**Non-volant mammals of the Serra da Macaca ParkRoad (SP-139), southeastern Brazil.**

*Francisco de Assis Alves1\*, Carlos Roberto Teixeira1, Luciano Barbosa2 & Jairo Alves Júnior3*

1. Universidade Estadual Paulista, Faculdade de Medicina Veterinária e Zootecnia, Departamento de Cirúrgia Veterinária e Reprodução Animal, Rua Prof. Doutor Walter Mauricio Correa, s/n, CEP: 18618-681, Botucatu, SP, Brasil.
2. Universidade Estadual Paulista, Departamento de Bioestatística, Instituto de Biociências, Rua Prof. Dr. Antônio Celso Wagner Zanin, 250, CEP: 18618-689, Botucatu, SP, Brasil.
3. Muriqui Consultoria Ambiental, Rua dos Bandeirantes, nº 461, Jardim Lemense, CEP: 13610-639, Leme, SP, Brasil.

E-mails: francisco.alves@unesp.br; cr.teixeira@unesp.br; luciano.barbosa@unesp.br; alves.jairo.jr@gmail.com

Running title: *Mamíferos da Estrada Parque (SP-139)*

**ABSTRACT**

Our objective was to survey non-volant mammals that use the Serra da Macaca ParkRoad (SP-139), particularly during daily closure. We used visual searches and camera traps to record mammals, and estimator Jackknife to estimate the expected richness. We also checked the roadkills of the non-volant mammals.

The dissimilarity of species between campaigns was evaluated through the Jaccard similarity index (*Cj*), while differences in diversity were calculated using the Shannon-Wiener index (*H’*). We recorded 12 species of non-volant mammals, four of them considered threatened in the State of São Paulo. A lower dissimilarity was observed between the results of the second and fourth sampling campaigns. An important find was the absence of some predominantly nocturnal species in the list of the roadkilled non-volant mammals. The results highlight the importance of the temporary road closures, during periods of high mammal movements.

**Keywords:** roadkill; mammalia; Atlantic Forest; road closures; protected areas.

The Atlantic Forest biome in Brazil is a world hotspot for biodiversity (Pinto *et al*., 2006), accounting for the second largest richness for mammal species in the country, with 298 species. Of these, 90 species are endemic to the biome (Paglia *et al*., 2012) and about 18% are officially considered threatened species (ICMBio, 2018). Even though mammals are seldom mentioned as bioindicators (Siddig *et al*., 2016), there are reports that the taxon is an early indicator of the incidence of alterations in natural areas (Carvalho *et al*., 2016).

Mammal surveys have a wide range of applications (Tobler *et al*., 2008). The most widespread techniques for studies of this nature include linear transections, direct observations, track search (tracks, fur, feces), captures and interviews (Voss & Emmons, 1996). On the other hand, the use of camera traps has gained popularity in the last decades (Tobler *et al*., 2008).

We aimed to perform a survey of mammals to obtain a list of species that use the Serra da Macaca ParkRoad (SP-139), focusing particularly during the period when the road is closed, between 8 p.m. and 6 a.m. of the following day.

Nequinho Fogaça Road (SP-139) was constructed in 1942 as an unpaved one-way road linking the highlands (São Miguel Arcanjo, State of São Paulo, in the Alto Paranapanemaregion) with the coastal plains (Sete Barras*,* State of São Paulo, in the Vale do Ribeira do Iguaperegion). This road crosses the Carlos BotelhoState Park (PECB), an integral protected area for the Atlantic Forest (Figure 1) that holds several rare endemic species (Beisiegel, 1999).

The project to pave and install improvements on the Serra da Macaca ParkRoad (SP-139), which is the popular name for the 33 km stretch of road crossing the PECB, was discussed during the establishment of the management plan for the Park. The paving project selected a type of ecological paving (using interlocked concrete blocks that allow better rainwater drainage and store less heat), installed a drainage system, regularized hillside protections and installed metallic protective fences. The work started on October 2013 and finished in November 2015. It was implemented a series of preventive measures to avoid roadkills. For instance, the road has a total of 16 overpasses (canopy bridges), 12 underpasses (bridges and culverts), speed limits of 40 km/h, nocturnal closure and two OCRs (Optical Character Recognition), at the entrance and exit of the PECB.

Data collection occurred between 2018 and 2019 during four different campaigns (10 days each): March 2018 (1st C), July 2018 (2nd C), November 2018 (3rd C), and February 2019 (4th C). We used “R” v. 3.5.1 (RSTUDIO TEAM, 2020) to conduct all the analyzes.

Visual searches on the road consisted in a slow car travel (approximately 20 km/h) in both ways by two observers in the morning (6 a.m. to 9:30 a.m.) and night (8 p.m. to 10:30 p.m.) periods, totaling 5.280 km travelled.

For the sampling with camera traps, 16 sampling points were selected in the studied area (Figure 1). The points were on average 2 km apart, approximately. A digital camara trap (Bushnel® Modelo ZT820) was installed at each point for five consecutive nights, always after the road was closed for traffic, totaling a sampling effort of 320 cameras/nights. The equipments were installed on vertical traffic signs at a height of 40 cm from the ground and were programmed to obtain 10-sec videos, with an interval of 10 seconds between triggers. We didn’t use any type of bait.

The species accumulation curve was plotted based on the records collected for non-volant mammals through a combination of visual searches and camera traps. Species richness estimates were obtained using the Jackknife estimator, performing 1.000 randomizations. Species dissimilarity between the sampling campaigns was measured through the Jaccard similarity index (*Cj*), which was adopted to build a dendrogram through the Unweighted Pair Group Method with Arithmetic (UPGMA), in order to check for temporal variations. The diversity of non-volant mammals observed during the four sampling campaigns was compared through the Shannon-Wiener Index (*H’*).

Also, for comparison purposes we recorded mammal roadkills data during the campaigns. In parallel, the State Forestry Foundation disclosed roadkills data collected by the fiscalization service of the Park, since the inauguration of the revitalized stretch of the road (November 2015).

The scientific names followed Abreu *et al*. (2021) and the species were identified according to Reis *et al.* (2011). Threat status was based on the List of Threatened Species in the State of São Paulo (São Paulo, 2018).

Twelve species of non-volant mammals were recorded during the study (Table 1 and Figure 2). The lowland tapir (*Tapirus terrestris* Linnaeus, 1758) is considered endangered in the State of São Paulo (São Paulo, 2018). Three other species were classified as vulnerable to extinction: the ocelot (*Leopardus pardalis* Linnaeus, 1758), the puma (*Puma concolor* Linnaeus, 1771) and the small red brocket (*Mazama bororo* Duarte, 1996). There was also the record of a near-threatened species, the agouti (*Cuniculus paca* Linnaeus, 1766), while the tapeti (*Sylvilagus brasiliensis* Linnaeus, 1758) is qualified as data deficient.

The species accumulation curve did not show the tendency towards complete stabilization with the sampling effort undertaken (Figure 3). This result indicates that a greater sampling effort would certainly result in the addiction of some species that were not sampled. The estimated species richness obtained through the non-parametric estimator Jackknife was 14.92 species (± 1.7).

The dissimilarity observed between campaigns two and four was the lowest (*Cj* = 0.20) – with eight species in common (Figure 4). On the other hand, campaign three was the most dissimilar (*Cj* = 0.68) and presented the lowest diversity (Table 2).

In turn, 21 carcasses of mammals belonging to 13 different species were recorded through the survey of roadkilled non-volant mammals (Table 3). Among these, the southeastern four-eyed opossum (*Philander quica* Temminck, 1824) had the highest relative frequency (19.05%). Besides that, the occurrence of another species vulnerable to the risk of extinction has been confirmed to the study area: the giant anteater (*Myrmecophaga tridactyla* Linnaeus, 1758).

The species richness observed in the Serra da Macaca ParkRoad (SP-139) corresponded to 22% of the species sampled by Brocardo *et al*. (2012), in the context of a survey of the non-volant mammals in the Carlos BotelhoState Park (PECB).

Among the species recorded, some are notable due to their conservation statuses, including felines such as puma (*Puma concolor*) and ocelot (*Leopardus pardalis*), as well as lowland tapir (*Tapirus terrestris*), which occur naturally in the wild in low densities (Saranholi, 2013; Affonso, 1998). An important find was the absence of these predominantly nocturnal species (Reis *et al.,* 2006), in the list of roadkilled non-volant mammals.

Also noteworthy is the presence of the small red brocket (*Mazama bororo*), the largest species of deer endemic to Brazil and possibly the deer species with the smallest geographic distribution among all neotropical deers (Duarte *et al*., 2017). During the assessed period we also recorded a specimen of small red brocket (*Mazama bororo)* roakilled.

The lowland tapir (*Tapirus terrestris*), despite the species populational attributes, was the second most abundant mammal during the sampling, just below the black-eared opossum (*Didelphis aurita* Wied-Neuwiedi, 1826). The largest non-volant mammal in Brazil (lowland tapir) was more commonly spotted on the higher parts of the road.

When considered isolated, visual searches led to the record of five species: the southern tamandua (*Tamandua tetradactyla* Linnaeus, 1758), the black-horned capuchin (*Sapajus nigritus*), the tapeti (*Sylvilagus brasiliensis* Linnaeus, 1758), the small red brocket (*Mazama bororo*) and ingram’s squirrel (*Guerlinguetus brasiliensis* Gmelin, 1788). Puma (*Puma concolor*) and ocelot (*Leopardus pardalis*) were detected exclusively by the camera traps, and the remaining non-volant mammals through both methods.

The dissimilarity of the third campaign in comparison to the others is related to the lower diversity observed. The data collected indicate that non-volant mammals do use the Serra da Macaca ParkRoad (SP-139). Felines and ungulates were recorded moving along the road, particularly at nighttime. On the other hand, we recorded agouti (*Cuniculus paca*), the crab-eating raccoon (*Procyon cancrivorus*) and the black-horned capuchins (*Sapajus nigritus*) just crossing the road. The primates benefited from the forest canopy to cross the road where the treetops touched over it.

The closure of the Serra da Macaca ParkRoad (SP-139) during nighttime makes it a unique road. The results observed allow us to infer that closing the road is essential for the conservation of nocturnal non-volant mammals that occur in the Carlos Botelho State Park.

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Legends:

Figure 1. Location of the 16 points sampled through the camera trap method along the Serra da MacacaPark Road (SP-139).

Figure 2. Records of non-volant mammals at the Serra da Macaca ParkRoad (SP-139). A = *Cuniculus paca*, B = *Tapirus terrestris*, C = *Procyon cancrivorus*, D = *Leopardus pardalis*, E = *Mazama bororo*, F = *Sapajus nigritus*, G = *Guerlinguetus ingrami*, H = *Philander quica*, I = *Didelphis aurita,* J = *Puma concolor.*

Figure 3. Rarefaction curve for the richness of non-volant mammals recorded through visual searches and camera traps at the Serra da Macaca ParkRoad (SP-139).

Figure 4. UPGMA dendrogram based on the species dissimilarity index between the four sampling campaigns (1st C = Mar / 2018, 2nd C = Jul / 2018, 3rd C = Nov / 2018 and 4th C = Feb / 2019) conducted at the Serra da Macaca ParkRoad (SP-139).

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| Table 1. Non-volant mammals recorded at the Serra da Macaca ParkRoad (SP-139). Types of records: CT = camera trap, VS = visual search. Conservation status in the State of São Paulo (São Paulo, 2018): EN = Endangered; VU = Vulnerable; NT = Near Threatened; DD = Data Deficient; LC = Least Concern. |
| **Taxon** | **Common Name** | **Status** | **1stC** | **2ndC** | **3rdC** | **4thC** |
| **Didelphimorphia** |  |  |  |  |  |  |
| Didelphidae |  |  |  |  |  |  |
|  *Didelphis aurita* Wied-Neuwied, 1826 | black-eared opossum | LC | VS | CT/VS | VS | VS |
|  *Philander quica* Temminck, 1824 | southeastern four-eyed opossum | LC | VS | CT/VS | VS | VS |
| **Pilosa** |  |  |  |  |  |  |
| Myrmecophagidae |  |  |  |  |  |  |
|  *Tamandua tetradactyla* Linnaeus, 1758 | southern anteater | LC | VS |  |  |  |
| **Primates** |  |  |  |  |  |  |
| Cebidae |  |  |  |  |  |  |
|  *Sapajus nigritus* Goldfuss, 1809 | black-horned capuchin | LC | VS | VS |  | VS |
| **Lagomorpha** |  |  |  |  |  |  |
| Leporidae |  |  |  |  |  |  |
|  *Sylvilagus brasiliensis* Linnaeus, 1758 | tapeti | DD |  |  | VS |  |
| **Carnivora** |  |  |  |  |  |  |
| Felidae |  |  |  |  |  |  |
|  *Leopardus pardalis* Linnaeus, 1758 | ocelot | VU | CT | CT |  | CT |
|  *Puma concolor* Linnaeus, 1771  | puma | VU |  | CT |  | CT |
| Procyonidae |  |  |  |  |  |  |
|  *Procyon cancrivorus* Cuvier, 1798 | crab-eating raccoon | LC | CT | VS |  | CT |
| **Perissodactyla** |  |  |  |  |  |  |
| Tapiridae |  |  |  |  |  |  |
|  *Tapirus terrestris* Linnaeus, 1758 | low-land tapir | EN | CT/VS | CT/VS | CT/VS | CT |
| **Cetartiodactyla** |  |  |  |  |  |  |
| Cervidae |  |  |  |  |  |  |
|  *Mazama bororo* Duarte, 1996 | small red brocket | VU |  | VS |  |  |
| **Rodentia** |  |  |  |  |  |  |
| Sciuridae |  |  |  |  |  |  |
|  *Guerlinguetus brasiliensis* Gmelin, 1788 | ingram’s squirrel | LC |  | VS |  |  |
| Cuniculidae |  |  |  |  |  |  |
|  *Cuniculus paca* Linnaeus, 1766 | agouti | NT | CT | CT/VS |   | VS |
| **Records in visual searches** |   |   | **5** | **8** | **4** | **4** |
| **Records in camera traps** |   |   | **4** | **6** | **1** | **4** |
| **Species total** |   |   | **8** | **10** | **4** | **8** |

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| Table 2. Shannon-Wiener Diversity Index calculated for the communities of non-volant mammals observed at the Serra da Macaca ParkRoad (SP-139). |
| Sampling Campaign | Shannon-Wiener (H') Index |
| 1st C | 1.859 |
| 2nd C | 1.865 |
| 3rd C | 1.220 |
| 4th C | 1.876 |

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| Table 3. List of non-volant mammals found dead in the Serra da Macaca ParkRoad (SP-139), number of records (N) and relative frequency (%). Conservation status in the State of São Paulo (São Paulo, 2018): EN = Endangered; VU = Vulnerable; NT = Near Threatened; LC = Least Concern; \* = Exotic. |
| **Taxon** | **Common Name** | **N** | **%** | **Status** |
| **Didelphimorphia** |  |  |  |  |
| Didelphidae |  |  |  |  |
|  *Didelphis aurita* Wied-Neuwied, 1826 | black-eared opossum | 3 | 14.28 | LC |
|  *Philander quica* Temminck, 1824 | southeastern four-eyed opossum | 4 | 19.05 | LC |
| **Pilosa** |  |  |  |  |
| Myrmecophagidae |  |  |  |  |
|  *Myrmecophaga tridactyla* Linnaeus, 1758 | giant anteater | 1 | 4.76 | VU |
|  *Tamandua tetradactyla* Linnaeus, 1758 | southern anteater | 1 | 4.76 | LC |
| **Cingulata** |  |  |  |  |
| Dasypodidae |  |  |  |  |
|  *Dasypus novemcinctus* Linnaeus, 1758 | nine-banded armadillo | 2 | 9.52 | LC |
| **Cetartiodactyla** |  |  |  |  |
| Cervidae |  |  |  |  |
|  *Mazama bororo* Duarte, 1996 | small red brocket deer | 1 | 4.76 | VU |
| **Primates** |  |  |  |  |
| Cebidae |  |  |  |  |
|  *Sapajus nigritus* Goldfuss, 1809 | black-horned capuchin | 2 | 9.52 | LC |
| **Carnivora** |  |  |  |  |
| Procyonidae |  |  |  |  |
|  *Nasua nasua* Linnaeus, 1766 | south american coati | 1 | 4.76 | LC |
| Mustelidae |  |  |  |  |
|  *Eira barbara* Linnaeus, 1758 | tayra | 1 | 4.76 | LC |
| **Rodentia** |  |  |  |  |
| Cricetidae |  |  |  |  |
|  *Oligoryzomys flavescens* Waterhouse, 1837 | yellow pygmy rice rat | 1 | 4.76 | LC |
| Cuniculidae |  |  |  |  |
|  *Cuniculus paca* Linnaeus, 1766 | agouti | 1 | 4.76 | NT |
| Sciuridae |  |  |  |  |
|  *Guerlinguetus brasiliensis* Gmelin, 1788 | ingram's squirrel | 2 | 9.52 | LC |
| **Lagomorpha** |  |  |  |  |
| Leporidae |  |  |  |  |
|  *Lepus europaeus* Pallas, 1778\* | european hare | 1 | 4.76 | LC |