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Article

Use of road underpasses by terrestrial tetrapods inside a protected area in the southeastern part of the State of São Paulo, Brazil

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Abstract: Roads, despite playing a key role in economy, begin or aggravate processes of forest fragmentation and lead to several species losing their natural habitats, acting as filters and barriers that impose challenges to animal locomotion. Wildlife crossings seek to reestablish the connectivity of the landscape. In general, after being installed, these passages need to be evaluated regarding their functioning. In this study, we present the results of monitoring the underpasses on Serra da Macaca Park Road (SP-139), which crosses Carlos Botelho State Park. The underpasses, constituted by bridges and culverts, were constructed after a license obtained to restore this section of the road. Camera traps were installed inside all crossing structures, and systematic samples were obtained through four field expeditions, with sampling efforts of 10 days each, between March 2018 and February 2019. The footprints found around the intersection structures were also considered. A two-sided Student's t-test was applied to verify differences in richness between the underpasses that have distinct types of margins. Differences in abundance were analyzed through the non-parametric Mann-Whitney test. A Whittaker diagram was generated according to the order of the species, from the most common to the rarest, while their diversity was estimated through Simpson's Diversity Index ($1 - D$). The Pearson correlation coefficient was used to study the interaction between diversity and altitude variation. We observed a richness of 16 species of terrestrial tetrapods, without any statistically significant differences between underpasses with dry or flooded margins. *Cuniculus paca* was the most abundant species, and higher altitudes presented the most diversity. We concluded that the underpasses located inside Permanent Preservation Areas (PPAs) of watercourses are essential to maintain the flow of animals beneath the road.
Keywords: *Wildlife crossing, mitigation, connectivity, PPA of watercourses.*

Uso de pontes e galerias rodoviárias por tetrápodes terrestres em uma área protegida na região sudeste do Estado de São Paulo

Resumo: As rodovias, apesar de exercerem um papel fundamental na economia, instauram ou agravam processos de fragmentação florestal e levam à perda de habitat. Atuam como filtros e barreiras, dificultando a movimentação animal. As passagens de fauna buscam restabelecer a conectividade na paisagem. Geralmente, após serem implantadas procede-se à avaliação do seu funcionamento. Nesse artigo nós apresentamos os resultados de um monitoramento do uso das passagens inferiores de fauna da Estrada Parque Serra da Macaca (SP-139), que atravessa o Parque Estadual Carlos Botelho. As passagens inferiores, representadas por pontes e galerias hidráulicas, foram concebidas através do licenciamento das obras de revitalização do trecho rodoviário. Armadilhas fotográficas foram instaladas no interior de todas as estruturas, e as amostragens sistemáticas ocorreram por meio de quatro expedições a campo, com um esforço amostral de 10 dias cada, entre março de 2018 e fevereiro de 2019. Os rastros encontrados no perímetro das estruturas de transposição também foram considerados. Os dados foram submetidos ao teste T de Student bicaudal, para verificar a diferença de riqueza entre as passagens inferiores com tipos distintos de margens. As diferenças de abundância foram analisadas pelo teste não paramétrico de Mann-Whitney. O diagrama de Whittaker foi gerado pela ordenação das espécies, a partir das mais comuns para as mais raras, enquanto a diversidade de espécies foi estimada pelo índice de Simpson ($1 - D$). O coeficiente de correlação de Pearson foi empregado para estudar a interação entre a diversidade e a variação de altitude. Detectamos uma riqueza de 16 espécies de tetrápodes terrestres e não houve diferença significativa entre as passagens com margens secas e alagadas. *Cuniculus paca* foi a espécie mais abundante. Altitudes mais elevadas concentraram as maiores diversidades. Concluímos que as passagens inferiores alocadas em APPs de curso d'água são essenciais para manter o fluxo da fauna sob a rodovia.
Palavras-chave: *Passagem de fauna, mitigação, conectividade, APP de curso d'água.*

Introduction

The construction of wildlife crossings has gained traction recently (Van der Grift et al. 2013). There are two distinct groups of wildlife crossings: underpasses (e.g., culverts, linear tunnels) and overpasses (e.g., green bridges and canopy bridges). This type of measure seeks to restore connectivity between sectioned habitats (Ascensão & Mira 2007); in addition, fences are built to direct vertebrate species towards underpasses (Dodd et al. 2004, Abra et al. 2020).

Roads may isolate these populations, affecting migrations and increasing mortality due to collisions with vehicles (Lesbarrères & Fahrig 2012). Furthermore, roads act as barriers for animal movement and may lead to genetic impoverishment of many species (Holderegger & Digiulio 2010). The combined use of wildlife crossings and guiding fences is considered the best method to decrease roadkill rates (Mastro et al. 2008).

Other solutions with less proven effectiveness have been described, including repellents, awareness campaigns, installation of roadway lighting, population control of target species, reduced speed limits, caution signs, and the use of animal detection systems (Mastro et al. 2008, Glista et al. 2009, Grilo et al. 2010). The cost of implementation is one of the main criteria to choose measures to mitigate collisions (Glista et al. 2009), but inconsistent proposals may be imprudent and hence aggravate the problem (Bager & Fontoura 2013).

Several factors influence the effectiveness of crossing structures. Vegetation covering their entrances has a positive effect as it favors the approach of animals (Ascensão & Mira 2007), and the conditions inside (luminosity, humidity, temperature, soil, and noise) also affect their behavior (Glista 2009). It is ideal that the underpasses be implemented on locations already identified as animal crossing spots in the roads, such as trails (Foster & Humphrey 1995, Grilo et al. 2008).

Yanes et al. (1994) analyzed the movements of vertebrates across 17 culverts distributed along roads and railroads for a period of one year in central Spain. They found that smaller passages inhibited the movement of medium-and large-sized mammals. Sawaya et al. (2013) described a learning curve for some species to find wildlife crossings and start using them. In a long-term monitoring performed in the Banff National Park, brown bears and wolves took years to feel comfortable using the structures to move around (Clevenger & Waltho 2003).

This study aims to assess the use of underpasses across Serra da Macaca Park Road (SP-139) by terrestrial tetrapods and to compare the richness and abundance of vertebrates between two types of wildlife crossing structures.

Materials and Methods

1. *Study area*

Carlos Botelho State Park (PECB) (24° 08' S; 47° 58' W) is located in the southeastern part of the State of São Paulo, Brazil (Figure 1) and covers an area of 37.644 ha of tropical rainforest (Veloso et al., 1991, Lima et al., 2011). The uneven topography of the area ranges from 30 to 1.000 m above sea level (Dias, 2005). Serra da Macaca Park Road (SP-139), within the jurisdiction of the Department of Roads and Highways of the State of São Paulo (DER/SP), is a popular name for the 33-km section of the road that crosses the PECB. A project for paving and improving this road has been discussed in the scope of a management plan of the Park, which was approved considering that the project would contribute to supervision and visitation in the Protected Area. The redevelopment project of the road network used a type of eco-friendly paving system, composed of interlocked concrete blocks that allow the rainwater to drain, trap less heat, and produce noise from the friction between tires and pavement to scare the fauna away. Additionally, the project implemented a drainage system, regularized the condition of hillsides, and installed metallic defenses. Work on the project started in October 2013 and finished in November 2015. Serra da Macaca Park Road (SP-139), being inside a region of great environmental concern, received a broad set of measures to help prevent collisions between animals and vehicles. Therefore, in order to ensure the sustainability of the local traffic of vehicles, the region has 16 overpasses, in the form of canopy bridges suspended by a system of interlaced cables and ropes (Teixeira et al. 2013), 12 underpasses (eight bridges and four culverts), 30 speed bumps distributed throughout the section, adequate vertical signaling, and speed limits of 40 km/h, in addition to two fixed OCR (Optical Character Recognition) devices to help monitor the road, one at the entrance and the other at the exit of PECB. Moreover, the road is closed during the night.

2. *Data collection and analysis*

This study was approved by the Ethics Committee on the Use of Animals of the School of Veterinary Medicine and Animal Science at the São Paulo State University (UNESP / Campus Botucatu), approval n. CEUA 0127/2019. The project was also approved by the techno-scientific committee of the Forestry Institute State of São Paulo – Letter Cotec n. 485/2019 – and is properly included in the Brazilian National Management System of the Genetic Heritage and Associated Traditional Knowledge – Register SisGen n. A18FA6A. All statistical analyses were performed using the software "R" v. 3.5.1 (R Core Team 2020).

Data collection was performed in four field expeditions distributed from March 2018 to February 2019. Samplings were carried out in the dry and wet seasons. A systematic sampling of terrestrial tetrapods in 12 wildlife crossings (Figure 2) was performed through camera traps. In addition, footprints found by chance inside or around the structures were considered as evidence of locomotion of medium-and large-sized mammals. In order to avoid biases, footprint records were not considered as a valid method for the estimates of abundance and diversity.

Underpasses in Serra da Macaca Park Road (SP-139) are waterway crossings with height exceeding 1 m, resulting in a group of eight bridges and four culverts (Figure 2) of varied shapes and sizes. The underpasses were subdivided in two categories according to the characteristics of their margins: dry (P5, P6, P7, P8, P10, and P11) and flooded (P1, P2, P3, P4, P9, and P12).

A camera trap (Bushnell Model ZT820) was positioned at each sampling location. The cameras were screwed to the inner walls of the underpasses at 40 cm above the ground and programmed to function continuously during the entire sampling period. The cameras were installed for 10 days per expedition, totalling a sampling effort of 480 cameras/days.

Videos of a single species obtained from the same camera trap in intervals below 20 minutes were considered as a single record of the species (except when the images captured showed two or more individuals together).

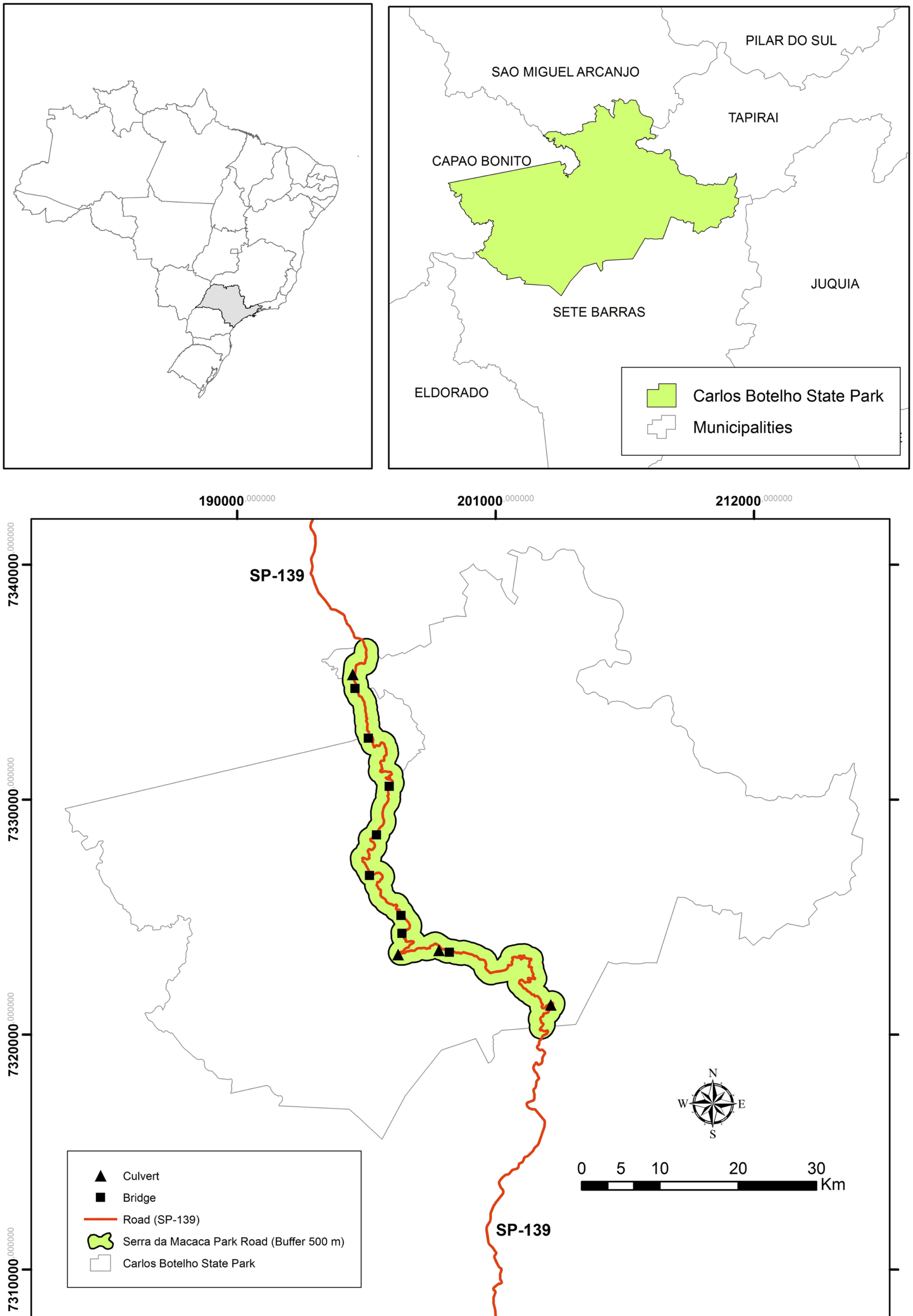

Figure 1. Location of the 12 wildlife crossings along Serra da Macaca Park Road (SP-139), which crosses Carlos Botelho State Park (PECB).

Figure 2. Existing wildlife underpasses along Serra da Macaca Park Road (SP-139). P1 – Circular culvert (Km 46+800), P2 – bridge (Km 56+200), P3 – rectangular culvert (Km 56+620), P4 – rectangular culvert (Km 58+714), P5 – bridge (Km 60+300), P6 – bridge (Km 61+300), P7 – bridge (Km 64+660), P8 – bridge (Km 67+100), P9 – bridge (Km 69+710), P10 – bridge (Km 73+850), P11 – bridge (Km 76+200), and P12 – circular culvert (Km 76+860).

A two-sided Student's t-test was applied to verify the differences in richness between underpasses with distinct types of margins, considering a 5% significance level. Assumptions of normality and homogeneity of the variances were also evaluated. The difference in abundance was analyzed based on the same parameters through a non-parametric Mann-Whitney test, as the data go against the normal distribution.

A Whittaker diagram was created to sort the species from the most common to the rarest, whereas species diversity in the underpasses was estimated according to Simpson's Diversity Index ($1 - D$). Pearson's correlation coeficiente was applied to study the interaction between species diversity and variations in elevation from the sea level. The P1 site was removed from this analysis, because there are no replicates for low altitudes.

The scientific names followed Piacentini et al. (2015), Abreu et al. (2021), and SBH (2021). Animals were identified based on existing literature (Sigrist 2009, Reis et al. 2006, Forlani et al. 2010). The conservation status of the species is in accordance with the List of Endangered Species of the State of São Paulo (São Paulo, 2018).

Results

We detected an abundance of 105 individuals in the underpasses during the study period, 8.57% of which were reptiles, whereas 15.24% were birds and 76.19% mammals. There were no records of amphibians. We observed a total richness of 16 species (Table 1 and Figure 3).

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Table 1. Taxonomic list (class, order, family, and species), registration method, abundance of individuals, richness of species, and Simpson's Diversity Index (1 – *D*) for the terrestrial tetrapods sampled in the wildlife crossings of Serra da Macaca Park Road (SP-139). Ct = Camera traps, F = Footprints. *Conservation status in the State of São Paulo (São Paulo 2018).

Taxon	Wildlife Crossings											
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12
	Altitude (m)											
	101	570	582	629	711	737	772	770	755	753	703	712
REPTILIA												
Squamata												
Teiidae												
Salvator merianae (Duméril & Bibron, 1839)											Ct	
BIRDS												
Galliformes												
Odontophoridae												
Odontophorus capueira (Spix, 1825)								Ct				
Gruiformes												
Ralidae												
Aramides saracura (Spix, 1825)			Ct					Ct			Ct	
Passeriformes												
Formicariidae												
Chamaeza campanisona (Lichtenstein, 1823)					Ct			Ct				
Turdidae												
Turdus rufiventris Vieillot, 1818			Ct		Ct	Ct						
MAMMALIA												
Didelphimorphia												
Didelphidae												
Chironectes minimus (Zimmermann, 1780)			Ct	Ct		Ct						
Didelphis aurita Wied-Neuwied, 1826							F		F			
Philander quica (Temminck, 1824)			Ct	Ct	Ct	Ct			Ct	Ct	Ct	Ct
Cingulata												
Dasypodidae												
Dasypus novemcinctus Linnaeus, 1758										F	Ct	
Carnivora												
Felidae												
Leopardus pardalis (Linnaeus, 1758)*		Ct				F		Ct, F				
Mustelidae												
Lontra longicaudis (Olfers, 1818)*						F						Ct
Procyonidae												
Procyon cancrivorus Cuvier, 1798			Ct	Ct				Ct	F			
Perissodactyla												
Tapiridae												
Tapirus terrestris (Linnaeus, 1758)*								Ct		F	F	
Cetartiodactyla												
Cervidae												
Mazama sp.						F		Ct				
Rodentia												
Sciuridae												
Guerlinguetus brasiliensis (Gmelin, 1788)						Ct				Ct		
Cuniculidae												
Cuniculus paca (Linnaeus, 1766)	F	Ct	Ct	Ct	Ct	F	F	Ct	Ct, F	Ct	Ct	Ct
Abundance of individuals	**0**	**6**	**28**	**7**	**7**	**9**	**0**	**10**	**3**	**4**	**26**	**5**
Richness of species	**1**	**2**	**6**	**4**	**4**	**8**	**2**	**8**	**4**	**5**	**6**	**3**
Simpson's Diversity Index (1 – *D*)	**0.00**	**0.44**	**0.67**	**0.61**	**0.69**	**0.64**	**0.00**	**0.86**	**0.44**	**0.62**	**0.74**	**0.64**

Figure 3. Images of tetrapods obtained in the wildlife crossings along Serra da Macaca Park Road (SP-139). *Leopardus pardalis* (a), *Aramides saracura* (b), *Procyon cancrivorus* (c), *Chamaeza campanisona* (d), *Odontophorus capueira* (e), *Tapirus terrestris* (f), *Mazama* sp. (g), *Cuniculus paca* (h), *Lontra longicaudis* (i), *Philander quica* (j), *Salvator merianae* (k), *Turdus rufiventris* (l), *Chironectes minimus* (m).

The Whittaker diagram (Figure 4) shows that the most abundant species in the underpasses were *Philander quica* (Temminck, 1824) (n = 32) and *Cuniculus paca* (Linnaeus, 1766) (n = 28). *Cuniculus paca* also presented the highest distribution, being recorded in all crossing structures.

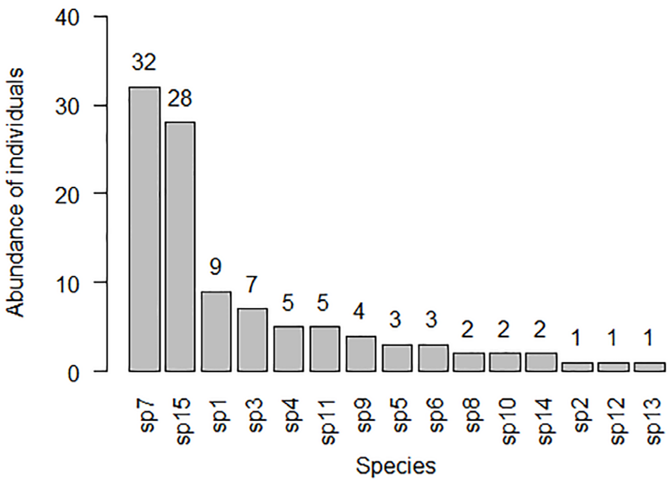

Figure 4. Whittaker diagram for the species of tetrapods sampled in the wildlife crossings along Serra da Macaca Park Road (SP-139): sp1 = *Salvator merianae*, sp2 = *Odontophorus capueira*, sp3 = *Aramides saracura*, sp4 = *Chamaeza campanisona*, sp5 = *Turdus rufiventris*, sp6 = *Chironectes minimus*, sp7 = *Philander quica*, sp8 = *Dasypus novemcinctus*, sp9 = *Leopardus pardalis*, sp10 = *Lontra longicaudis*, sp11 = *Procyon cancrivorus*, sp12 = *Tapirus terrestris*, sp13 = *Mazama* sp., sp14 = *Guerlinguetus brasiliensis*, sp15 = *Cuniculus paca.*

Among all underpasses studied, P1 and P7 showed the lowest Simpson Diversity Index, whereas P8 presented the highest (Table 1).

The underpasses with dry margins presented an average of ~2.2 more species than the passages with flooded margins (Figure 5), but this result is not statistically significant according to the t-test (t = -1.813, g.l.= 10, p = 0.100). There were also no statistically significant differences between the two types of passage regarding abundance (p = 0.574) (Figure 6).

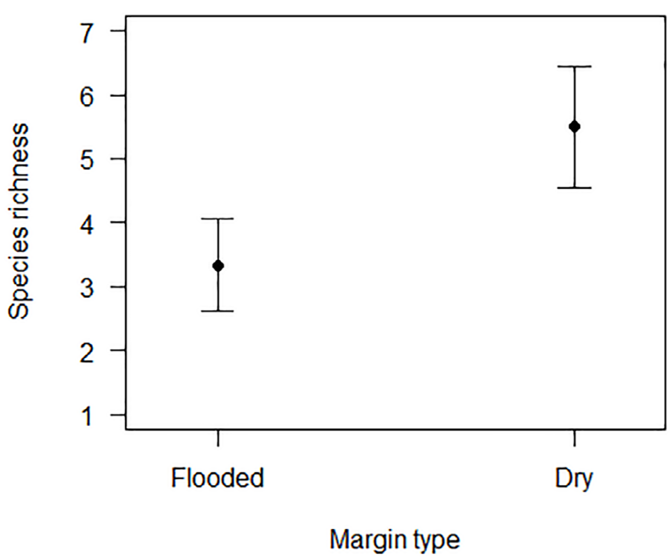

Figure 5. Mean (dot) and standard deviation (bar) for the richness of tetrapod species observed in the wildlife crossings along Serra da Macaca Park Road (SP-139), according to the type of margin. The differences are not statistically significant according to the t-test (t = -1.813, g.l. = 10, p = 0.100).

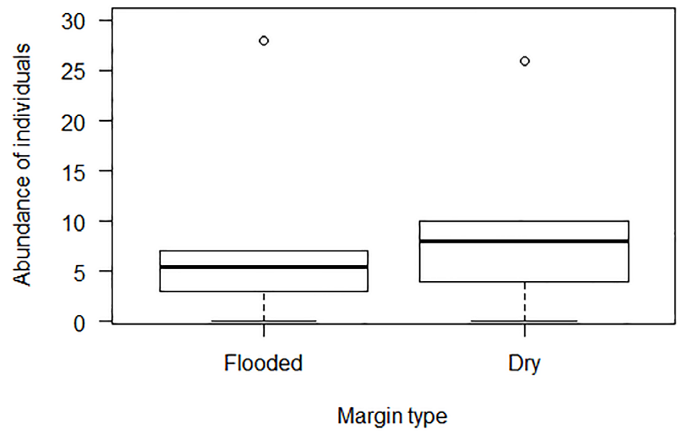

Figure 6. Boxplot of the variation in the abundance of individuals registered in the two groups of underpasses analyzed in Serra da Macaca Park Road (SP-139). The differences are not statistically significant according to the Mann-Whitney test (p = 0.574).

Based on the data obtained, we didn't observe correlation between diversity and altitude variables for the underpasses (Figure 7).

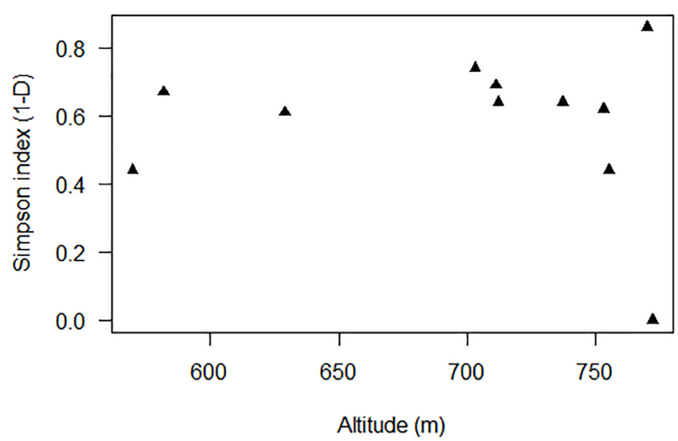

Figure 7. Correlation between the diversity of species and the altitude of the underpasses along Serra da Macaca Park Road (SP-139): r = -0.107 and p > 0.05.

Discussion

A bridge is a type of structure built to connect two points separated by valleys. Its main function is to facilitate the drainage of waterways and improve the flow. Secondarily, it allows the passage of animals beneath the roads (Van der Ree et al. 2007). Likewise, culverts and drainage galleries serve the same purpose for the local fauna (Clevenger et al. 2001).

One common characteristic to the underpasses of Serra da Macaca Park Road (SP-139) is the environment in which they are inserted. The structures were constructed in Permanent Preservation Areas (PPAs) of rivers and permanent streams, whose margins are valuable for connecting animal populations and consequently maintaining gene flow (Coutinho et al. 2013).

Even in the flooded crossing structures, the water level remained low during the samplings (0.15 m), and we believe this may explain why no statistically significant differences were observed when comparing the underpasses with dry and flooded margins regarding the abundance of individuals and richness of species.

The most expressive results obtained for medium and large mammals, when compared with other terrestrial tetrapods, are related to the sampling methods chosen.

Regarding the conservation status of recorded taxa, two of the species sampled are considered Vulnerable to extinction in the State of São Paulo: *Leopardus pardalis* (Linnaeus, 1758) and *Lontra longicaudis* (Olfers, 1818). *Tapirus terrestris* (Linnaeus, 1758) is Endangered, whereas *Odontophorus capueira* (Spix, 1825), *Chironectes minimus* (Zimmermann, 1780), and *Cuniculus paca* are Near Threatened.

Concerning the most abundant species, agoutis (*Cuniculus paca*) typically inhabit forested areas, living near watercourses. They have the habit of burrowing in hillsides and are common in locations where hunting them is forbidden (Reis et al. 2006). The southeastern four-eyed opossum (*Philander quica*) is also a forest species, positively associated to litter cover and watercourses (Moura et al. 2005).

The underpasses of Serra da Macaca Park Road (SP-139) do not have any guiding fences to direct animal passage through them. The choice for not installing this accessory measure was to prevent medium and large mammals from having their escape routes towards the PPAs blocked, during the day. At night the road is closed to traffic.

Simpson's Diversity Index measures the probability of two individuals, randomly chosen from the community, belonging to different species (Melo, 2008). According to this index, P1 and P7 were the underpasses with the lowest diversity. We recorded only one mammal species at P1, through the footprint method. A possible explanation for this result is the tourism in the location, which is easily accessible and highly sought in PECB.

Brocardo et al. (2012) stated that the high portion of PECB shows a lesser degree of anthropization in comparison with the rest of the protected area, which faces illegal extraction of heart of palm and hunting as the main challenges to the conservation of its biota. The greatest diversities were observed in the underpasses P8 and P11 respectively, both located in the highest parts of the mountain range. However, as shown, we did not find a correlation between the diversity of species and the altitude where the structures are located.

These results highlight the ecological importance of having underpasses on roads crossing Permanent Preservation Areas (PPAs) along watercourses. The presence of a thin layer of water on the ground of the crossing structures did not interfere with their use by the tetrapods, but it is worth noting that this type of environment was more favorable for species with higher affinity to wet areas. Therefore, we highlight the need to implement dry causeways inside flooded passages. Abra et al. (2020) found that some species of Brazilian mammals exclusively used passages with running water, whereas others preferred dry ones.

If, on the one hand, the absence of fencing did not inhibit the use of the bridges and culverts by the animals, on the other human presence in the crossing structures may compromise their functionality.

Acknowledgements

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Author Contributions

Francisco de Assis Alves: Substantial contribution towards the idealization and design of the study; contribution in collection, analysis and interpretation of the data; contribution in the preparation of the manuscript.

Carlos Roberto Teixeira: Contribution towards the idealization and design of the study; contributions with critical reviews, aggregating intellectual content.

Luciano Barbosa: Contribution in the analysis and interpretation of the data; contributions with critical reviews of the analyses.

Jairo Alves Júnior: Contribution with data collection.

Conflicting Interests

The authors declare they have no conflicting interests in what pertains to the publication of this manuscript.

Ethics

This study did not envolve experimentation with or collection of animals.

Data Availability

All information necessary to replicate this study is present in the text of the manuscript.

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