladiadora	2	0.004
Pithecopus nordestinus (Caramaschi 2006)**	Leaf Frog/perereca-das-folhagens	1	0.002
Family Leptodactylidae			
Leptodactylus latrans (Steffen 1815)*	Buttter Frog/caçote	8	0.017

Table 1. Continued on next page…

Table 1. ...Continued

Taxon	Common name/Local name	N	Road-kill ratio (ind/ km/day)
REPTILIA			
Order Squamata			
Family Amphisbaenidae			
Amphisbaena alba Linnaeus 1758	Red Worm Lizard/cobra-de-duas-cabeças	3	0.006
Family Boidae			
Epicrates assisi (Machado 1945)*	Caatinga Rainbow Boa/salamanta	1	0.002
Family Dipsadidae			
Dipsas sp.		1	0.002
Erythrolamprus almadensis (Wagler 1824)	Almaden Groud Snake/cobra-d'água	4	0.008
Oxyrhopus trigeminus (Duméril, Bibron & Duméril 1854)	Brazilian False Coral Snake /falsa-coral	11	0.024
Oxyrhopus petolarius (Linnaeus 1758)**	Forest Flame Snake /falsa-coral	2	0.004
Philodryas patagoniensis (Girard 1858)	Patagonia Green Racer /corredeira	2	0.004
Sibynomorphus neuwiedi (Ihering 1911)	Eastern Slug-eater /dormideira	11	0.024
Family Elapidae			
Micrurus ibiboboca (Merrem 1820)	Caatinga Coral Snake /coral-verdadeira	2	0.004
Family Viperidae			
Bothrops sp.*		1	0.002
not identified			
sp1		1	0.002
sp2		1	0.002
sp3		1	0.002
sp4		1	0.002
Family Teiidae			
Tupinambis sp.*		1	0.002
Family Polychrotidae			
Polychrus acutirostris (Spix 1825)	Brazilian Bush Anole /papa-vento	1	0.002
BIRDS			
Family Caprimulgidae			
Chordeiles acutipennis (Hermann 1783)	Lesser Nighthawk /bacurau-de-asa-fina	6	0.013
Family Columbidae			
Columbina talpacoti (Temminck 1811)	Ruddy Ground-Dove /rolinha-roxa	1	0.002
Columbina picui (Temminck 1813)**	Picui Ground-Dove/rolinha	1	0.002
Family Cuculidae			
Coccyzus euleri Cabanis 1873	Pearly-breasted Cuckoo/papa-lagarta-de-euler	1	0.002
Coccyzus melacoryphus Vieillot 1817	Dark-billed Cuckoo/papa-lagarta-acanelado	1	0.002

Table 1. Continued on next page…

Table 1. ...Continued

Taxon	Common name/Local name	N	Road-kill ratio (ind/ km/day)
Guira guira (Gmelin 1788)**	Guira Cuckoo /anu-branco	1	0.002
Crotophaga ani Linnaeus 1758**	Smooth-billed Ani/anu-preto	1	0.002
Tapera naevia (Linnaeus 1766)*	Striped Cuckoo/saci	1	0.002
Family Donacobiidae			
Donacobius atricapilla (Linnaeus 1766)	Black-capped Donacobius/japacanim	1	0.002
Family Estrildidae			
Estrilda astrild (Linnaeus 1758)	Common Waxbill /bico-de-lacre	1	0.002
Family Falconidae			
Milvago chimachima (Vieillot 1816)*	Yellow-headed Caracara/carrapateiro	1	0.002
Family Furnariidae			
Synallaxis frontalis Pelzeln 1859	Sooty-fronted Spinetail/petrim	1	0.002
Synallaxis hypospodia Sclater 1874	Cinereous-breasted Spinetail/joão-grilo	2	0.004
Family Icteridae			
Icterus jamacaii (Gmelin 1788)**	Campo Troupial/sofrê	1	0.002
Family Parulidae			
Setophaga pitiayumi (Vieillot 1817)**	Tropical Parula/mariquita	1	0.002
Family Passerellidae			
Ammodramus humeralis (Bosc 1792)	Grassland Sparrow/tico-tico-do-campo	1	0.002
Family Passeridae			
Passer domesticus (Linnaeus 1758)	House Sparrow/pardal	1	0.002
Family Picidae			
Colaptes campestris (Vieillot 1818)**	Campo Flicker/pica-pau-do-campo	1	0.002
Family Polioptilidae			
Polioptila plumbea (Gmelin 1788)	Tropical Gnatcatcher/balança-rabo-de-chapéu- preto	1	0.002
Family Thraupidae			
Conirostrum speciosum (Temminck 1824)	Chestnut-vented Conebill/figuinha-de-rabo-castanho	1	0.002
Coryphospingus pileatus (Wied 1821)	Pileated Finch/tico-tico-rei-cinza	1	0.002
Sporophila angolensis (Linnaeus 1766)	Chestnut-bellied Seed-finch/curió	1	0.002
Sporophila nigricollis (Vieillot 1823)	Yellow-bellied Seedeater/baiano	4	0.008
Sporophila plumbea (Wied 1830)	Plumbeous Seedeater/patativa	1	0.002
Volatinia jacarina (Linnaeus 1766)	Blue-black Grassquit/tiziu	9	0.020
Sporophila caerulescens (Vieillot 1823)**	Double-collared Seedeater/coleirinha	1	0.002
Tangara sayaca (Linnaeus 1766)	Sayaca Tanager/sanhaçu-cinzento	1	0.002
Family Tityridae			
Laniisoma elegans (Thunberg 1823)	Shrike-like Cotinga/chibante	1	0.002

Table 1. Continued on next page…

Table 1. ...Continued

Taxon	Common name/Local name	N	Road-kill ratio (ind/ km/day)
Family Trochilidae			
Chlorostilbon lucidus (Shaw 1812)	Glittering-bellied Emerald/ besourinho-de-bico-vermelho	2	0.004
Eupetomena macroura (Gmelin 1788)	Swallow-tailed Hummingbird/beija-flor-tesoura	1	0.002
Glaucis hirsutus (Gmelin 1788)	Rufous-breasted Hermit/balança-rabo-de-bico-torto	1	0.002
Heliomaster furcifer (Shaw 1812)	Blue-tufted Starthroat/bico-reto-azul	1	0.002
Phaethornis sp.		1	0.002
Thalurania sp.		1	0.002
Family Troglodytidae			
Troglodytes musculus Naumann 1823	Southern House Wren/corruíra	3	0.006
Family Tyrannidae			
Euscarthmus meloryphus Wied 1831	Tawny-crowned Pygmy-tyrant/ barulhento	1	0.002
Lathrotriccus euleri (Cabanis 1868)	Euler's Flycatcher/enferrujado	8	0.017
Legatus leucophaius (Vieillot 1818)	Piratic Flycatcher/bem-te-vi-pirata	1	0.002
Myiophobus fasciatus (Statius Muller 1776)	Bran-colored Flycatcher/filipe	3	0.006
Tyrannus melancholicus (Vieillot 1819)	Tropical Kingbird/suiriri	1	0.002
Family Vireonidae			
Hylophilus amaurocephalus (Nordmann 1835)	Gray-eyed Greenlet/vite-vite-de-olho-cinza	1	0.002
not identified			
sp.1		1	0.002
MAMMALIA			
Order Primates			
Family Callitrichidae			
Callithrix penicillata (É. Geoffroy 1812)	Black-pencilled Marmoset/sagui-do-tufo-branco	2	0.004
Order Cingulata			
Family Dasypodidae			
Dasypus sp.		1	0.002
Order Didelphimorphia			
Family Didelphidae			
Didelphis albiventris Lund 1840*	White-eared Opossum/gambá-de-orelha-branca	3	0.006
Order Chiroptera			
Family Phyllostomidae			
Carollia perspicillata (Linnaeus 1758)	Seba's Short-tailed Bat/morcego	1	0.002

Table 1. Continued on next page…

Table 1. ...Continued

Taxon	Common name/Local name	N	Road-kill ratio (ind/km/day)
Glossophaga soricina (Pallas 1766)	Pallas's Long-tongued Bat/morcego-beija-flor	1	0.002
Platyrrhinus lineatus (E. Geoffroy 1810)	White-lined Broad-nosed Bat/morcego	1	0.002
Family Molossidae			
Molossus molossus (Pallas 1766)**	Pallas's Mastiff Bat/morcego	1	0.002
Family Vespertilionidae			
Lasiurus sp.		3	0.006

genus level, and only one could not be identified. Two bats were identified only to the genus level.

Three bird species were the most recorded: *Chordeiles acutipennis* (N = 6), *Lathrotriccus euleri* (N= 8) and *Volatinia jacarina* (N = 9). Most bird species (71.4%), however, were represented by only one specimen in the present study. One of the species with only one road-kill record, *Crotophaga ani,* was nonetheless frequently observed occupying the vegetation and crop areas adjacent to the road. In addition to the greatest richness, birds also composed the greatest number of road-killed individuals (N = 64; 0.14 ind/km/day).

The second group with the highest road-kill rates was reptiles (N = 42; 0.09 ind/km/day), represented by 15 species (Table 1). The two most common species of reptiles were *Sibynomorphus neuwiedi* (N = 11) and *Oxyrhopus trigeminus* (N = 11), which together constituted 52.3% of the road-killed reptiles. Although we did not register any *Tropidurus hispidus* road-killed on the studied stretch, we observed several individuals of this species moving along the shoulders of the road, close to rocky edges.

The amphibians represent the third most affected group, having three road-killed species in total: *Rhinella jimi* (n = 18), *Leptodactylus latrans* (n = 8) and *Boana crepitans* (n = 2). Mammals were represented by seven species, four of which were Chiroptera (7 individuals), corresponding to a road-kill rate of 0.026 ind/km/day (Table 1). Compared to the other groups, fewer mammals were road-killed. The mammal species with the highest number of road-kills were the marsupial *Didelphis albiventris* (n = 3) and the bat *Lasiurus* sp. (n = 3).

The accumulation curve increased without reaching an asymptote. The value obtained by Jackknife I (98.85 ± 7.65) corresponded to 61% of our observed dataset (Figure 2), meaning that 43 additional field trips (= 550 km) would be necessary to potentially register all wildlife at risk of being road-killed.

Seasonality

A larger number of individuals of all studied vertebrate groups (amphibians, reptiles, birds and mammals) were road-killed per kilometer during the rainy season (Figure 3), and we found a significant difference between the dry season and rainy season in terms of the abundance of road-killed individuals (U = 51.5; p = 0.004). When consider separately, only amphibians (U= 77.0; p= 0.028) and mammals (U= 85.0; p= 0.041;) presented a significant seasonal pattern in road-kill.

Hotspots

We recorded 26 hotspots for vertebrate road-kill, which were concentrated at different points along km 728, 729, 735, 736, 737, 739 and 740 (Table 2). We registered the highest number of road-kill events close to patches of Caatinga, Atlantic Forest, ecotone areas between these two domains, swamps and a reservoir. We registered the fewest road-kill events close to areas characterized by small remnants of forest interspersed with areas of "cabruca", pasture and housing.

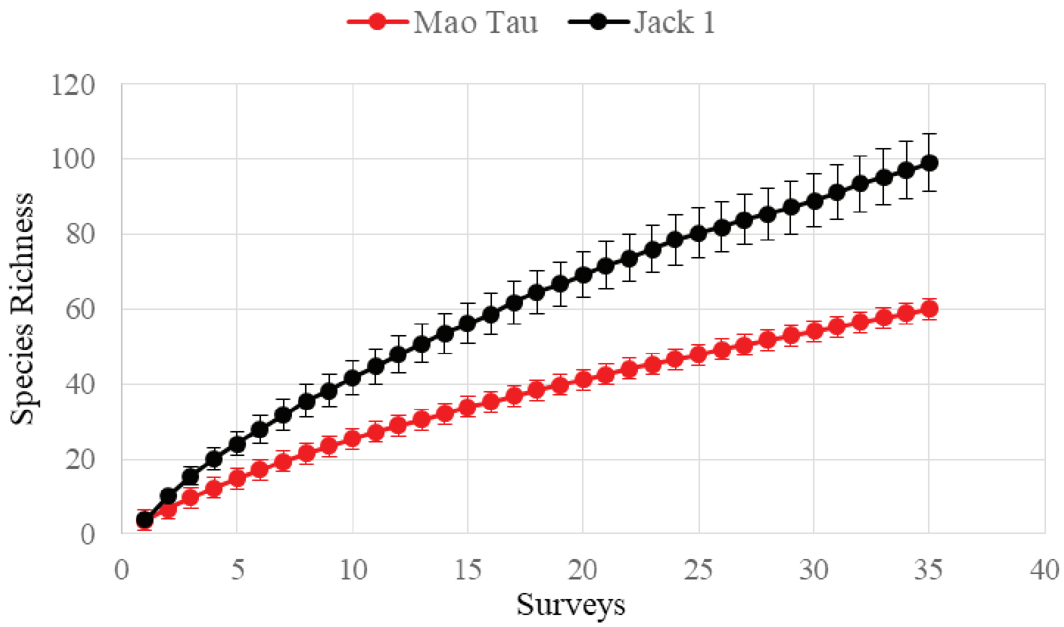

Figure 2. Road-killed species accumulation curve (Mao Tau) and corresponding richness values estimated by Jaccard's coefficient. Vertical bars correspond to standard deviation.

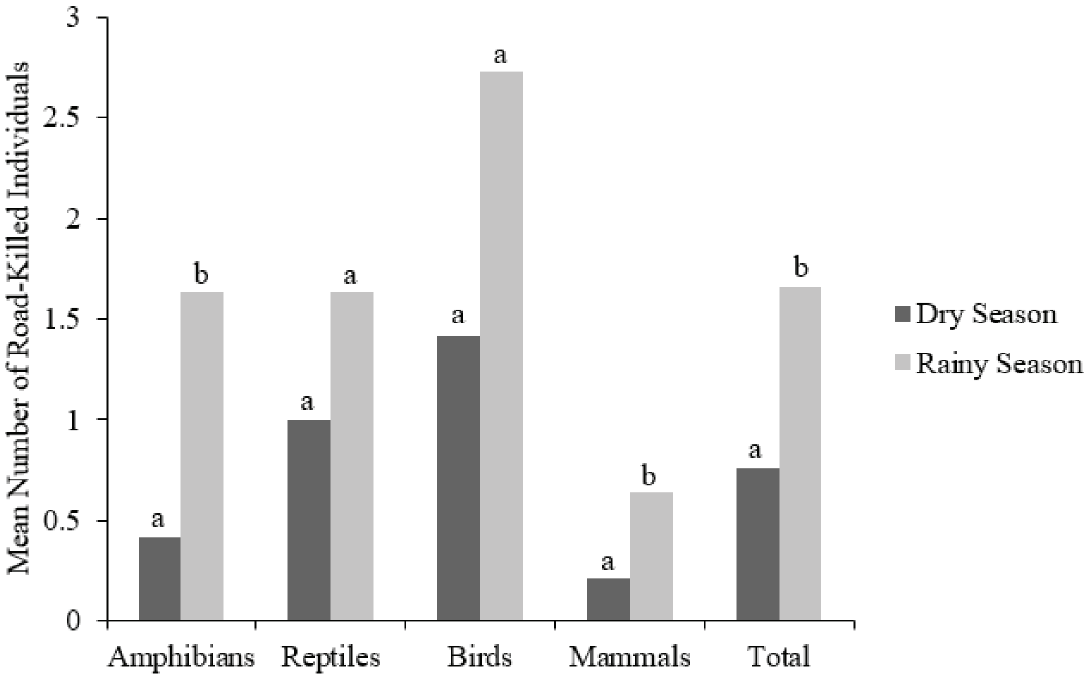

Figure 3. Mean number of road-killed individuals of amphibian, reptile, birds and mammal species per kilometer, during the rainy season and dry season between May/2012 and August/2013, on BR-330 in the municipality of Jequié, state of Bahia. Different letters correspond to statistical difference among groups.

Table 2. Road-kill hotspots along the studied stretch of BR-330 (km 728.1–740.9), municipality of Jequié, state of Bahia, from May 2012 to August 2013; identified through 2D Ripley's K-statistic test. HS: function value; UCL: upper confident interval; LCL: lower confidence limit.

Km	Latitude (X)	Longitude (Y)	HS	UCL	LCL
728.227	-13.966	-39.959	1.928	0.929	-1.071
728.278	-13.965	-39.959	1.899	0.899	-1.101
728.736	-13.963	-39.962	1.916	0.916	-1.084
728.787	-13.962	-39.963	2.885	0.885	-1.115
728.838	-13.962	-39.963	2.868	0.868	-1.132
729.042	-13.961	-39.965	1.896	0.9	-1.091
729.093	-13.961	-39.965	1.86	0.868	-1.116
729.755	-13.957	-39.969	1.898	0.898	-1.102
729.806	-13.956	-39.969	1.894	0.894	-1.106
729.907	-13.956	-39.97	1.911	0.911	-1.089
735.915	-13.919	-40.009	1.928	0.928	-1.072
735.966	-13.918	-40.01	3.88	0.906	-1.077
736.017	-13.918	-40.01	3.907	0.916	-1.078
736.068	-13.917	-40.01	2.871	1.88	-1.094
736.119	-13.917	-40.01	2.905	0.91	-1.084
736.934	-13.911	-40.015	1.989	0.989	-1.011
736.985	-13.911	-40.015	1.947	0.947	-1.053
737.036	-13.911	-40.016	2.876	0.909	-1.057
737.086	-13.911	-40.016	1.9	0.914	-1.058
739.276	-13.892	-40.023	1.964	0.968	-1.025
739.327	-13.892	-40.023	2.922	0.925	-1.073
739.378	-13.892	-40.024	3.896	0.907	-1.086
739.429	-13.892	-40.024	2.879	1.879	-1.121
740.396	-13.886	-40.031	1.891	0.898	-1.087
740.447	-13.886	-40.031	1.891	0.891	-1.109
740.498	-13.885	-40.031	2.896	0.896	-1.104

DISCUSSION

Small animals (species with less than 400 g) represented the largest proportion of our road-kill records. This finding can be explained by the high representation of bird and reptile groups and the low velocity of monitoring by bicycle, which allows higher detection of the smaller carcasses (Rosa *et al.* 2012, Pracucci *et al.* 2012, Santos *et al.* 2016), as observed in similar studies (Pracucci *et al.* 2012, Pinheiro & Turci 2013).

The higher number of road-kills among birds may be explained by the greater richness and abundance of bird species in areas adjacent to roads, compared to other animal groups, an evident fact in other vertebrate communities throughout Brazil (e.g. Sabino & Prado 2003). It is also worth emphasizing that most of the bird species sampled here are tolerant to the environmental changes caused by roads and sometimes even benefit from structural changes associated with roads (see Fahrig & Rytwinski 2009, Morelli *et al.* 2014). Three bird species can be considered, locally, the most affected by road-kill: *Chordeiles acutipennis*, *Lathrotriccus euleri* and *Volatinia jacarina*, the last one being the most road-killed species in other studies as well (see Dornas *et al.* 2017). These species' high road-kill rates may be related to their diet, foraging methods, and use of roads to feed. *Chordeiles acutipennis* and *L. euleri* are insectivorous birds (Sick 2001, Sigrist 2013). Along the studied stretch, we observed several insects, alive and dead, that are commonly eaten by generalist birds living near roads (Erritzoe *et al.* 2003). *Chordeiles acutipennis* forages mainly by visual perception and is most active during the twilight and on clear (moonlit) nights (Sick 2001). The light provided by car headlights increases the visibility of insects and may therefore create a possible collision course for *C. acutipennis* (see Jackson 2003). *Lathrotriccus euleri* usually occurs at high densities in degraded areas, and it presents daytime activity (Sigrist 2013), which can result in higher road-kill rates due to the greater flow of vehicles during the day. The pattern of activity and the use of weeds and grassy areas that surrounds the roads as foraging sites (Sigrist 2013) can also explain the high road-kill rate of *V. jacarina*.

The second group most affected by road-kills in our study was reptiles, particularly snakes. We listed four possible reasons that may contribute to the road-kill rate among snakes: 1) use of the road as a thermoregulatory surface (Sullivan 1981, Mccardle & Fontenot 2016, Gonçalves *et al.* 2018); 2) motionlessness as a defensive tactic used by some species (Andrews & Gibbons 2005); 3) intentional road-killing of snakes (Secco *et al.* 2014); and 4) locomotion limitations imposed by low friction surface of roads, compared to natural ground (D.C. Ward, W. F. Bien, I. Telenchev *et al.*, unpublished data).

The higher abundance (or activity pattern) may explain the higher road-kill rates among the snakes *Oxyrhopus trigeminus* and *Sibynomorphus neuwiedi*. Those species are commonly registered in the region and are habitat generalists (J. Zina, personal observation). Therefore, we assume that these species have more opportunity to be affected by road-killing, as also observed by Gonçalves *et al.* (2018). I. Telenchev *et al.* (unpublished) pointed out that locomotory performance associated with habitat preferences accounts for the higher rates in road-kill rates for reptiles than amphibian species.

Although reptiles presented the second highest richness of road-killed species, this number may still be underestimated. For example, the lizard *Tropidurus hispidus* presented no road-kill record, despite being registered on the roadside. Additionally, at least eight snake species and seven lizard species are known to occur in the vicinity of the road (J. Zina, personal observation) but were not registered in our dataset of road-killed species.

Amphibians were the third group most affected in the present study. Although at least 30 species of this group occurs in this area (Lantyer-Silva *et al.* 2013), only four were negatively impacted on the studied stretch of road. *Leptodactylus latrans* and *Boana crepitans* represent the most abundant species in the municipality of Jequié and are frequently recorded in human-modified areas (J. Zina, personal observation). Therefore, the areas of high population density attract these species increasing road-killing rates. In addition, both use temporary, semi-permanent and permanent environments for reproductive activity (Lantyer-Silva *et al.* 2013), which increase the potential areas to be used by both species. Additionally, both species are frequently observed moving from one spot to another (J. Zina, personal observation), especially during the wet season, probably in search for suitable water bodies for reproduction. This behavior increases their chances to be in collision routs. This assertion is corroborated by the high rates of anurans struck near areas close to aquatic environments. *Rhinella jimi*, however, is a species that, despite its low population density (J. Zina, personal observation), is commonly associated with anthropic environments (Andrade & Carnaval 2004). According to Dornas *et al.* (2017), toads of the genus *Rhinella* are among the most road-killed amphibian species. Many individuals can be found foraging under light poles (Coelho *et al.* 2012), which may explain why this species had the highest road-kill rate among all species recorded in the present study.

Mammals were the group least affected by road-kill in our study area. One explanation for this result is that the diversity of mammal species may be low in the surroundings of the studied section of road. However, given the lack of studies of the group in the region, we cannot affirm this statement.

Surprisingly, the most affected order of mammals was bats, a group that has enormous richness and may occur in a myriad of environments (Reis *et al.* 2007). These two characteristics, combined with man-made structures near the road (which may be attractive for bat species) and the presence of artificial lighting (which attracts insects, main food resource for bats), may explain the greater prevalence of chiropterans in the present study (see Rydell 2006). According to Novaes *et al.* (2018), most publications on wildlife road-kill in Brazil do not report bats, which could give the false idea that roads pose no risk to this group. These same authors believe that, because bats are road-killed during flight, most individuals can be projected off the road, leading to an underestimation of the number of road-killed bats (Secco *et al.* 2017).

The species accumulation curve showed an underestimation of species at risk of being be road-killed along the studied stretch. Besides secondary causes, such as carcass removal (Teixeira *et al.* 2013, Ratton *et al.* 2014), desiccation (Pereira *et al.* 2018), and projection of animals upon collision with vehicle (Novaes *et al.* 2018), the first explanation for the underestimation in our data may be related to the short distance sampled and the relatively few field surveys. However, as road-kill represents a sporadic event for most species, even long-term studies or monitoring over longer stretches may neglect some potential at-risk taxa (Bager & Rosa 2011). Therefore, we believed that it is possible, to an extent, to identify general patterns (such as seasonality and the most affected species and groups) that can be used to ground mitigation actions.

We observed an increase in the number of road-killed individuals during the rainy season for all vertebrate groups, which indicates a seasonal pattern of road-killing similar to that observed in other studies (Machado *et al.* 2015, Garriga *et al.* 2017). This is not surprising, since the remarkable climatic variability of semi-arid regions directly influences the activity/record of vertebrate species in the region (J. Zina, personal observation). The increase in resources (food and water) in the road's surroundings during the rainy period may be responsible for the higher road-kill rates during this season (Machado *et al.* 2015). In the case of amphibians, the rainy season usually corresponds with a time of higher reproductive activity among these species (see Toledo *et al.* 2003, Zina *et al.* 2007). Mammals present a significant seasonal road-kill pattern. Contrary to Bueno & Almeida (2010), we registered more road-killed mammals during the rainy season. Bueno & Almeida argue that the dry season is a period when individuals and groups of mammals move more in search of resources, especially food. We suggest that the movement of individuals or groups during the dry season must be a trade-off between the possibility of finding food and the risk of dehydration; thus, road-kill events are particular to each morphoclimatic region.

Some areas along the studied stretch of BR-330 presented higher numbers of road-kills and were considered hotspots by our analysis. The common aspect among them is their proximity to water bodies in both Caatinga and Atlantic Forest areas. Possibly, the presence of the water attracted various species of the groups that use this resource (Junior *et al.* 2012), especially in semi-arid environments, for reproduction (e.g. anurans), feeding, or as passage routes (Bueno *et al.* 2013, 2015, Freitas *et al.* 2015).

The studied stretch of highway presents great regional and national importance, which makes the study of the impacts of vertebrate road-kill extremely important for the conservation of the local wildlife. In addition, the study was conducted in an ecotone, an area of recognized ecological importance. However, the absence of baseline studies for most of the vertebrate groups considered here hinders the determination of the highway's actual impact on population dynamics. Fortunately, a simple inventory of the most affected species and the road-kill hotspots can provide meaningful input for future decision-making regarding mitigation measures, such as fences and wildlife passages, signaling and control signs, and traffic reduction during animal breeding periods (Fahrig & Rytwinski 2009, Van der Grift *et al.* 2013).

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