WORKING PAPER Nº IDB-WP-01778

Effectiveness of Crossing Structures for Wildlife on Two Roads Associated with Biological Corridors in Costa Rica

Fernanda Z. Teixeira
Daniela Araya-Gamboa
Susana Gutiérrez Acuña
Igor P. Coelho
Yosette Araya Jiménez
Erick Víquez Alvarado
Esther Pomareda García
Silvio Boyat
Roberto Salom-Pérez
Esmeralda Arévalo Huezo
Priscila Picado Valenzuela
Valentina Saavedra Gómez
Esperanza González Mahecha
María Irene Gauto Espínola
Andrea Ávila Alfaro
Jaime Quirós Calderón

Inter-American Development Bank
Climate Change Solutions Division

February 2026

Effectiveness of Crossing Structures for Wildlife on Two Roads Associated with Biological Corridors in Costa Rica

Fernanda Z. Teixeira
Daniela Araya-Gamboa
Susana Gutiérrez Acuña
Igor P. Coelho
Yosette Araya Jiménez
Erick Víquez Alvarado
Esther Pomareda García
Silvio Boyat
Roberto Salom-Pérez
Esmeralda Arévalo Huezo
Priscila Picado Valenzuela
Valentina Saavedra Gómez
Esperanza González Mahecha
María Irene Gauto Espínola
Andrea Ávila Alfaro
Jaime Quirós Calderón

Inter-American Development Bank
Climate Change Solutions Division

February 2026

Cataloging-in-Publication data provided by the
Inter-American Development Bank
Felipe Herrera Library
Effectiveness of crossing structures for wildlife on two roads associated with biological corridors in Costa Rica / Fernanda Z. Teixeira, Daniela Araya-Gamboa, Susana Gutiérrez Acuña, Igor P. Coelho, Yosette Araya Jiménez, Erick Víquez Alvarado, Esther Pomareda García, Silvio Boyat, Roberto Salom-Pérez, Esmeralda Arévalo Huezo, Priscila Picado Valenzuela, Valentina Saavedra Gómez, Esperanza González-Mahecha, Maria Irene Gauto Espínola, Andrea Ávila Alfaro, Jaime Quirós Calderón.
p. cm. — (IDB Working Paper Series ; 1778)
Includes bibliographical references.
1. Wildlife crossings-Costa Rica. 2. Nature conservation-Costa Rica. 3. Animal-Effect of roads on-Costa Rica. 4. Corridors (Ecology)-Costa Rica. I. Teixeira, Fernanda Z. II. Araya-Gamboa, Daniela. III. Gutiérrez Acuña, Susana. IV. Coelho, Igor P. V. Araya Jiménez, Yosette. VI. Víquez Alvarado, Erick. VII. Pomareda García, Esther. VIII. Boyat, Silvio. IX. Salom-Pérez, Roberto. X. Arévalo Huezo, Esmeralda. XI. Picado Valenzuela, Priscila. XII. Saavedra Gómez, Valentina. XIII. González-Mahecha, Esperanza. XIV. Gauto Espínola, Maria Irene. XV. Ávila Alfaro, Andrea. XVI. Quirós Calderón, Jaime. XVII. Inter-American Development Bank. Climate Change Solutions Division. XVIII. Series.
IDB-WP-1778

Keywords: Mitigation effectiveness, crossing structures, follow up monitoring, road mortality, connectivity, space use, camera traps.
JEL Codes: Q54, Q57, L73, L92

http://www.iadb.org

Copyright © 2026 Inter-American Development Bank (IDB). This work is subject to a Creative Commons Attribution 4.0 International Public License CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/legalcode). The terms and conditions indicated in the URL link must be met and the respective recognition must be granted to the IDB.

Any and all disputes arising under this license that cannot be settled amicably shall be resolved in accordance with the following procedure. Pursuant to a notice of mediation communicated by reasonable means by either you or the licensor to the other, the dispute shall be submitted to non-binding mediation conducted in accordance with the World Intellectual Property Organization (WIPO) Mediation Rules. Any dispute that cannot be settled amicably shall be submitted to arbitration pursuant to the United Nations Commission on International Trade Law (UNCITRAL) rules. The use of the IDB name for any purpose other than the respective recognition and the use of the IDB logo are not authorized by this license and require an additional license agreement.

Note that the URL link includes terms and conditions that are an integral part of this license.

The opinions expressed in the work are those of its authors and do not necessarily reflect the views of the IDB, its Board of Executive Directors, or the countries they represent.

BID
PANTHERA

DATA PAPER

EFFECTIVENESS OF CROSSING STRUCTURES FOR WILDLIFE ON TWO ROADS ASSOCIATED WITH BIOLOGICAL CORRIDORS IN COSTA RICA

Fernanda Z. Teixeira, Daniela Araya-Gamboa, Susana Gutiérrez Acuña, Igor P. Coelho, Yosette Araya Jiménez, Erick Víquez Alvarado, Esther Pomareda García, Silvio Boyat, Roberto Salom-Pérez, Esmeralda Arévalo Huezo, Priscila Picado Valenzuela, Valentina Saavedra Gómez, Esperanza González Mahecha, María Irene Gauto Espínola, Andrea Ávila Alfaro y Jaime Quirós Calderón

Effectiveness of crossing structures for wildlife on two roads associated with biological corridors in Costa Rica

Highlights

- Mitigation effectiveness should be assessed in road projects
- Wildlife crossing structures allow the safe movement of a variety of species
- Road mortality was higher on road sections with incomplete crossing structures
- Mitigation measures should be completed and maintained

Abstract: After implementing mitigation measures on road projects, it is critical to assess whether they effectively address the targeted impacts—namely, restrict wildlife movement and mortality from wildlife-vehicle collisions. In this study, we used a control-impact design to answer two key questions: (1) Is the probability of use by terrestrial and arboreal animals similar in crossing structures compared to surrounding forest sites? (2) Is wildlife mortality lower on road sections with mitigation measures than on those without? Our study was conducted on two roads in Costa Rica – National Route 160 and National Route 1 – focusing on underpasses and canopy bridges. To address the first question, we applied single-season occupancy models to camera trap data for ground-dwelling and arboreal mammals and reptiles, collected both at crossing structures and in surrounding forest. To address the second question, we compared roadkill data collected through vehicle surveys between road segments with and without crossing structures. Our results show that multiple taxa, including ground-dwelling and arboreal species, used structures such as underpasses, box culverts, bridges adapted with dry ledges, and canopy bridges. The probability of use suggests that some species are relatively well adapted to these structures, although confidence intervals remain broad. However, certain species were never recorded using any crossing structures, and, on Route 1, the amount of roadkill was higher for road segments with crossing structures. In conclusion, while wildlife crossing structures show promise in facilitating animal movement across roads, they are insufficient on their own to prevent wildlife mortality, showing that the mitigation system needs improvement. Based on existing best practices, we recommend extending and upgrading the exclusion fence for the underpasses, retrofitting box culverts with dry ledges and exclusion fence and maintenance activities to improve the effectiveness of this mitigation system.

Keywords: Mitigation effectiveness, crossing structures, follow up monitoring, road mortality, connectivity, space use, camera traps.

Introduction

Linear infrastructure, such as roads, railways, power lines, and water canals, poses a series of threats to wildlife, such as wildlife-vehicle collisions and a restriction on wildlife movement (Van Der Ree, Smith, & Grilo, 2015). Direct road mortality on roads reduces population abundance, genetic diversity and can increase genetic isolation, thus reducing population persistence (Jackson & Fahrig, 2011). Reduced animal movement across roads generates a barrier effect, increasing genetic isolation, and this limited movement and access to resources can reduce population abundance and likely reduce population persistence (Teixeira, Rytwinski, & Fahrig, 2020).

Measures to reduce wildlife-vehicle collisions and allow wildlife safe movement are increasingly being implemented as environmental safeguards during projects for road construction or upgrading. These measures include building crossing structures such as dedicated wildlife crossings (underpasses, overpasses, and canopy bridges) for animal movement or adapting bridges and water culverts with dry ledges (Soanes et al., 2024). The implementation of fences to block animal access to the road and guide them to crossing structures is recommended as best practice (Rytwinski et al., 2016). Road signage is also a frequent measure, although usually ineffective in reducing road impacts (Huijser et al. 2022).

Following the implementation of crossing structures, it is critical to determine if they are successful in mitigating the targeted impacts, namely wildlife-vehicle collisions and restriction of wildlife movement. This involves monitoring the species use of crossing structures, road mortality or species movement, and analyzing the characteristics of crossing structures associated with species use. Research on this topic is geographically biased, with more studies in North America and Europe than in other continents and mostly focused on ungulates (Rytwinski et al., 2016; Soanes et al., 2024). This results in a knowledge gap related to the success of crossing structures for a wide range of species, especially in the tropics (Pinto, Clevenger, & Grilo, 2020).

Wildlife crossing structures are increasingly being integrated into road infrastructure projects across Latin America, aiming both to reduce wildlife-vehicle collisions and to maintain wildlife movement. A variety of mitigation solutions have been implemented in different ecological and geographic contexts, including underpasses and culverts with dry ledges designed for terrestrial species (e.g. González-Gallina et al. 2018; Abra et al. 2020), canopy bridges for arboreal fauna (Teixeira et al. 2022), and wildlife overpasses intended for the safe passage of large mammals (e.g. Varela 2015). Additionally, targeted fencing has been applied to both large mammals and smaller species such as treefrogs (Zank et al. 2019), helping to guide animals toward safe crossing points and reduce road mortality due to vehicle collisions. Monitoring efforts to assess the use and effectiveness of these structures are underway in many locations across the region, employing tools such as camera traps (e.g. Abra et al. 2020) and track beds.

In Costa Rica, numerous scientific studies have documented direct mortality due to wildlife-vehicle collisions as a negative impact of roads, but the impact of wildlife movement restriction by roads has not. In parallel, there are ongoing projects aimed at expanding and upgrading the national road network, which could further exacerbate road impacts if not properly managed. Based on increasing research on road ecology in Costa Rica, several mitigation measures – such as underpasses, bridges with ledges and canopy bridges – have been recommended and are being implemented (Pomareda-García et al. 2016; Arévalo-Huezo et al. 2020). Nonetheless, empirical studies evaluating the use and effectiveness of crossing structures remain limited (Araya-Jiménez. 2019; Panthera. 2019; Villalobos-Hoffman et al., 2022; Panthera, 2022), highlighting a critical gap in the application of adaptive management and evidence-based infrastructure planning.

In this paper, we assess the effectiveness of crossing structures (underpasses, culverts, bridges adapted with dry ledges, and canopy bridges) on two roads in Costa Rica in facilitating wildlife movement and mitigating wildlife–vehicle collisions. We employed camera traps in the crossing structures and surrounding forest areas to investigate what species use crossing structures in relation to the local species pool. We also compared the use of crossing structures by species to the use of surrounding forest sites, given that low use can indicate that the structure can be better designed for the species movement. To test the effectiveness of crossing structures in reducing road mortality, we collected roadkill data during car surveys and tested if roadkills of the species that use crossing structures are lower in road sections with crossing structures than without them.

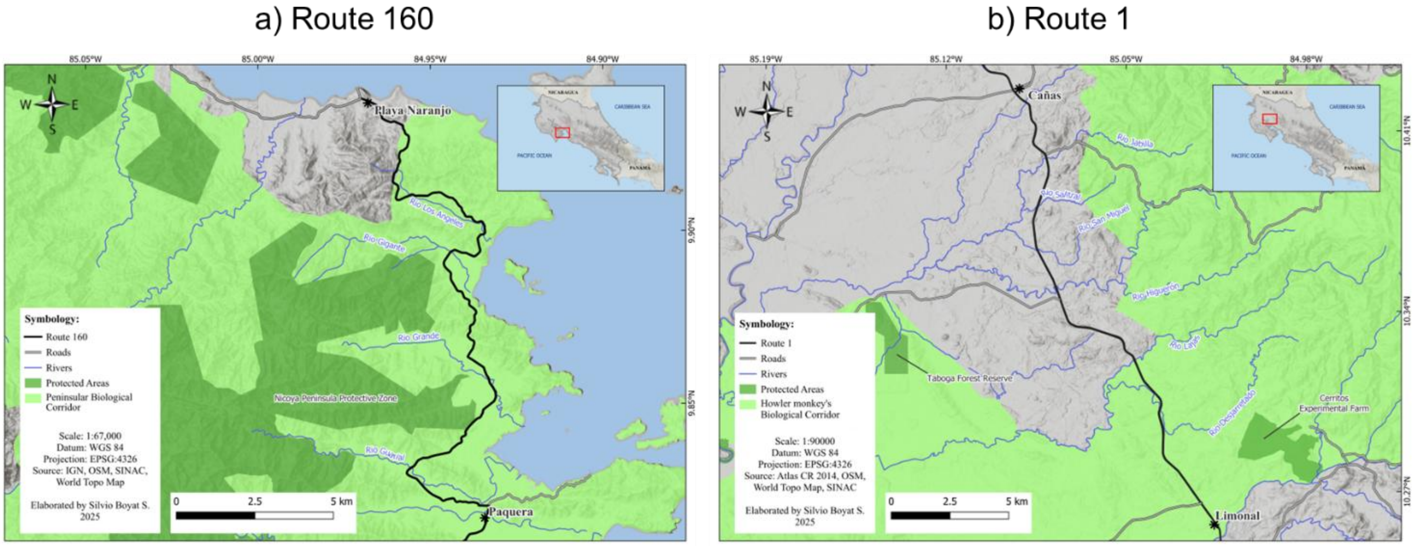
Based on the results of these comparisons, we recommend the improvement of the mitigation system implemented.

Methods

Study area

Costa Rica is a global model in biodiversity conservation, with an extensive network of protected areas and officially designated biological corridors. The road network in the country comprises around 36 thousand kilometers, a quarter of which is paved (MOPT, 2011). Our study was carried out on two roads with existing crossing structures in Costa Rica, Route 160 and Route 1 (Figure 1).

The National Route 160, section Playa Naranjo-Paquera, is located in Puntarenas and Paquera districts in the Nicoya Peninsula. It has a total length of 24 km, with two paved lanes, one in each direction, and includes bus bays, shoulders, gutters, among other infrastructure elements, and a maximum speed of 50 km/h. It has eight underpasses and four canopy bridges installed (Figure 2). Underpasses are comprised of 2x2m or 2.5x2.5m box culverts (varying 15-24 m in length) with dry ledges for wildlife (50-cm wide), with approximately 100-m guiding fences on each roadside. Canopy bridges are 50-cm wide with 15 to 25 m of length, installed at approximately 10-m height. The study area related to Route 160 lies within the Tempisque National Conservation Area and mostly comprises the Tropical Humid Forest, according to the Ecological Map of Costa Rica (Bolaños, Watson & Tosi, 2005). The Tropical Humid Forest is characterized by an annual precipitation range between 1950 and 3000 mm and an annual mean temperature between 24 and 27º C. The yearly dry period is highly variable, from 0 to 5 months. Land cover and uses present in this area are mango, teak and guava plantations, cattle pastures, secondary forest, riparian forest, mangroves, coastal areas, and urban settlements. It is located within the Península de Nicoya Key Biodiversity Area (Eken, 2004) at the edge of the Peninsular Biological Corridor, which covers about 60,000 ha and promotes connectivity with many other protected areas. The connection of Route 160 with other parts of the country is through the Paquera and Playa Naranjo Ferries to the west or through roads going south and north into the Peninsula.


a) Route 160

b) Route 1

Fig. 1. Map of the study area showing the location of the two paved roads where the effectiveness of underpasses and canopy bridges were studied in Costa Rica. (a) National Route 160, section Playa Naranjo-Paquera, a two-lane road. (b) National Route 1, section Limonal to Cañas, a four-lane highway. An elevation layer is displayed for context.

Fig. 2. Example of a) an underpass (square culverts with dry ledges for wildlife), b) fencing associated with the underpasses, and c) canopy bridges installed on Route 160.

The National Route 1, section Limonal to Cañas, is in Guanacaste, between Abangares and Cañas. It has a total length of 20.2 km, with four paved lanes, two in each direction, and a maximum speed of 80 km/h. Its width can vary between approximately 25 m and 60 m, divided by a concrete median barrier or New Jersey barrier in some sections. It includes bus bays, shoulders, gutters, turnarounds, and other infrastructure elements. It has six bridges, which have been adapted with dry ledges for wildlife (without fencing), four box culverts that do not have dry ledges or fences to direct wildlife, and six canopy bridges (Figure 3). Underpasses are comprised of 2x2m or 2.7x2.7m box culverts (varying 29-52 m in length). Canopy bridges are 60-cm wide with 27 to 34 m of length, installed at approximately 8 to 10-m height. Route 1 is located within the Arenal-Tempisque Conservation Area and crosses the Howler Monkey Biological Corridor and six important rivers. It presents three life zones: Tropical Humid Forest, Humid Forest transitioning to Dry, and Premontane Humid Forest transitioning to Basal (Bolaños, Watson & Tosi, 2005). Land cover and uses present in the area are agriculture, teak plantations, patches of secondary and riparian forests, cattle pasture and urban settlements.

Fig. 3. Examples of a) box culvert, b) bridge adapted with ledges for wildlife, and c) canopy bridge installed on Route 1.

Data collection for assessing effectiveness

Wildlife movement: Camera trap surveys

We used camera traps in the study areas to detect mammal, bird and reptile species that could potentially use and benefit from the road crossing structures, namely ground-dwelling, scansorial or arboreal. We deployed one camera trap per site to compare the use of crossing structures with surrounding forest sites located at varying distances from the road (0.1–5.3 km), including both sites associated with crossing structures and sites from a regular grid (Figure 4). Since camera traps were installed in the center of the underpasses, culverts and bridges, and on the top of poles targeting the center of the canopy bridges, we considered any species record as evidence of crossing use. On Route 160, a total of 38 camera trap stations were set, eight in underpasses, 18 on the forest ground (50 cm high), four on canopy bridges, and eight in the forest canopy. On Route 1, 42 camera trap stations were set, four in box culverts, six under bridges with dry ledges, 19 on the forest ground (50 cm high), six on canopy bridges, and seven on the forest canopy. We deployed the camera traps between September 2024 to March 2025, with sites varying from 35 to 227 sampling days, totaling 6,924 sampling days on Route 160 and 5,691 on Route 1 (Table S1). We defined independent records using a 1-h interval. Consecutive photographs of the same species taken within 1 h were considered a single record, regardless of the number of individuals observed. Data from camera trap images (Table S2) and the number of records per species per site (Tables S3 and S4) are available as supplementary files.

Wildlife mortality caused by vehicle collisions: Roadkill surveys

We carried out roadkill surveys twice a day (immediately after sunrise and around 7 pm, after sunset) for three consecutive days separated by approximately 30 days for 6 months, between August 2024 and January 2025. A total of 36 surveys were conducted on Route 160 (18 in the early morning and 18 in the evening), and 35 surveys were conducted on Route 1 (18 in the early morning and 17 in the evening).

Surveys were carried out in a vehicle at 30 km/h with 2 observers plus the driver. Route 160 has two lanes and both lanes were monitored in every survey. Route 1 has 4 lanes divided by a New Jersey barrier, so the two lanes in each direction were monitored in each survey. Observers recorded any vertebrate carcasses spotted on the road during car surveys, but here we only used data from ground-dwelling, scansorial and arboreal mammals and reptiles. These were the species with potential use of the existing crossing structures that can be detected by camera traps. Carcasses of reptiles were removed from the road after being recorded to avoid double-counting, while mammal carcasses were marked and not removed until the last day of each monthly survey, aiming to estimate carcass detection and persistence in future studies.

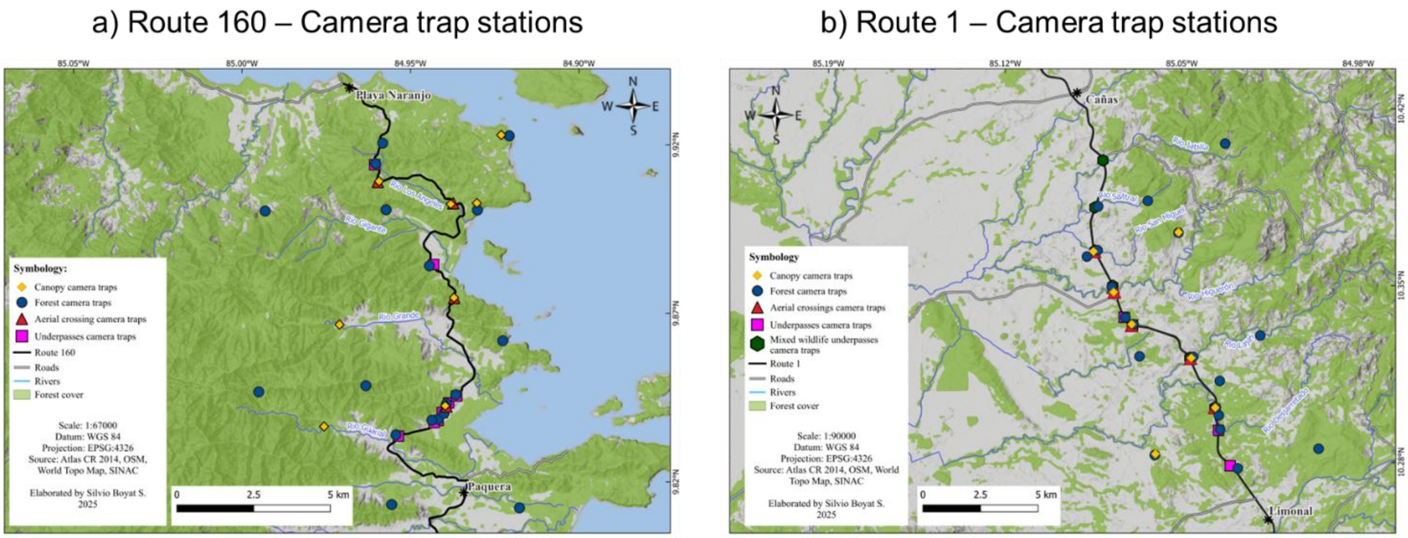


Fig. 4. Camera traps set in the forest ground (blue circles), in the underpasses, culverts or bridges (purple squares), on the canopy bridges (red triangles) and in the forest canopy (yellow diamonds) in the study areas associated with Route 160 and Route 1.

Analysis

Wildlife movement: Species use of crossing structures and surrounding areas

Species-independent records from camera traps are influenced by many factors, such as sampling effort (number of camera stations and sampling days), species' abundance and movement patterns, camera trap setup, and habitat characteristics, challenging their use as indexes to compare sites or species (Sollmann et al., 2013; Sollmann, 2018). We used single-season occupancy models to estimate the species' probability of use while accounting for detection (Mackenzie et al., 2002; Mackenzie et al., 2017). Occupancy models are hierarchical models that combine the sampling process (p, the probability of detecting the species) and the biological process (Ψ, the probability that the species occurs at a site, or occupancy). Model parameters are estimated using data from repeated visits (sampling occasions) at multiple sites, a matrix with binary species detection/non-detection.

To compare the probability of use of species in crossing structures and forest sites, we tested the effect of site type (underpass or forest ground on Route 160; culvert, bridge or forest ground on Route 160; and canopy bridge or forest canopy in both roads) using single-season occupancy models in the R (R Core Team, 2023) package "unmarked" (Fiske et al., 2011; Kellner et al., 2023). We carried out the analysis for each species recorded at least once in the crossing structures, so species only recorded in forest sites were not included in these analyses. We considered one sampling occasion as five consecutive days of camera operation at a given site, preparing the data using the "camtrapR" R package (Niedballa et al., 2016). We assessed the models' fit to the data using the goodness-of-fit test for single-season occupancy models based on Pearson's chi-square (MacKenzie and Bailey, 2004) in the R package "AICcmodavg" (Mazerolle, 2023). R codes are available as supplementary material (Rcode S1 and Rcode S2).

Wildlife mortality caused by vehicle collisions

To evaluate if mortality is lower on road sections with crossing structures compared to road sections without them, we tested if the number of roadkills in each road segment was associated with the presence or absence of crossing structures in that segment. Because of the few roadkill records of species that used the crossing structures, we carried out the analysis only for the group of species that were recorded using culverts and bridges along Route 1, totaling 67 roadkill records. We divided the road into 100-m segments classified according to the presence or absence of culverts and bridges (hereafter referred to as underpasses). The number of roadkills on segments was compared using a generalized linear model (GLM) with family Poisson in R (R Core Team, 2023). The model goodness of fit was tested by dividing the residual deviance by the residual degrees of freedom. R code is available as supplementary material (Rcode S3). Amphibians were not included in the study as there were moments where it was almost impossible to count them, because of the large number of individuals.

Results

Wildlife movement: Species use of crossing structures and surrounding sites

On Route 160, we recorded 12 species using the underpasses, 48 % of the 25 species recorded at the forest ground in the road surroundings (Table S5). The coyote (*Canis latrans*), ocelot (*Leopardus pardalis*), common spiny-tailed iguana (*Ctenosaura similis*), and opossum (*Didelphis* sp.) showed high probability of using the underpasses in periods of five days (over 0.5; Table 1). For the coyote, the use of underpasses was significantly higher than the forest ground sites. The nine-banded armadillo (*Dasypus novemcinctus*) and white-nosed coati (*Nausa narica*) showed significantly lower use of underpasses than forest ground sites (Table 1). Four different arboreal species used canopy bridges, 50 % of the eight species recorded using the forest canopy (Table S5). Except for the variegated squirrel (*Sciurus variegatoides*), species showed probabilities of using canopy bridges under 0.5, but no significant differences with the use of forest canopy were observed (Table 1). Except for the models for coyote, opossum, and variegated squirrel, models showed strong under- or overdispersion, indicating a poor fit to the data.

On Route 1, from a total of nine species using crossing structures, we recorded five using box culverts and eight using bridges with ledges, representing 18 % and 29 % of the 27 species recorded in the forest ground at surrounding sites (Table S6). For the nine species analyzed, the probability of using culverts for periods of five days was lower than 0.4 (Table 2). Though there is no significant difference observed, the probability of species using bridges showed higher values than culverts, except for the paca (*Cuniculus paca*) and Northern racoon (*Procyon lotor*). The common spiny-tailed iguana, common green iguana (*Iguana iguana*) and mexican hairy porcupine (*Coendou mexicanus*) showed a high probability of using bridges (over 0.5; Table 2). The only significant difference observed was the higher use of forest ground than bridges by the white-nosed coati (Table 2). For arboreal and scansorial animals, two species used canopy bridges; 15 % of the 13 species recorded using the forest canopy in the Route 1 area (Table S6). The Central America wooly opossum (*Caluromys derbianus*) and variegated squirrel showed a low probability of using canopy bridges, under 0.31, but no significant difference from the use of forest canopy was observed (Table 2). Except for the model for the ocelot, models showed strong under- or overdispersion, indicating a poor fit to the data.

Table 1.
Comparison of species use of crossing structures (culverts with fencing and dry ledge) versus forest surrounding sites in Route 160. Estimated probability of use in periods of five days and 95% confidence intervals (in parentheses). P values under 0.05 indicate significant differences. All species recorded at least once in the crossing structures were included in these analyses, species only recorded in forest sites were not.

Species	Underpass	Forest ground	P	Canopy bridge	Forest canopy	P
Alouatta palliata				0.25 (0.03 - 0.76)	0.5 (0.2 - 0.8)	0.41
Canis latrans	0.78 (0.35 - 0.95)	0.29 (0.12 - 0.54)	**0.04**			
Cebus imitator				0.5 (0.12 - 0.87)	0.75 (0.37 - 0.93)	0.39
Ctenosaura similis	0.62 (0.28 - 0.87)	0.28 (0.12 - 0.52)	0.1			
Cuniculus paca	0.12 (0.01 - 0.53)	0.16 (0.05 - 0.41)	0.78			
Dasypus novemcinctus	0.25 (0.06 - 0.62)	0.84 (0.59 - 0.95)	**0.008**			
Didelphis sp.	0.62 (0.28 - 0.87)	0.8 (0.54 - 0.93)	0.3			
Eira barbara	0.14 (0.01 - 0.58)	0.52 (0.26 - 0.77)	0.12			
Iguana iguana	0.25 (0.06 - 0.63)	0.11 (0.02 - 0.36)	0.38			
Leopardus pardalis	0.65 (0.28 - 0.89)	0.78 (0.57 - 0.97)	0.25			
Nasua narica	0.5 (0.2 - 0.79)	0.9 (0.63 - 0.97)	**0.04**			
Potos flavus				0.25 (0.03 - 0.77)	0.63 (0.28 - 0.88)	0.24
Procyon lotor	0.5 (0.2 - 0.8)	0.28 (0.12 - 0.53)	0.29			
Sciurus variegatoides				0.99 (0 - 1)	0.87 (0.46 -0.98)	0.85
Tamandua mexicana	0.29 (0.07 - 0.7)	0.48 (0.22 - 0.75)	0.45			
Urocyon cinereoargenteus	0.37 (0.12 - 0.71)	0.28 (0.12 - 0.52)	0.63			

Table 2.

Comparison of species use of crossing structures (culverts with no dry ledge and bridges with dry ledge) versus forest surrounding sites in Route 1. Estimated probability of use in periods of five days and 95% confidence intervals (in parentheses). When P values were different between the pairwise comparisons, three P values are shown for the same species, representing comparisons between culvert and bridge, culvert and forest, and bridge and forest, respectively. P values under 0.05 indicate significant differences. All species recorded at least once in the crossing structures were included in these analyses, species only recorded in forest sites were not.

Species	Culvert	Bridge	Forest ground	P	Canopy bridge	Forest canopy	P
Caluromys derbianus					0.3 (0.02 - 0.87)	0.46 (0.06 - 0.92)	0.7
Ctenosaura similis	0.27 (0.03 - 0.79)	0.56 (0.08 - 0.94)	0.48 (0.27 - 0.7)	> 0.2			
Cuniculus paca	0.36 (0.03 - 0.91)	0.3 (0.03 - 0.84)	0.16 (0.05 - 0.41)	> 0.4			
Didelphis sp.	0.23 (0.03 - 0.797)	0.47 (0.09 - 0.88)	0.86 (0.61 - 0.96)	> 0.1			
Iguana iguana	0.27 (0.03 - 0.79)	0.53 (0.08 - 0.92)	0.37 (0.19 - 0.6)	> 0.5			
Leopardus pardalis	0.002 (0 - 0.99)	0.28 (0.03 - 0.82)	0.49 (0.27 - 0.7)	> 0.4			
Nasua narica	0.0004 (0 - 1)	0.2 (0.02 - 0.69)	0.79 (0.55 - 0.92)	0.7, 0.22, **0.03**			
Procyon lotor	0.27 (0.03 - 0.8)	0.0003 (0 - 1)	0.37 (0.19 - 0.6)	> 0.7			
Sciurus variegatoides					0.17 (0.02 - 0.63)	0.99 (0 - 1)	0.7
Coendou mexicanus	0.0007 (0 - 1)	0.99 (0 - 1)	0.912 (0.009 - 0.68)	> 0.8			
Tamandua mexicana	0.0003 (0 - 1)	0.31 (0.03 - 0.85)	0.73 (0.47 - 0.9)	> 0.2			

Wildlife mortality caused by vehicle collisions

We recorded a total of 22 roadkills from seven species (six of which were snakes) on Route 160 (Table 3). The most recorded species was the snake, *Leptodeira rhombifera* (n = 11). Of all species recorded in camera traps, only the opossum was also recorded as roadkill, representing 6 % of the 16 species recorded by camera traps on crossing structures and 3 % of the 28 species at forest ground or canopy at surrounding sites. No road-killed snake species were recorded by camera traps. On Route 1, we recorded 124 roadkills from 20 species (Table 3). The most recorded species were the opossum (n = 30), nine-banded armadillo (n = 18), Northern tamandua (*Tamandua mexicana*, n = 12), Central American lyre snake (*Trimorphodon quadruplex*, n = 12), and northern racoon (n = 10). Of all road-killed species, eight were also recorded by camera traps, representing 72 % of the 11 species using crossing structures and 26 % of the 30 species using ground or canopy forest at surrounding sites. Ten species were recorded road-killed but not by camera traps: the Eastern cottontail rabbit (*Sylvilagus floridanus*) and nine species of snakes. On Route 1, the number of roadkills (of those species recorded using culverts or bridges) was higher on 100-m segments with crossing structures than on segments without them (Figure 1).

Table 3.

Roadkills recorded at two roads in Costa Rica between August 2024 and January 2025. Asterisks for species recorded using crossing structures.

Species	Route 160	Route 1
Boa imperator	2	5
Cabassous centralis	0	1
Caluromys derbianus	0	2 *
Crotalus simus	2	0
Ctenosaura similis	0	7 *
Dasypus novemcinctus	0	18
Didelphis sp.	1 *	30 *
Iguana iguana	0	3 *
Leptodeira rhombifera	11	9
Leptophis mexicanus	0	1
Loxocemus bicolor	0	3
Masticophis mentovarius	0	1
Micrurus nigrocinctus	1	1
Nasua narica	0	4 *
Procyon lotor	0	10 *
Scolecophis atrocinctus	0	1
Sibon anthracops	0	1
Coendou mexicanus	0	1 *
Stenorrhina freminvillii	1	0
Sylvilagus floridanus	0	2
Tamandua mexicana	0	12 *
Trimorphodon quadruplex	6	12
Total	22	124
Species richness	7	20

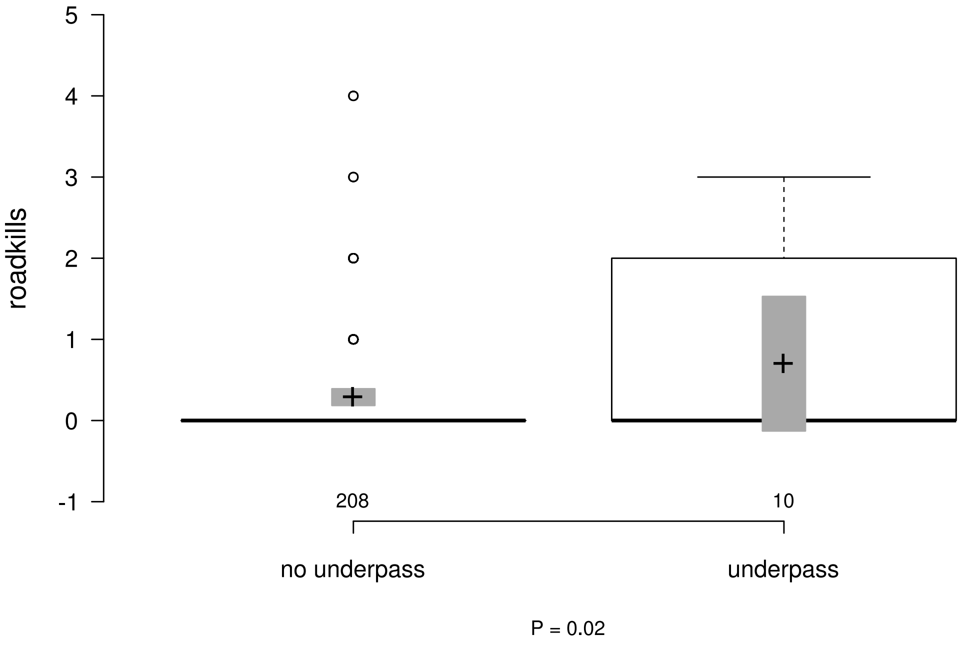


Fig. 5. Boxplot comparisons of the number of roadkills of the species recorded using culverts or bridges (here combined as "underpass") on 218 100-m road segments with (n=10) and without "underpasses" (n=208) on Route 1. Center lines show the medians; box limits indicate the 25th and 75th percentiles as determined by R software; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles, outliers are represented by dots; crosses represent sample means; bars indicate 95% confidence intervals of the means.

Discussion

Our results demonstrate that crossing structures are being used by various species to move across roads, supporting their potential to facilitate wildlife movement (Soanes et al. 2024). Camera trap data revealed that multiple taxa, including ground-dwelling and arboreal species, use structures such as box culverts, bridge ledges, underpasses (culverts with dry ledges) and canopy bridges. For example, species such as coyote, ocelot, common spiny-tailed iguana, and opossum showed a high probability of using underpasses in five-day periods on Route 160. The higher probability of coyotes using underpasses compared to forest sites can indicate behavioral adaptation to these structures, and this species also prefers open vegetation to forest areas in the region.

For most ground-dwelling species and for all arboreal species analyzed, the probability of use of crossing structures was comparable to forest sites, suggesting that these species are also relatively well adapted to the structures. These findings are consistent with earlier studies showing that crossing structures can support diverse wildlife use (Andis et al. 2017; Soanes et al. 2024; Denneboom et al. 2021). However, the estimates obtained should be interpreted with caution, given their relatively low precision and the wide confidence intervals associated with them, probably due to limited sample size. Even with uncertainty, models allow us to account for imperfect detection and provide estimates of use that are less biased than simple encounter frequencies (Mackenzie et al., 2002; Mackenzie et al., 2017; Sollmann et al., 2013; Sollmann, 2018).

Despite the encouraging patterns of structure use found, certain species were never recorded using any of the available crossings, even though they were present in adjacent forests. For example, nine-banded armadillo (*Dasypus novemcinctus*), collared peccary (*Pecari tajacu*), puma (*Puma concolor*), margay (*Leopardus wiedii*) and Jaguarundi (*Herpailurus yagouaroundi*) were all recorded on the forest sites but not on crossing structures. On Route 160, about half of the species detected were observed in underpasses or canopy bridges, suggesting that culverts with dry ledges and fences, shorter canopy bridges, a narrower road and lower traffic volumes create conditions more favorable for diverse species to use the structures. In contrast, use was much lower on Route 1, where only 18% of species were recorded in culverts, 29% in bridges, and 15% on canopy bridges. Several factors may explain this discrepancy. Culverts on Route 1 lacked dry ledges, and both culverts and bridges lacked fences, which likely reduced their functionality (Huijser, 2008; Rytwinski et al. 2016). In addition, the greater width of the highway requires canopy bridges to span longer gaps, potentially deterring species sensitive to exposure. These results may reflect species-specific avoidance behaviors as well as structural and environmental constraints—such as canopy gap width, noise, light pollution, temperature, and traffic intensity—that discourage use and could lead to genetic isolation of populations on either side of the road. Altogether, the contrasting characteristics of the two roads highlight how both structure design and broader road context might influence patterns of wildlife crossing use.

Notably, roadkill data did show that mortality rates were higher on road segments with crossing structures in comparison to road segments without them on Route 1. This highlights a key challenge in road mitigation: while crossing structures enable movement, they may not prevent mortality unless access to the road itself is blocked (e.g., with fencing) and a dry ledge is provided (Rytwinski et al. 2016). Ideally, monitoring to disentangle all these effects and assess effectiveness should apply Before-After-Control-Impact study designs, with adequate reference comparisons (Soanes et al. 2024). In line with the lower use of crossing structures on Route 1, they may be less effective at reducing wildlife mortality compared to those on Route 160. For Route 160, comparisons of roadkill numbers between segments with and without crossing structures were not possible due to the low number of recorded roadkill. Additionally, the mortality patterns observed for Route 1 may reflect the fact that these structures were installed in areas with higher expected wildlife presence. The placement of crossings without fencing and without dry ledges in hotspots of animal activity may have coincided with higher underlying mortality, complicating the interpretation of their effectiveness. Thus, higher mortality might be expected in these segments, as they coincide with areas of higher wildlife activity identified during the environmental impact assessment.

Lastly, in the absence of effective exclusion measures, such as continuous fencing, animals can still access the road directly, even if they also use the structures. This highlights the critical importance of incorporating exclusion fencing in road mitigation design, as supported by a large body of literature (Huijser, 2008; Rytwinski et al., 2016). On Route 1, no fences were implemented around the underpasses, and on Route 160, existing fences were short (less than 100 m) and incomplete. Without adequate fencing, animals are free to approach the road, increasing the likelihood of collision (Huijser et al. 2016). Beyond simply extending fence length, it is essential to improve fence design to prevent access by a broader range of ground-dwelling species, including small-bodied animals, which are often overlooked in mitigation planning. The inclusion of dry ledges on box culverts at Route 1 will allow wildlife movement even if water is flowing through most of the time.

In conclusion, while wildlife crossing structures show promise in supporting animal movement across roads, they are insufficient on their own to prevent road mortality. Effective road mitigation must integrate well-placed and species-appropriate structures with exclusion fencing, dry ledges and landscape-scale planning. The mitigation measures should be improved, including the construction of dry ledges where they are not yet present (on Route 1) and the implementation of fences to block animals from accessing the road. Fence design can also be improved to target a larger range of ground-dwelling species, especially animals with smaller body sizes, such as snakes that were recorded road-killed and could potentially use crossing structures. Furthermore, maintenance of the mitigation measures is also key to maintaining their functionality. We identify the need for specific methodology to measure the impact of roads on amphibians.

Acknowledgments

The authors thank the partner institutions and technical teams, including MOPT, PROGAS, CONAVI, VAVS, LACTWG, COSEVI, MINAE-SINAC, ICE, COMEX and CSV, for their collaboration. We also appreciate the valuable insights provided by Fernando Quirós (TSP) and Dayana Ugalde (ESG) from the IDB Group, as well as local committees, workshops and consultation participants from communities along Routes 160 and 1, whose contributions enriched this work. This publication was funded by the IDB under project RG-E1990, Evaluating Environmental Measures for Wildlife Protection on Costa Rica's Road Network.

Supplementary material

Complete datasets and R codes for analysis are available at https://osf.io/6fe83/files/osfstorage. Tables S5 and S6 are included after the references section.

References

Abra FD, Canena A da C, Garbino GST, Medici EP (2020) Use of unfenced highway underpasses by lowland tapirs and other medium and large mammals in central-western Brazil. Perspect Ecol Conserv 18:247–256. https://doi.org/10.1016/j.pecon.2020.10.006

Andis AZ, Huijser MP and Broberg L (2017) Performance of Arch-Style Road Crossing Structures from Relative Movement Rates of Large Mammals. Front. Ecol. Evol. 5:122. doi: 10.3389/fevo.2017.00122

Araya-Jiménez, Y. (2019). Efectividad de estructuras para el paso de fauna silvestre en la Ruta Nacional No 4, Bajos de Chilamate – Vuelta Kooper, Costa Rica. Universidad Latina de Costa Rica, San José, Costa Rica. 53pp.

Arévalo-Huezo E, Pomareda-García E, Araya-Gamboa D (2020) Ruta Nacional 253, Amigable con la Vida Silvestre. Carrillo, Guanacaste Costa Rica. Informe Final.

Bolaños, R.; Watson, V., y Tosi, J. (2005). Mapa ecológico de Costa Rica (Zonas de Vida), según el sistema de clasificación de zonas de vida del mundo de L.R. Holdridge), Escala 1:750 000. Centro Científico Tropical, San José, Costa Rica.

Denneboom, D, Bar-Massada, A. and Shwartz, A. (2021) Factors affecting usage of crossing structures by wildlife – a systematic review and meta-analysis, Science of the Total Environment 777 (2021) 146061, https://doi.org/10.1016/j.scitotenv.2021.146061

Eken, G., Bennun, L., Brooks, T. M., Darwall, W., Fishpool, L. D., Foster, M., ... & Tordoff, A. (2004). Key biodiversity areas as site conservation targets. BioScience, 54(12), 1110-1118.

González-Gallina A, Hidalgo-Mihart MG, Castelazo-Calva V (2018) Conservation implications for jaguars and other neotropical mammals using highway underpasses. PLoS One 13:1–20. https://doi.org/10.1371/journal.pone.0206614

Huijser, M. 2008. “Wildlife-Vehicle Collision Reduction Study: Report to Congress.” US Department of Transportation, Federal Highway Administration. McLean, VA.

Huijser, MP, Fairbank, ER, Camel-Means, W, Graham, J, Watson, V, Basting, P, Becker, D. (2016) Effectiveness of short sections of wildlife fencing and crossing structures along highways in reducing wildlife–vehicle collisions and providing safe crossing opportunities for large mammals. Biological Conservation 197, 61-68, https://doi.org/10.1016/j.biocon.2016.02.002.

Huijser, M. P., Ament, R. J., Bell, M., Clevenger, A. P., Fairbank, E. R., Gunson, K. E., & McGuire, T. (Eds.). (2022). Animal-vehicle collision reduction and habitat connectivity pooled fund study – Literature review. Nevada Department of Transportation.

Ian Fiske, Richard Chandler (2011). unmarked: An R Package for Fitting Hierarchical Models of Wildlife Occurrence and Abundance. Journal of Statistical Software, 43(10), 1-23. URL https://www.jstatsoft.org/v43/i10/.

Jackson, N. D., & Fahrig, L. (2011). Relative effects of road mortality and decreased connectivity on population genetic diversity. Biological Conservation, 144(12), 3143–3148. https://doi.org/10.1016/j.biocon.2011.09.010

Jürgen Niedballa, Rahel Sollmann, Alexandre Courtiol, Andreas Wilting (2016). camtrapR: an R package for efficient camera trap data management. Methods in Ecology and Evolution 7(12), 1457-1462.

Kellner KF, Smith AD, Royle JA, Kery M, Belant JL, Chandler RB (2023). The unmarked R package: Twelve years of advances in occurrence and abundance modelling in ecology. Methods in Ecology and Evolution, 14(6), 1408-1415. URL https://doi.org/10.1111/2041-210X.14123

Mackenzie, D. I., J. D. Nichols, G. B. Lachman, S. Droege, J. A. Royle, and C. A. Langtimm. 2002. Estimating the Occupancy Rate When Detection Probabilities Are Less Than One. Ecology 83, no. 8: 2248–2255.

MacKenzie, D. I., Bailey, L. L. (2004) Assessing the fit of site-occupancy models. Journal of Agricultural, Biological, and Environmental Statistics 9, 300–318.

MacKenzie, D. I., & Royle, J. A. (2005). Designing occupancy studies: General advice and allocating survey effort. Journal of Applied Ecology, 42 (6), 1105–1114. https://doi.org/10.1111/j.1365-2664.2005.01098.x.

Mackenzie, D. I., J. D. Nichols, J. A. Royle, K. H. Pollock, L. L. Bailey, and J. E. Hines. 2017. Occupancy Estimation and Modeling. Inferring Patterns and Dynamics of Species Occurrence. 2nd Edition. Elsevier.

Mazerolle MJ (2023) AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c)_. R package version 2.3.3, <https://cran.r-project.org/package=AICcmodavg>.

Niedballa J, Sollmann R, Courtiol A, Wilting A (2016). camtrapR: an R package for efficient camera trap data management. Methods in Ecology and Evolution 7(12), 1457-1462.

MOPT (Ministerio de Obras Públicas y Transportes de Costa Rica). (2011). Plan Nacional de Transportes de Costa Rica 2011-2035. Retrieved August 9, 2021, from Web Archive: https://web.archive.org/web/20210809041046/https://www.mopt.go.cr/wps/wcm/connect/a0214712-9291-485d-b05a-62640daedaf3/Metodologia.pdf

Panthera (2019). Monitoreo del jaguar, otros felinos silvestres y sus especies presa en el cantón central de Limón, Costa Rica. Informe final para CCT-APM Terminals. Costa

Rica. 58pp.

Panthera (2022). Aplicación de la Guía Ambiental: Vías Amigables con la Vida Silvestre en la Ruta 35, Sección: Florencia-Ciudad Quesada. Identificación del impacto en la fauna silvestre y propuesta de medidas para reducirlo. Costa Rica. 53 pp.

Pinto, F. A. S., Clevenger, A. P., & Grilo, C. (2020). Effects of roads on terrestrial vertebrate species in Latin America. Environmental Impact Assessment Review, 81(November 2019), 106337. https://doi.org/10.1016/j.eiar.2019.106337

Pomareda-García E, Araya-Gamboa D, E. A-H (2016) Informe técnico científico: Verificación de las Medidas ambientales para disminuir el impacto en la fauna silvestre, de la ampliación en la carretera nacional, Ruta 32, Limón, Costa Rica. Costa Rica

R Core Team (2023). R: A Language and Environment for Statistical Computing_. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.

Rytwinski, T., Soanes, K., Jaeger, J. A. G., Fahrig, L., Findlay, C. S., Houlahan, J., … Van Der Grift, E. A. (2016). How effective is road mitigation at reducing road-kill? A meta-analysis. PLoS ONE, 11(11), 1–25. https://doi.org/10.1371/journal.pone.0166941

Soanes, K., Rytwinski, T., Fahrig, L., Huijser, M. P., Jaeger, J. A. G. G., Teixeira, F. Z., … van der Grift, E. A. (2024). Do wildlife crossing structures mitigate the barrier effect of roads on animal movement? A global assessment. Journal of Applied Ecology, 61(3), 417–430. https://doi.org/10.1111/1365-2664.14582

Sollmann R. A gentle introduction to camera-trap data analysis. Afr J Ecol. 2018; 56: 740–749. https://doi.org/10.1111/aje.12557

Teixeira, F. Z., Rytwinski, T., & Fahrig, L. (2020). Inference in road ecology research: What we know versus what we think we know. Biology Letters, 16, 20200140.

Teixeira FZ, Gonçalves da Silva L, Abra F, et al (2022) A review of the application of canopy bridges in the conservation of primates and other arboreal animals across Brazil. Folia Primatol 93:479–492. https://doi.org/10.1163/14219980-20211210

Van Der Ree, R., Smith, D. J., & Grilo, C. (2015). The Ecological Effects of Linear Infrastructure and Traffic: Challenges and Opportunities of Rapid Global Growth. In R. Van Der Ree, D. J. Smith, & C. Grilo (Eds.), Handbook of Road Ecology (First Edit, pp. 1–9). John Wiley & Sons, Ltd. Retrieved from www.wiley.com%5Cgo%5Cvanderree%5Croadecology

Varela D (2015) Ecología de Rutas en Misiones. Evaluación de la efectividad de los pasafaunas y ecoductos. Informe con resultados para el período 2011-2014

Villalobos-Hoffman, R.; Ewing, J.E.; Mooring, M.S. (2022) Do Wildlife Crossings Mitigate the Roadkill Mortality of Tropical Mammals? A Case Study from Costa Rica. Diversity 14, 665. https://doi.org/10.3390/d14080665

Zank C, Beduschi J, Pereira D, et al (2019) Avaliação da mortalidade por atropelamento e proposição de medidas mitigadoras para anfíbios em um hotspot de biodiversidade no Rio Grande do Sul. Rev Estrad 24:26–32

Table S5. Number of camera trap records (images within 1h interval) of species that potentially use crossing structures in underpasses, canopy bridges and surrounding forest sites in Route 160 area. Consecutive photographs of the same species taken within 1 h were considered a single record, regardless of the number of individuals observed.

Species	**Underpass**	**Forest Ground**	**Forest Canopy**	**Canopy Bridge**
Alouatta palliata	0	1	9	37
Aramides cajaneus	0	1	0	0
Caluromys derbianus	0	0	1	0
Canis latrans	22	19	0	0
Cebus imitator	0	6	30	22
Conepatus semistriatus	0	2	0	0
Crypturellus cinnamomeus	0	3	0	0
Ctenosaura similis	31	7	5	0
Cuniculus paca	8	9	0	0

Dasyprocta punctata	0	30	0	0
Dasypus novemcinctus	1	47	0	0
Dicotyles tajacu	0	3	0	0
Didelphis sp.	20	21	0	0
Eira barbara	5	5	0	0
Herpailurus yagouaroundi	0	4	0	0
Iguana iguana	10	6	0	0
Leopardus pardalis	5	21	0	0
Leopardus wiedii	0	9	0	0
Nasua narica	14	28	0	0
Odocoileus virginianus	0	5	0	0
Potos flavus	0	0	21	8
Procyon lotor	7	24	0	0
Puma concolor	0	10	0	0
Sciurus variegatoides	0	1	34	30
Coendou mexicanus	0	0	1	0
Spilogale putorius	0	18	0	0
Tamandua mexicana	1	9	2	0
Urocyon cinereoargenteus	29	12	0	0
Species Richness	**12**	**25**	**8**	**4**
Camera Sites	**8**	**18**	**8**	**4**
Camera Days	**1514**	**3247**	**1452**	**711**

Table S6. Number of camera trap records (images within 1h interval) of species that potentially use crossing structures in underpasses, canopy bridges and surrounding forest sites in Route 1 area. Consecutive photographs of the same species taken within 1 h were considered a single record, regardless of the number of individuals observed.

Species	**Culvert**	**Bridge**	**Forest Ground**	**Canopy Bridge**	**Forest Canopy**
Alouatta palliata	0	0	1	0	10
Cabassous centralis	0	0	2	0	0
Caluromys derbianus	0	0	0	3	2
Canis latrans	0	0	12	0	0
Cebus imitator	0	0	0	0	1
Conepatus semistriatus	0	0	14	0	0
Crax rubra	0	0	1	0	0
Crypturellus cinnamomeus	0	0	5	0	0
Ctenosaura similis	2	8	33	0	18
Cuniculus paca	2	3	8	0	0
Dasyprocta punctata	0	0	34	0	0
Dasypus novemcinctus	0	0	60	0	0

Dicotyles tajacu	0	0	5	0	0
Didelphis sp.	1	5	29	0	13
Eira barbara	0	0	2	0	0
Herpailurus yagouaroundi	0	0	1	0	0
Iguana iguana	1	9	34	0	45
Leopardus pardalis	0	1	10	0	0
Mephitis macroura	0	0	12	0	0
Nasua narica	0	1	41	0	1
Odocoileus virginianus	0	0	1	0	0
Philander opossum	0	0	79	0	0
Potos flavus	0	0	0	0	2
Procyon lotor	1	0	9	0	0
Puma concolor	0	0	3	0	0
Sciurus variegatoides	0	0	6	2	7
Coendou mexicanus	0	1	1	0	45
Spilogale putorius	0	0	18	0	0
Tamandua mexicana	0	2	20	0	11
Urocyon cinereoargenteus	0	0	7	0	0
Species Richness	**5**	**8**	**27**	**2**	**11**
Camera Sites	**4**	**6**	**19**	**6**	**7**
Camera Days	**357**	**275**	**3349**	**766**	**944**