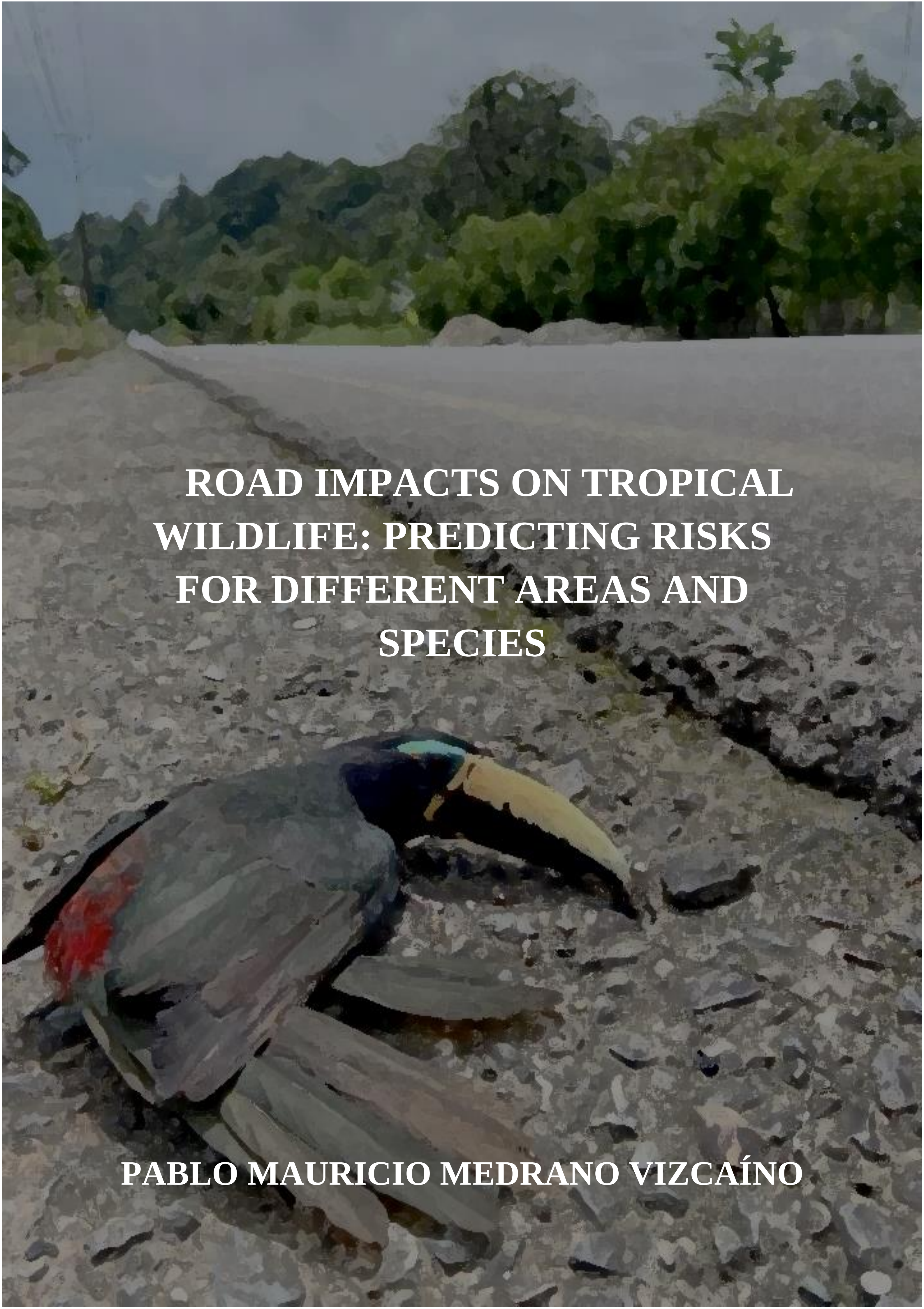


ROAD IMPACTS ON TROPICAL WILDLIFE: PREDICTING RISKS FOR DIFFERENT AREAS AND SPECIES

PABLO MAURICIO MEDRANO VIZCAÍNO

ROAD IMPACTS ON TROPICAL WILDLIFE: PREDICTING RISKS FOR DIFFERENT AREAS AND SPECIES

A thesis submitted for the degree of Doctor of Philosophy

School of Biological Sciences

Ecology and Evolutionary Biology

University of Reading

Pablo Mauricio Medrano Vizcaíno

Main supervisor: Manuela González-Suárez

Co-supervisor: Clara Grilo

December 2022

Declaration

I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged

Pablo Mauricio Medrano Vizcaíno

Acknowledgments

This PhD journey has been one of the most enriching experiences of my life. I have learnt lots of new things, visited fantastic places and mainly met wonderful people.

One of my biggest life dreams was to do a PhD in England. I knew this was something very challenging to do, especially due to lack of scholarships for Ecuadorians. At the end of 2018 I read that every year the University of Reading awards a full scholarship for PhD studies. I applied, however, being a worldwide competition, I saw it as something so difficult to achieve, but I wanted to try!. Months later, on April 24, 2019, I received an email informing me that I was the winner of the full scholarship. I was so happy to know that this dream was about to begin. Therefore, I am deeply thankful to the University of Reading, for allowing this to come true.

Manuela, your constant guidance and support during these three years and three months have been key for reaching this point. You were very supportive since my first day in the UK: picking me up in the airport, providing exhaustive revisions to my work, and helping me to develop my ideas. I feel very happy and lucky to have had you as my supervisor, you have been always ready to help and I feel that because of you, my scientific perspective and goals are now more ambitious. My work improves significantly with your active engagement, and it is great that we have more ongoing collaborations. Thanks for being an amazing supervisor and friend.

Clara, even though our connection was mainly through videoconferences, and just met in person once, talking with you was always very helpful. All our working meetings were very nicely accompanied by your kindness, and sense of humor. Thanks for being so nice and for providing excellent feedback on my work, which really helped to improve the quality of manuscripts.

Mom, you are the most important person of my life. Everything I have achieved is because of you. Although making this dream come true implied being away, you always understood how important this is for me. There are no words to describe how thankful I am for all the things you have done for me. Your unconditional and pure love is the main engine for my life. Nothing would be possible without you.

Dad, I wish you could enjoy this moment with us. I know you would be very happy to see me accomplishing my goals. Now I can do this because of your guidance and support since I was born. I will always miss you.

Ñaña, thanks for being always nearby. You have supported my ideas and projects since I was a child, and this has been very helpful to reach my goals. I am very happy that together with Cristian, you allow lovely moments in family bringing Cami, Estefy and Samu to life. This work was also supported by the love provided by all of you.

Ñaño Marco, COVID times allowed us to spend more time during lockdown. Since then, we have had a closer connection as a family and hope we can have so many more moments of happiness and fun.

Lola, thanks for always being worried about my wellbeing since I was a child. It is great to feel how happy you are for every success in my life.

Valeria, your love has showed me a different face of life. Having you beside me, living our dreams is such an ineffable experience. We have been together just for one year and a half, but it feels like much more time. Despite of the short time, we have now lived together in two different cities, and have visited so many places. This strong and beautiful story that we have constructed, will surely take us to know many other places, and to live more wonderful adventures. Knowing your mom is part of the great thanks to you. Ursulita has supported us so much in many aspects, and I feel very grateful to her. Valeria, I am so happy to feel our families as one. Thanks for bringing light and magic to my days.

David, your friendship, and great effort collaborating during fieldwork was key for this work. We had very hard work in Tena, but that allowed conducting more research and parallel projects such as our REMFA and the parasites project. Besides work, we also had very enjoyable moments. I remember pleasantly those beers and wine days listening to music and playing chess.

My lab mates: Tom and Chanida, you were the first people that I met in the University. I remember having a little trouble to understand British accent those first days in Reading, but both of you were always very nice. Thanks for your friendship, I really enjoyed meeting you. Kerry, you have been a very good friend. I enjoyed our time in the congress of Prague and will always be very grateful for your help when moving to Birmingham. Brooke, you are a lovely friend, your kindness and good vibes are present in every conversation we have.

To the friends I met in Reading (Jorge A., Ricardo, Julián, Rubén, Danilo, Jorge, Rubén, Esmeralda, Diana), you did this time in the UK more enjoyable and fun. Those days of Purple turtle, football, and pub tours were very fun!.

Ana and Sandra, thanks for being so open when I reached to you with the idea about studying wildlife parasites as a parallel project of this PhD. Now, that is a reality and we have done an amazing job, which will surely provide very interesting results in the future.

Pablo Jarrín, thanks for being very supportive during fieldwork. Your help facilitating a lab in IKIAM University was very valuable for dissections work.

Joy moments with my cousins, aunts, uncles, and friends were very important to make this a more pleasant journey.

Agradecimientos

Este viaje de doctorado ha sido una de las experiencias más enriquecedoras de mi vida. Aprendí muchas cosas nuevas, visité lugares fantásticos y sobre todo conocí gente maravillosa.

Uno de mis mayores sueños de vida era cursar un PhD en Inglaterra. Sabía que sería algo difícil de conseguir, especialmente por la falta de becas para ecuatorianos. A finales del 2018 leí que anualmente la Universidad de Reading otorgaba una beca completa para estudios de PhD. Apliqué, sin embargo al ser una competencia a nivel mundial, lo vi como algo tan difícil de alcanzar, ¡pero quería intentarlo!. Meses después, el 24 de abril del 2019, recibí un email informándome que fui el ganador de la beca completa. Fue una alegría enorme saber que este sueño estaba por iniciar. Por lo tanto, expreso mi profundo agradecimiento a la Universidad de Reading, por haber permitido que todo esto sea una realidad.

Manuela, tu guía y apoyo constante durante estos tres años y tres meses han sido clave para llegar a este punto. Tu apoyo estuvo ahí desde mi primer día en el Reino Unido: desde ir a encontrarme al aeropuerto, tus revisiones exhaustivas de los manuscritos y tu soporte para desarrollar mis ideas. Me siento muy feliz y afortunado de haberte tenido como mi supervisora, siempre has estado dispuesto a ayudar y siento que, gracias a ti, mi perspectiva científica y objetivos son ahora más ambiciosos. Mi trabajo mejora significativamente con tu compromiso activo y es genial que tengamos más colaboraciones en curso. Gracias por ser una supervisora y amiga increíble.

Clara, aunque nuestra conexión fue principalmente a través de videoconferencias, y solo nos conocimos en persona una vez, hablar contigo siempre fue muy útil. Todas nuestras reuniones de trabajo estuvieron muy bien acompañadas por tu amabilidad y sentido del humor. Tus revisiones han ayudado a mejorar la calidad de los manuscritos.

Mami, eres la persona más importante de mi vida. Todo lo que he logrado es gracias a ti. Aunque hacer realidad este sueño implicaba estar lejos, siempre entendiste lo importante que era esto para mí. No tengo palabras que describan cuan agradecido estoy por todo lo que has hecho por mí. Tu amor puro e incondicional es el principal motor para mi vida. Nada sería posible sin ti.

Papi, ojalá pudieras disfrutar este momento con nosotros. Sé que estarías muy feliz de verme lograr mis metas. Ahora puedo hacer esto gracias a tu guía y apoyo desde que nací. Siempre te voy a extrañar.

Ñaña, gracias por estar siempre cerca. Has apoyado mis ideas y proyectos desde que era niño, y esto ha sido de gran ayuda para alcanzar mis metas. Me alegra mucho que, junto a Cristian, permitas lindos momentos en familia habiendo traído a Cami, Estefy y Samu a nuestras vidas. Este trabajo fue apoyado por el amor brindado por todos ustedes.

Ñaño Marco, los tiempos de COVID nos permitieron pasar más tiempo durante el encierro. Desde entonces, hemos tenido una conexión más cercana como familia y espero tener muchos más momentos de felicidad y diversión.

Lola, gracias por estar siempre preocupada por mi bienestar desde niño. Es genial sentir lo feliz que eres por cada éxito en mi vida.

Valeria, tu amor me ha mostrado una cara diferente de la vida. Tenerte a mi lado, vivir nuestros sueños es una experiencia inefable. Hemos estado juntos solo por un año y medio, pero se siente como mucho más tiempo. A pesar del poco tiempo, ya hemos vivido juntos en dos ciudades diferentes y hemos visitado muchos lugares. Esta fuerte y hermosa historia que hemos construido, seguramente nos llevará a conocer muchos otros lugares, y a vivir más aventuras maravillosas. Conocer a tu mamá es parte del gran agradecimiento hacia ti. Ursulita nos ha apoyado mucho en varios aspectos y su cariño ha estado siempre presente. Valeria, estoy muy feliz de sentir a nuestras familias como una sola. Gracias por traer luz y magia a mis días.

David, tu amistad y gran esfuerzo colaborando durante el trabajo de campo fue clave para este trabajo. Tuvimos labores arduas en Tena, pero eso permitió hacer más investigaciones y proyectos paralelos como nuestra REMFA y el proyecto de parásitos. Además del trabajo, también pasamos momentos muy agradables. Recuerdo gratamente aquellos días de cervezas y vino escuchando música y jugando al ajedrez.

Mis compañeros de laboratorio: Tom y Chanida, ustedes fueron las primeras personas que conocí en la Universidad. Recuerdo que tuve un poco de dificultad para entender el acento británico esos primeros días en Reading, pero ustedes siempre fueron muy amables. Gracias por su amistad, disfruté mucho conocerles. Kerry, has sido una muy buena amiga. Fue genial haber compartido momentos en el congreso de Praga y siempre estaré muy agradecido por tu ayuda para la mudanza a Birmingham. Brooke, eres una amiga encantadora, tu amabilidad y buena onda están presentes en cada conversación que tenemos.

A los amigos que conocí en Reading (Jorge A., Ricardo, Julián, Rubén, Danilo, Jorge, Rubén, Esmeralda, Diana), hicieron que este tiempo en UK sea más ameno y divertido. ¡Esos días de Purple turtle, fútbol y tours de pubs fueron muy divertidos!

Ana y Sandra, gracias por ser tan abiertas cuando les propuse la idea de estudiar los parásitos de la vida silvestre como un proyecto paralelo de este doctorado. Ahora, eso es una realidad y hemos hecho un trabajo increíble, que seguramente dará resultados muy interesantes en el futuro.

Pablo Jarrín, gracias por ser un gran apoyo durante el trabajo de campo. Tu ayuda facilitando el uso de laboratorios en la Universidad IKIAM fue muy valiosa para el trabajo de disecciones.

Los momentos de alegría con mis primos, tías, tíos y amigos fueron muy importantes para hacer de este un viaje más placentero.

Impact statement

Three of the four chapters of this thesis are already published:

Medrano-Vizcaíno, P., Grilo, C., Silva Pinto, F.A., Carvalho, W.D., Melinski, R.D.,Schultz, E.D. & González-Suárez, M. (2022) Roadkill patterns in Latin American birds and mammals. *Global Ecology and Biogeography,* 31, 1756–1783.

Medrano-Vizcaíno, P., Grilo, & González-Suárez, M. (2023) Research and conservation priorities to protect wildlife from collisions with vehicles. *Biological Conservation,* 280, 109952.

Medrano-Vizcaíno, P., Brito-Zapata, D., Rueda, A., García-Carrasco, J. M., Medina, D., Aguilar, J., Acosta, N. & Gonzalez-Suarez, M. (2023) First national assessment of wildlife mortality in Ecuador: an effort from citizens and academia to collect roadkill data at country scale. *Ecology and Evolution,* 13, e9916.

One chapter is in review:

Medrano-Vizcaíno, P., Grilo, C., Brito-Zapata, D. & González-Suárez, M. (In review) The road of> 1000 corpses: landscape and road-related features that promote mortality in the Amazon. *Biodiversity and Conservation*.

Part of the data collected during fieldwork was used to write three additional manuscripts: one scientific paper published, one popular science article published, and one scientific manuscript submitted:

Medrano-Vizcaíno, P. & Brito-Zapata, D. (2021) Filling biogeographical gaps through wildlife roadkills: New distribution records for six snake species from Ecuador (*Anilius scytale, Drymarchon corais, Erythrolamprus breviceps, Micrurus lemniscatus, Oxyrhopus vanidicus, Trilepida anthracina*). *Neotropical Biodiversity,* 7(1), 554-559.

Medrano-Vizcaíno, P. (2022) Mortalidad de fauna silvestre en las carreteras de Ecuador: una realidad desapercibida que requiere atención. *Catálisis Revista Digital,* 4(8), 22-25.

Coba, M., **Medrano-Vizcaíno, P**., Enríquez, S., Brito-Zapata, D., Martin-Solano, S., Ocaña, S., Carrillo, G., González-Suárez, M. & Poveda, A. (Submitted) Roadkill animals can be a valuable source of genetic data to monitor diversity without additional impact on wildlife.

PLOS One.

Additional research published during this PhD (September 2019-December 2022):

Link, A., Alvarez-Solas, S., Blake, J., Campos, F., Espinosa, S., **Medrano-Vizcaino, P**., ... & Valenzuela, L. (2022) Insights into the habits of the elusive nocturnal curassow (*Nothocrax urumutum*). *Ornitología Neotropical*, 33(1), 74-78.

Tipantiza-Tuguminago L. & **Medrano-Vizcaíno P.** (2021) *Bothrocophias campbelli* (Campbell's Toad-headed Pitviper). Male combat. *Herpetological Review*. 52 (3): 658–659.

Tipantiza-Tuguminago L., Villareal-Mejía D. & **Medrano-Vizcaíno P.** (2021) *Riama unicolor* (Unicolored Lightbulb-lizard). Limb malformation and ectrodactyly. *Herpetological Review*. 52 (3): 653.

Medrano-Vizcaíno P. & Espinosa, S. (2021) Geography of roadkills within the Tropical Andes Biodiversity Hotspot: Poorly known vertebrates are part of the toll. *Biotropica* 53 (3): 820–830.

Tipantiza-Tuguminago L. & **Medrano-Vizcaíno P.** (2021) *Dendropsophus rhodopeplus* (Red-skirted Treefrog) Cloacal Prolapse. *Herpetological Review*. 52 (2): 369.

Tipantiza-Tuguminago L, Auqui E. & **Medrano-Vizcaíno P.** (2020) *Rhinella marina* (Cane toad). Morphological abnormalities. *Herpetological Review*. 51 (3): 570

Medrano-Vizcaíno P. & Gutiérrez P. (2020) Current and future suitable habitat areas for *Nasuella olivacea* (Gray, 1865) in Colombia and Ecuador and analysis of its distribution across different land uses. *Biodiversity Data Journal*. 8: e49164.

Medrano-Vizcaíno P., Bedoya J. & Ortiz-Cadena H. (2020) Dinámica de la distribución y hospederos de *Molothrus bonariensis* (Passeriformes: Icteridae) en Ecuador. *Caldasia*. 42 (1): 38–49.

Tipantiza-Tuguminago L. & **Medrano-Vizcaíno P**. (2020) *Pristimantis kichwarum*. Predation. *Herpetological Review*. 51 (1): 100.

Tipantiza-Tuguminago L., **Medrano-Vizcaíno P**., Arguello P. & Auqui E. (2019) *Erythrolamprus miliaris crysostomus* (Military ground snake). Diet. Herpetological Review. 50 (4): 800.

Tipantiza-Tuguminago L., Brito-Zapata D. & **Medrano-Vizcaíno P**. (2019) *Anolis fitchi*

(Fitch's Anole). Tail bifurcation. *Herpetological Review* 50 (4): 781–782.

<u>Additional research and a book chapter in review:</u>

Rahnal, N., Pinto, F., **Medrano-Vizcaíno, P**., Francisco, C., and Bruno, S. (In review) Assessing Brazilian reptiles’ road-kill risks using trait-based models. *Austral Ecology*.

González-Suárez, M., **Medrano-Vizcaíno, P**. & Grilo, C. (In review) Species traits in road ecology: defining intrinsic vulnerability. In: Road Ecology: Synthesis and Perspectives. (Eds: D'Amico M., Barrientos R., Ascensão F.). Springer International Publishing AG, Cham (Switzerland).

Authors contributions

For published and submitted manuscripts, author contributions are detailed below.

Chapter 2 - *Roadkill patterns in Latin American birds and mammals*

PMMV depurated a previously compiled database, compiled new data, performed analyses, and wrote the manuscript. CG revised and edited the manuscript. FASP, WDC, RDM and EDS collected a previous database, and MGS led the supervision of the research, guided model design, and revised and edited the manuscript.

Chapter 3 - *Research and conservation priorities to protect wildlife from collisions with vehicles*

PMMV collected and analysed the data, performed analyses, and wrote the manuscript. CG revised and edited the the manuscript, and MGS led the supervision of the research, guided model design, and revised and edited the manuscript.

Chapter 4 - *The road of> 1000 corpses: landscape and road-related features that promote mortality in the Amazon.*

PMMV collected and analysed the data, performed analyses, and wrote the manuscript. DBZ collected data. CG revised and edited the manuscript, and MGS led the supervision of the research, revised and edited the manuscript.

Chapter 5 - *First national assessment of wildlife mortality in Ecuador: an effort from citizens and academia to collect roadkill data at country scale.*

PMMV collected and curated data, performed analyses, and wrote the manuscript. DBZ collected and processed data, AR processed and curated data. JMGC provided data, DM, JA, and NA provided data. MGS led the supervision of the research, revised and edited the manuscript.

“Our loyalties are to the species and the planet, we speak for Earth. Our obligation to survive and flourish is owed not just to ourselves but also to that Cosmos ancient and vast from which we spring”.

Carl Sagan (1934-1996)

Abstract

Social and economic development are often accompanied by expansion in infrastructure including roads. The presence of roads is prevalent globally with general plans for expansion particularly in megadiverse areas such as Latin America. Given the growing road network and the extraordinary biodiversity in this region, many species could be impacted by mortality due to vehicle collisions. Nevertheless, deficient road ecology research has resulted in limited knowledge of these impacts, which also hinders the development of conservation and mitigation strategies. For this thesis, I bring together different approaches at different spatial scales to evaluate roadkill impacts on Latin American and Caribbean wildlife. In the first chapter of this thesis, I present the close relation that humans have had with roads since ancient times, and how the increasing mortality of species on roads has called the attention of researchers since early 1920's until current times, leading to the development of sophisticated methods for data analysis and new mitigation measures. For the second chapter, I developed predictive traits-based models for Latin American and Caribbean birds and mammals, which revealed a higher mortality for larger birds and medium-sized mammals with early maturity ages, more clutches/litters per year, higher population densities, and diet and habitat generalist habits. Spatial predictions revealed that roads across Central America, northern Andean regions, eastern Brazil, Uruguay, central-eastern Argentina, and southern Chile harbor higher mortality. For the third chapter, using predicted roadkill rates for birds and mammals from chapter 2, information on road network, species conservation status, and availability of road ecology studies, I identified priority areas and species for research and conservation in road ecology in Latin America and the Caribbean. For the fourth chapter, I analysed the spatial distribution of 1,125 roadkill records (that I collected across 240 km of roads in the Ecuadorian Amazon) in relation to landscape and road-related features. Results showed that wildlife mortality is more likely in straight sections of roads near rivers, and that for birds and mammals, proximity to forests and herbaceous-shrubby vegetation can also increase mortality. I also identified taxa-specific roadkill hotspots across the study area. For the fifth chapter, I conducted an assessment of wildlife road mortality at country level in Ecuador presenting a citizen science project I founded. For the assessment I compiled 5,010 roadkill records from systematic and non-systematic surveys, and identified the species and areas for which, further research and conservation actions are necessary. The final chapter presents a general discussion. Overall, this thesis constitutes a comprehensive evaluation of the impacts of roadkill on wildlife in Latin America, which can guide research and

conservation actions for species and areas. As the applied approaches are widely replicable, this document can be also conceived as a reference for future works in other regions of the world.

Table of Contents

Chapter 1. Introduction

1.1 Roads across human history

Roads are involved in almost all our daily activities such as commerce, education, health care, and tourism, which are essential aspects for the social and economic development of nations (Van Der Ree *et al.*, 2015b). These infrastructures have been important components of human society since ancient times connecting small settlements and big cities across the globe. The oldest road in the world dates from the neolithic period, about 6,000 years ago in Shapwick Heath-England and connected two islands through a wooden walkway of 1,800 m., where people presumably transported wood, tools, hunted animals and plants (Coles, 1989). There is evidence of other ancient roads constructed in China, India, Persia, and Rome, which together with the popularization of wheeled vehicles facilitated human activities such as hunting, and military and religious activities (Jacobson, 1940; Avilova & Gey, 2018). The benefits obtained by humans through time have led to a continuous and significant expansion of the road network in the planet (which currently exceeds 64 million of km) (Van Der Ree *et al.*, 2015b); however, this has generally occurred without an adequate assessment of the impacts on the environment and wildlife.

When roads are constructed, these become permanent components of the landscape. For example, the oldest known stone road was constructed with limestone, sandstone, petrified wood, and mafic sill debris about 5,000 years ago in Egypt. Originally it had a length of 11 km and a width of 2 m, and parts of it still remain today (Khalaf & El-Kheir, 2022). The decision of what types of road are built and where, carries social and environmental consequences, which can be long-lasting (Van Der Ree *et al.*, 2015b). Additionally, the construction of roads is usually accompanied by increased human development activities that can act as catalysts for land-use change and ecosystem degradation causing significant negative effects on biodiversity (Young, 1994; Curatola Fernández *et al.*, 2015). When roads are constructed, the colonization of land by humans near these infrastructures increases over time, leading also to greater pollution (chemical, light, and noise), spread of invasive species, and overexploitation of resources through the intensification of hunting, agricultural practices, and logging, which can be dramatically high (95% of all deforestation can occur near roads) (Berthinussen & Altringham, 2012; Barber *et al.*, 2014; Laurance *et al.*, 2014; Kleinschroth & Healey, 2017; Lemke *et al.*, 2019; Paterson *et al.*, 2019).

1.2 A history of roadkill

All issues previously mentioned are inherent aspects of the presence of roads; however, one of the most visible effects of roads is wildlife mortality by vehicle collision (roadkill), which seems to have been occurring since early times. A Greek marble funerary stele from the 2nd-3rd century showing a pig crushed by the wheels of a chariot (Wypustek, 2021) (Figure 1.1) is possibly the oldest known roadkill record. No other similar documented reports can be found from early centuries, maybe because these events were infrequent due to the small extension of road infrastructure, and low vehicular traffic. Therefore, roadkill could have been a minor problem in the past, but the exponential growth of the road network and associated vehicular traffic since the twentieth century has increased wildlife mortality on roads (Kroll, 2015).

Early documented roadkill records appeared in 1920, when zoologist Joseph Grinnell recorded roadkills of jack rabbits, cottontails, kangaroo rats, squirrels, skunks, dogs, cats, meadowlarks, bullock orioles, and mockingbirds while travelling along the California state highway in 1920 (Kroll, 2015). Later, in 1925, Dayton Stoner, a North American naturalist published the first road ecology scientific paper in the journal *Science* reporting 225 roadkilled individuals from 29 species during a four-days (482.8 km) trip from Iowa to West Lake Okoboji in the United States (Stoner, 1925).

Figure 1.1 Funerary stele representing a roadkilled pig during the 2nd-3rd century. Modified from Csapo (2013), this stele includes an epitaph that translated into English is read as: “Here I lie: a pig, everyone’s friend, a young quadruped. I abandoned the soil of Dalmatia after being offered as a gift. I walked through Dyrrachium and, longing for Apollonia, I crossed the whole land on foot, alone, unharmed. But by the force of a wheel I have now left the light, yearning to see Emathia and the chariot of the Phallic procession. Now I lie here, owing nothing to death anymore”.

Subsequently, Stoner published another roadkill paper in 1935, and some other studies were conducted in the early 20th century (Kroll, 2015). Beyond the United States, Beadnell (1937) estimated that ten thousand animals were being roadkilled daily on roads of Great Britain. While Pickles (1942) reported 687 roadkilled individuals from 42 species on a road of 4.82 km that was surveyed during the whole year of 1938 in the United Kingdom. These types of reports in the United Kingdom and the United States were key to promote some of the first actions focused on the reduction of mortality. During the 60’s and 70’s, the very high numbers of deer killed on roads of the United States, resulting also in financial costs of vehicles reparation and threat to driver and passengers’ lives, were noted by wildlife biologists and road engineers that developed strategies to mitigate this impact, such as the installation of fences during 1976-1979, which indeed reduced the mortality of deer in California-United States (Ford, 1980; Kroll, 2015).

1.3 Road ecology: a new scientific field has emerged

In 1981, the German ecologist Heinz Ellenberg and his collaborators used for the first time the term road ecology (in German: Straßenökologie) (see Ellenberg *et al.*, 1981) to refer to the effects of roads on the environment, vegetation, wildlife and the fragmentation of landscapes. However, it was only in 2003, that this term was widely popularized across the world by the landscape ecologist Richard Forman in his book entitled “Road ecology: Science and solutions” (Forman *et al.*, 2003). In this book, road ecology is presented as a field where the integration of landscape ecology, and road and automotive engineering are necessary for the design of road systems. This book sparked an interest in this topic that is reflected on the growing number of scientific publications on road ecology during the last two decades (see Coffin *et al.*, 2021) (Figure 1.2).

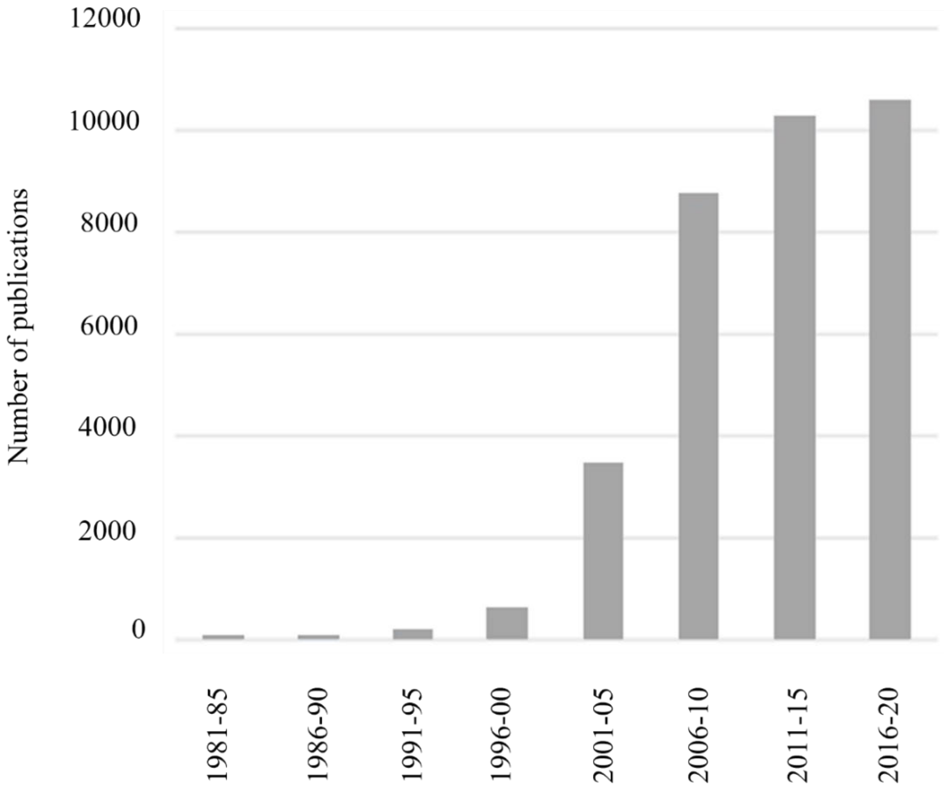


Figure 1.2 The increasing number of road ecology publications in English over the last two decades. Modified from Coffin *et al.* (2021).

1.3.1 *Road ecology: a further and wider step*

The development of ecological and spatial analyses has allowed scientists to improve the quality and the scope of road ecology studies that were initially largely counts of dead animals, species and locations (Forman *et al.*, 2003; Coffin *et al.*, 2021). Recent work has contributed to improve our understanding on how this mortality influences population and species dynamics and has revealed sublethal impacts. For example, proximity to roads has led to changes in the vocal sexual displays of some birds to cope with road traffic noise (Barrero *et al.*, 2021). Small mammals can alter their movement patterns to avoid crossing roads (Ji *et al.*, 2017). Some reptiles can also limit their movements to avoid roads, but others have been found to decrease their home range instead (Hibbitts *et al.*, 2017; Peaden *et al.*, 2017; Paterson *et al.*, 2019). This avoidance behaviour can prevent road mortality, but it is not innocuous, as roads can become barriers leading to fragmentation, isolation of populations, and loss of genetic diversity (Clark *et al.*, 2010a; Jackson & Fahrig, 2011). Roads can even lead to evolutionary changes. Brown & Bomberger Brown (2013) studied cliff swallows (*Petrochelidon pyrrhonota*) for 30 years in Southwestern Nebraska-USA and found that roadkills decreased over time while population size increased. Also noting a change in wing length overtime, they proposed that the selection pressure caused by collisions had favored individuals with longer wings, which are better able to escape, and thus overtime road mortality was reduced, and the population increased.

1.3.2 *Roads and wildlife mortality*

Wildlife-vehicle collisions are one of the main anthropogenic causes of mortality for several vertebrates in the planet (Hill *et al.*, 2019; Taylor-Brown *et al.*, 2019). Annually, millions of animals are killed on roads across Europe (Grilo *et al.*, 2020), America (Loss *et al.*, 2014; González-Suárez *et al.*, 2018), and Australia (Englefield *et al.*, 2018), and major impacts have been also reported in Asia and Africa (Seo *et al.*, 2013; Périquet *et al.*, 2018; Lala *et al.*, 2021; Wang *et al.*, 2022). Therefore, understanding which species and areas are most susceptible to road impacts, especially to the main threat of direct mortality (roadkill), is key to ensure sustainable development, particularly in tropical areas where biodiversity levels are high and road network expansion is occurring rapidly (Myers *et al.*, 2000; Laurance *et al.*, 2014).

1.4 Latin America: road impacts and lack of research

Latin America englobes areas like the Tropical Andes, Mesoamerica, Caribbean, Brazil's Cerrado, the Choco Darien Western Ecuador and Central Chile, all of them biodiversity hotspots with high levels of endemism and that contain some of the last pristine forests in the planet (Myers *et al.*, 2000; Meijer *et al.*, 2018). Across these areas some of the major road developments in the world are planned by 2050 (Meijer *et al.*, 2018). Only in the Amazon (which harbors 10-15 % of global biodiversity) more than 10,000 kilometers of official roads will be opened or improved in the next five years (Nobre *et al.*, 2016; Vilela *et al.*, 2020). This can be alarming, as these future roads might lead to the building of additional secondary, tertiary and illegal roads, which can triple the length of official roads (Meijer *et al.*, 2018; Vilela *et al.*, 2020) and lead to a great mortality of wildlife. It has been already reported that roads can threaten the viability of populations of the giant anteater (*Myrmecophaga tridactyla*), jaguar (*Panthera onca*), maned wolf (*Chrysocyon brachyurus*), and northern tiger cat (*Leopardus tigrinus*) in Brazil (Diniz & Brito, 2015; Cullen *et al.*, 2016; Grilo *et al.*, 2021), and lava lizards (*Microlophus albemarlensis*) in the Galapagos Islands of Ecuador (Tanner & Perry, 2007). Still, road ecology research is scarce or inexistent for many areas across this region. A compilation by Pinto *et al.*, (2020) identified 68 road mortality studies representing only seven out of the 33 countries in Latin America and the Caribbean. Additionally, 69% of the existing studies have been conducted in Brazil, revealing large gaps of information in the rest of Latin America. Wider research efforts are necessary to fill the information gaps and to aid in the identification of the areas and organisms most affected by road mortality.

1.5 What influences road mortality

Road ecology studies commonly identify the most roadkilled species and the areas with higher concentrations of mortality (e.g., González-Gallina *et al.*, 2013; Bravo-Naranjo *et al.*, 2019). Several of these species show a high mortality across different study areas. Likewise, certain characteristics of roads and landscapes seem to promote an increased mortality (Clevenger *et al.*, 2003; Cuyckens *et al.*, 2016; Bastos *et al.*, 2019). These mortality patterns have not been explored at large scales; however, a few studies have identified particular characteristics of species, landscapes and roads that have led to a higher mortality in specific study areas.

1.5.1 Road mortality and species traits

Traits have been associated with road mortality by a few studies in Latin America, mostly conducted at local scales. In Mato Grosso do Sul-Brazil nocturnal mammals with low locomotion showed a higher mortality (Caceres, 2011). In Sao Paulo State-Brazil, mortality was increased for generalist mammals with large home ranges (Ciocheti *et al.*, 2017). In the state of Rondônia-Brazil, habitat and diet generalist mammals showed a higher mortality (Caires *et al.*, 2019). A similar result was found for omnivorous non-volant mammals in the state of Goiás-Brazil (de Araújo *et al.*, 2019), and for habitat generalist birds in Azuay-Ecuador (Aguilar *et al.*, 2019). In addition, there has been a larger scale study focused in Brazil (González-Suárez *et al.*, 2018) which showed generalized traits associated with increased roadkill rates, particularly they observed higher rates among nocturnal species with larger and intermediate body sizes, and earlier maturity ages. Similarly, mammals with small home ranges, and short life-spans, and birds with wider habitat breadths showed a higher mortality. While these studies show these traits are associated to road mortality, it is unclear whether they directly influence mortality or instead drive local abundance which can in turn increase roadkill numbers (Caceres, 2011; Leonan *et al.*, 2018; Silva *et al.*, 2019).

Not surprisingly, common species are often found as roadkill. Opossums *Didelphis spp*. are abundant marsupials in Latin America (Boullosa *et al.*, 2021) that have been reported as the most roadkilled animals across different areas: Misiones-Argentina, Rio Grande do Sul-Brazil, Espírito Santo state-Brazil, Rio de Janeiro-Brazil, Napo-Ecuador, Middle Magdalena Valley-Colombia, and Antiquia-Colombia (Delgado-V, 2007; Coelho *et al.*, 2008; Bueno *et al.*, 2015; Bauni *et al.*, 2017; Meza-Joya *et al.*, 2019; Ferreguetti *et al.*, 2020; Medrano-Vizcaíno & Espinosa, 2021). Similarly, the cane toad *Rhinella marina*, an abundant species with an expanding distribution range (Solís *et al.*, 2009) was the most roadkilled species in a study conducted in a road between *Bolivar* and Sucre localities in Colombia, and in Departamento del Cauca-Colombia (Castillo-R *et al.*, 2015; Monroy *et al.*, 2015). A similar case for the crab-eating fox (*Cerdocyon thous*), a common canid (Lucherini, 2015), that was the most roadkilled species in Mato Grosso do Sul-Brazil, Santa Catarina-Brasil, Município de Quirinópolis-Brazil, Paraná state-Brazil, and Sucre-Colombia (Cherem *et al.*, 2007; Costa & Dias, 2013; Belao *et al.*, 2014; De La Ossa & Galván-Guevara, 2015; Ascensão *et al.*, 2017).

While locally abundant animals are frequent victims of collisions, rare organisms can also be highly affected by road mortality. Caecilians, poorly known vertebrates due to their fossorial habits (Wilkinson, 2012), have been commonly found as roadkilled in Ecuador and Mexico (Medrano-Vizcaíno & Espinosa, 2021; Salazar-Sánchez, 2022). *Atractus spp*. snakes, another group of hardly known vertebrates (Cisneros-Heredia, 2005), have shown high mortality in two studies in Ecuador (Filius *et al.*, 2020; Medrano-Vizcaíno & Espinosa, 2021). Even threatened species can be affected. For the Florida panther (*Felis concolor coryi*), the last surviving puma subspecies in the east of the United States, the main cause of mortality (79%) was collision with vehicles (FWC, 2016). Road mortality accounted for 16.7% of the Red List Endangered Iberian lynx (*Lynx pardinus*) in Parque Nacional de Doñana, Spain (Ferreras *et al.*, 1992). While in France, 12.5% of the mortality of the Critically Endangered European minks *Mustela lutreola* was caused by vehicle collisions (Lodé *et al.*, 2001). A high mortality for species of conservation concern can have critical effects for their populations, as even low roadkill rates can increase their risk of extinction (Grilo *et al.*, 2021). Therefore, researching further aspects linked to a higher mortality is necessary to identify species for which road mortality could threaten their viability, and also the areas where this mortality is higher.

1.5.2 Road mortality and landscape and road-related features

The composition and configuration of landscapes influence animal movement, as individuals visit different areas to fulfill their needs to feed, find shelter and mates, and avoid predators (Avgar *et al.*, 2015). When roads occur in areas with resources or intersect the corridors linking these resources, mortality can be higher. For example, the mortality of amphibians has found to be related to the presence of water resources (Carvalho & Mira, 2011; Coelho *et al.*, 2012; Bastos *et al.*, 2019), reptile mortality to pasturelands (Quintero-Ángel *et al.*, 2012; Medrano-Vizcaíno & Espinosa, 2021), bird mortality to pastures (Braz & Rodrigues, 2016), and mammalian mortality to agricultural lands (Cuyckens *et al.*, 2016). These effects may vary across areas due to differences in the animal communities and landscape composition and configuration (Ciocheti *et al.*, 2017; Medrano-Vizcaíno & Espinosa, 2021). For example. in Sao Paulo, Brazil, roadkill of the maned wolf (*Chrysocyon brachyurus*) was more likely in urban areas, while crab-eating foxes (*Cerdocyon thous*) were more likely to die on roads near forests (Freitas *et al.*, 2015). Likewise, in the states of Rio de Janeiro and Minas Gerais-Brazil, jaguarundi (*Herpailurus yagouaroundi*) mortality was higher across areas with more herbaceous vegetation and less crops, while for the ocelot (*Leopardus pardalis*), mortality increased with more coverage of forest and crops (Costa *et al.*, 2022). Moreover, the density and configuration of roads can module the magnitude of impacts on biodiversity (Van Der Ree *et al.*, 2015a). In a study conducted in Sao Paulo-Brazil, road widening led to a higher mortality for the European hare *Lepus europaeus* and armadillos *Dasypus sp.*, but to a lower mortality for the black-horned capuchin *Sapajus nigritus* (Ciocheti *et al.*, 2017). In Minas Geraes and Sao Paulo-Brazil, it was found that a straight road design, together with native vegetation across roads, increased the mortality of the giant anteater (*Myrmecophaga tridactyla*) by 40.1% (Freitas *et al.*, 2014).

1.6 Research is the base for conservation actions

Conducting integral approaches that allow the identification of species and areas susceptible to road mortality can be useful to inform on potential measures to reduce mortality (Clevenger & Kociolek, 2013; Girardet *et al.*, 2015; Raymond *et al.*, 2021). For example, in Florida-United States, after installing drift fences, turtle mortality decreased from 11.9 to 0.09 ind./km/day (Aresco, 2005). In Ontario-Canada, the mortality of the massasauga rattlesnake (*Sistrurus catenatus*) was reduced by applying mitigation systems that also provided movement corridors through road transected habitats (Colley *et al.*, 2017). Likewise, the installation of small road tunnels and fences in Sweden increased successful crossings of amphibians by 25-340% and decreased their mortality by 85-100% (Helldin & Petrovan, 2019). While in Tasmania, installing virtual fences (devices that emit sound and light to scare away animals when a vehicle is approaching) reduced mortality in general by 50% (Fox *et al.*, 2019).

In Latin America, scarce research on road ecology has limited the application of effective mitigation systems (Pinto *et al.*, 2020). For example, static warning signs, known to be ineffective, are widespread as mitigation measures in this region (Payán *et al.*, 2013; Huijser *et al.*, 2015), likewise, systems such as underpasses have failed to reduce wildlife roadkill in Brazil (see Bager & Fontoura, 2013; Ciocheti *et al.*, 2017). In order to plan cost-effective management systems that allow the reduction of wildlife mortality and the maintenance of populations connectivity, more information is needed, and this can be obtained via traditional research studies but also with the participation of the public in citizen science projects.

1.7 Citizen science and roadkill

Citizen science projects can contribute vital information on biodiversity and impacts while also raising awareness of nature and environmental problems, which can promote support for conservation actions (Bíl *et al.*, 2020). One of the first efforts to quantify road mortality through citizen science took place in the United Kingdom during 1960-1961, when ornithologists and members of the general public were provided with questionnaires and forms to participate in a national survey that reported 5,269 roadkill records from 88 birds species (Hodson & Snow, 1965). Nowadays the use of electronic devices has facilitated the compilation of roadkill data through citizen science initiatives (Vercayie & Herremans, 2015), such as The Road Lab project (previously known as Project Splatter) in the United Kingdom (https://www.theroadlab.co.uk/), TaiRON Project in Taiwan (https://roadkill.tw/en/app/report), CROS system in the United States (https://www.wildlifecrossing.net/california/), and project Roadkill in Austria (https://roadkill.at/en/). The maintenance of these projects over time has provided useful data to quantify the effects of roads at large spatial scales. For example, citizen science efforts have contributed to a better understanding of road impacts in Taiwan, the United Kingdom, and South Africa (Périquet *et al.*, 2018; Chyn *et al.*, 2019; Raymond *et al.*, 2021) and provided key information to inform mitigation plans (Yue *et al.*, 2019).

In Latin America, citizen science initiatives to collect roadkill data have recently started with projects in Colombia (http://www.recosfa.com/), and Brazil (https://sistemaurubu.com.br/dados/) that have helped identified movement corridors for Brazilian felids (Cerqueira *et al.*, 2021), and wildlife roadkill hotspots in Colombia (González-Vélez *et al.*, 2021). Additional citizen science initiatives in other Latin American countries can offer a way to increase awareness of the impact of roads on wildlife and gather higher quantities of data at a large spatial and temporal scale that can be key to mitigate road mortality, and also to increase the knowledge of wildlife species.

1.8 Overall purposes and thesis outline

Roads impact wildlife worldwide, but particularly, biodiverse areas such as Latin America, where road network is expanding rapidly (Meijer *et al.*, 2018) can see important losses of animal populations. Road ecology research is scarce in this region and studying the main drivers of wildlife mortality can provide vital information to identify mortality patterns. In this thesis I analysed how biological traits, habitat preferences, and landscape and road-related features can be used to identify the areas and species that currently harbor higher mortality, but also that could be highly impacted with the construction of new roads. I also identify spatial and taxa information gaps in Latin America that need special research efforts to assess the impacts of roads. Moreover, I present how joint efforts from researchers, citizens and government can be highly beneficial to consolidate big databases that allow large scale assessments, potentially useful for the development of conservation plans. I address these topics in four research chapters, as following:

Chapter 2: Roadkill patterns in Latin American birds and mammals

For this chapter, I compiled roadkill data of 346 birds species and 159 mammals species from 85 studies in Latin America and applied random forest models to predict mortality rates considering species traits, habitat preferences and the geographical coordinates of each study where data was collected (to control for local and regional variation). These models besides providing information about the main species traits and habitat preferences that drive wildlife mortality on roads, were also useful to generate spatial predictions of mortality across Latin American roads.

Chapter 3: Research and conservation priorities to protect wildlife from collisions with vehicles

For this chapter I used predicted mortality rates for birds and mammals (from chapter 2), road density, species conservation status, and availability of road ecology studies to define priority areas and taxa for research and conservation in Latin America. Priority areas for research were unstudied areas with high road density and where species have high predicted road mortality. Priority areas for conservation were those with a high predicted mortality but currently low road density (i.e., areas where future road developments could lead to a great wildlife mortality). Additionally, based on mortality rates, availability of studies and conservation status, I identified some taxa for which, research or conservation efforts should be addressed. This chapter presents a widely applicable framework that could help addressing efforts for conservation and research in road ecology for other areas and organisms.

Chapter 4: The road of >1000 corpses: landscape and road-related features that promote mortality in the Amazon

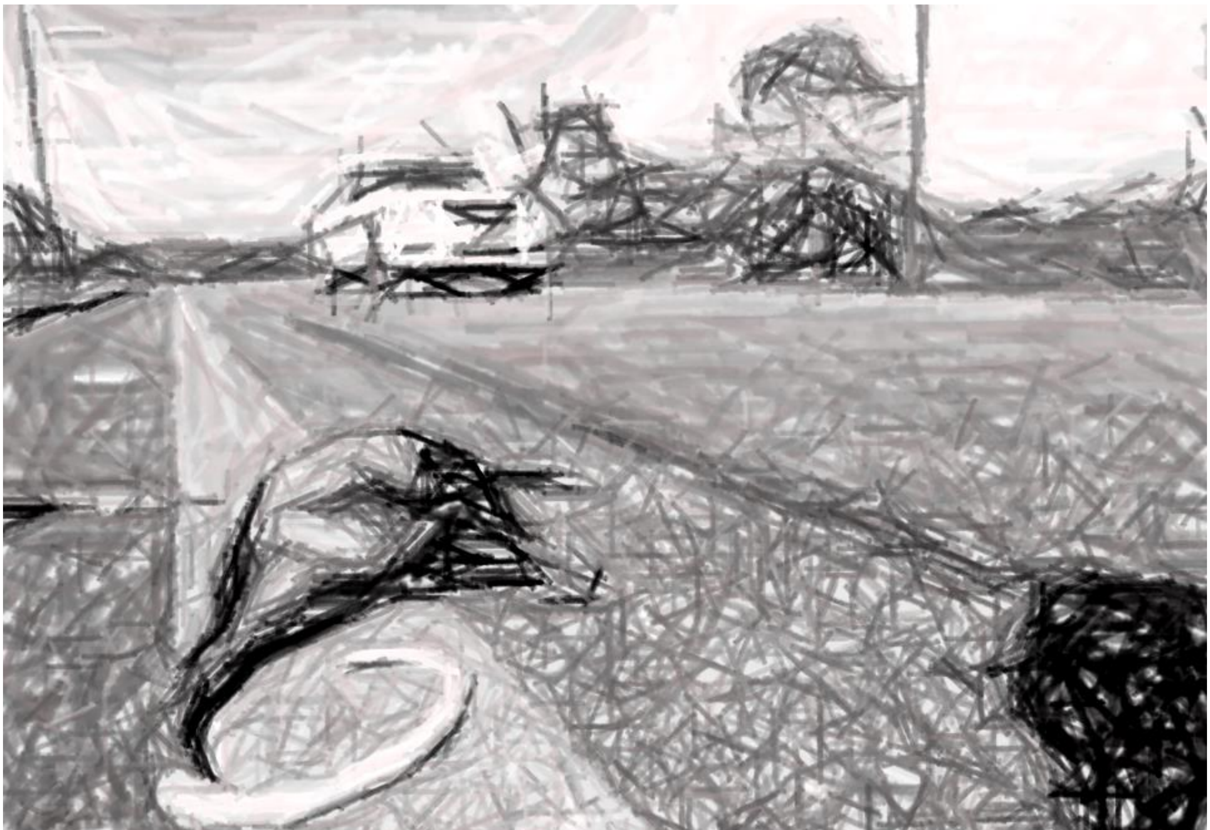
For this chapter, from September 2020 to March 2021, I conducted fieldwork looking for carcasses of wild vertebrates across 240 km of roads of the Ecuadorian Amazon (Napo Province). I georeferenced each roadkill record and collected some specimens for further analyses and further taxonomic identification. Later, using Random Forest models, I studied how different land cover types and road sinuosity can drive different mortality rates across taxa. I also identified roadkill hotspots, which are areas where further research and mitigation could be necessary to reduce road mortality.

Chapter 5: First national assessment of wildlife mortality in Ecuador: an effort from citizens and academia to collect roadkill data at country scale.

For this chapter, I collected roadkill data from published studies with systematic roadkill surveys in Ecuador, and also from non-systematic surveys such as the citizen science project that I founded and lead in Ecuador: Red Ecuatoriana para el Monitoreo de Fauna Atropellada (hereafter REMFA) (https://remfa.webnode.co.uk/), also from iNaturalist (https://www.inaturalist.org/), and sporadic records from scientific literature not gathered via systematic surveys. With these data I was able to identify species and areas affected by road mortality in Ecuador, and also knowledge gaps in road ecology within this country. Joining efforts from citizens, researchers and government, I provide a database of more than 5,000 roadkill records (4244 georeferenced), which hopefully can be a reference for conservation actions and the sustainable planning of road developments in this country.

Finally, in **Chapter 6** I discuss the combined relevance of these findings, and how taking these topics from a holistic perspective can be useful for planning research-based strategies that reduce wildlife mortality on roads, and that can guide sustainable roads not only in Latin America, but also in other regions and for other taxa.

Chapter 2. Roadkill patterns in Latin American birds and mammals



2.1 Abstract

Roads are a major threat for wildlife, degrading habitat and causing mortality via wildlife-vehicle collisions. In Latin America, the conjunction of high biodiversity and a rapidly expanding road network is reason for concern. We introduce an approach that combines species traits and habitat preferences to describe vulnerability and map high roadkill risk areas. Thus, we present the first assessment of roadkill impacts for Latin American birds and mammals. We compiled data from 85 roadkill surveys from Latin America that provided 1,691 roadkill rate estimates for 346 bird and 159 mammalian species, from which, 520 rates from 249 birds and 457 rates from 103 mammals were used for analyses. We applied Random Forest models to predict roadkill rates considering species' traits, habitat preferences, and the geographic coordinates of each study to control for local and regional variation. Fitted models were used to predict spatial risks in Latin American roads for roadkilled birds and mammals across their areas of habitat. We found higher roadkill rates in larger birds and medium-sized mammals with faster reproduction (more clutches/litters per year and early maturity ages), higher population densities, and wider use of habitats that included anthropized areas. In mammals, scavengers and those with diets based on invertebrates showed higher rates. Spatial predictions revealed higher rates in roads across Central America, northern Andean regions, eastern Brazil, Uruguay, central-eastern Argentina, and southern Chile. This first comprehensive assessment for Latin America explores various drivers of roadkill risk for birds and mammals and identifies species and areas where existing roads can impact wildlife. Trait-based models fine-tuned with realistic spatial information that accounts for habitat suitability provide a valuable tool for the assessment of human impacts including roads and traffic.

Keywords: Body mass, Diet, Neotropics, Maturity age, Longevity, Random Forest, Road ecology, Central and South America, Traits, Vertebrates.

2.2 Introduction

Transportation is key for many human activities including commerce, tourism and education, which are essential for social and economic development (Forman *et al.*, 2003). Unfortunately, this dependence on roads and vehicles can cause environmental destruction (Van Der Ree *et al.*, 2015b). Wildlife-vehicle collisions (hereafter roadkill) were found to be the second highest source of anthropogenic mortality for large and medium-sized mammals in North America, only surpassed by hunting (Collins & Kays, 2011). Road infrastructure can disrupt movement (Chen & Koprowski, 2016; Bischof *et al.*, 2017; Cayuela *et al.*, 2019) and prevent gene flow leading to fragmentation, isolation of populations, loss of genetic diversity, and even genetic differentiation of populations (Lesbarrères *et al.*, 2006; Clark *et al.*, 2010b; Jackson & Fahrig, 2011).

Latin America is a highly biodiverse region that includes seven global biodiversity hotspots (The Tropical Andes, Mesoamerica, Caribbean, Brazil’s Atlantic Forest, Choco/Darien/Western Ecuador, Brazil’s Cerrado and Central Chile) with very high concentrations of endemic vertebrates (Myers *et al.*, 2000). This region currently has almost 3.5 million km of roads, and is expected to have the highest rate of new road development in the near future (Meijer *et al.*, 2018). For example, plans include construction and expansion of 12,000 km of roads in the Amazon basin in the next five years (Vilela *et al.*, 2020), which could lead to increased roadkill rates and affect local populations. The combination of high biodiversity and expanding infrastructure makes it urgent to assess how roads affect Latin American wildlife to facilitate sustainable development and effective mitigation.

Different habitat types and configurations have shown to influence the risk of wildlife-vehicle collision. For example, amphibians are frequently roadkilled near forests (Braz & Rodrigues, 2016), birds are more affected in roads bordering pasturelands and fragmented forests (Medrano-Vizcaíno & Espinosa, 2021), some species of mammals, such as *Cerdocyon thous* and *Lepus europaeus*, are more vulnerable near water bodies (Freitas *et al.*, 2015), while mortality of snakes is higher near pasturelands (Quintero-Ángel *et al.*, 2012). Although habitat composition and configuration are important, they do not seem to fully explain wildlife mortality patterns. Species that use similar habitats can have different roadkill rates. For example, the crab-eating fox *Cerdocyon thous* and the white-eared opossum *Didelphis albiventris* are both habitat generalists that tolerate humanized areas (Cantor *et al.*, 2010; De Barros Ferraz *et al.*, 2010), but are affected very differently by roads: in a study conducted in

Misiones-Argentina the white-eared opossum comprised the 38.8% of total roadkills while *C. thous* represented just 3.3% (Bauni *et al.*, 2017). Although variation in local abundance or density can partly explain differences in roadkill rates (Delgado-V, 2007; Caceres, 2011; Medrano-Vizcaíno, 2015), rare species can also suffer high mortality. The western mountain coati (*Nasuella olivacea*), an elusive carnivore whose ecology is scarcely known (Medrano-Vizcaíno & Gutiérrez-Salazar, 2020), was the second most roadkilled species in Envigado-Colombia (Delgado-V, 2007).

Diet, life-history, and morphology have shown to influence roadkill risk in some regions of Latin America (Caceres 2011, González-Suárez et al., 2018). Large body size, ground-foraging behaviour, and a wider diet breadth have been associated with roadkill risk in Brazilian birds (González-Suárez *et al.*, 2018), while mammals at higher risks were those with diurnal habits, intermediate body masses, slow movements, smaller home ranges and a scavenging behaviour (Caceres, 2011; González-Suárez *et al.*, 2018). These types of trait-based predictive models can offer initial assessment tools to identify vulnerable species and areas. A few previous studies have used this approach at local (Leonan et al., 2018; Rincón-Aranguri et al., 2019) and national scales (González-Suárez *et al.*, 2018) within Latin America, but local inferences may not be applicable to all regions and diverse wildlife communities in the Neotropics. Here, we present the first continental-scale assessment of roadkill risk for Latin American birds and mammals.

We compiled a comprehensive database of roadkill rates for over 500 species of birds and mammals across Latin America and the Caribbean and applied machine learning methods to develop predictive models of roadkill vulnerability. Predictors included diet, life-history, and morphological traits that we hypothesized *a priori* could influence roadkill rates (Table 2.1). Going a step beyond previous work we refined spatial predictions using habitat preferences to provide a more realistic assessment of roadkill risk. Our analyses identify generalized traits that make Latin American birds and mammals more vulnerable to roadkill and offer a continental risk assessment that reveals both vulnerable species and areas where road mortality rates may be particularly high.

2.3 Methods

We searched peer-reviewed publications and grey literature (e.g., academic theses and dissertations) to locate systematic roadkill surveys published before July 2020 using the keywords “roadkill”, “wildlife vehicle collision”, “road mortality” “road ecology”, “birds roadkills”, “mammals roadkills” plus the name of each country in Latin America. We also searched for these terms in Spanish and Portuguese to identify additional sources in those languages. From each identified study reporting roadkill data, we collected for each species the number of individual carcasses found as roadkill, descriptors of the survey design (time between surveys, total sampling period from first to last survey, length of road surveyed), and the geographical coordinates of the study area (central point). We used the IUCN nomenclature and synonyms to standardize all species names after correcting identified spelling mistakes. We assumed correct taxonomic identification of roadkill individuals from each study.

Roadkill rates per species and study were calculated as the total number of carcasses found divided by the length of the road surveyed in kilometers and the study duration in days. This daily rate was then converted to number of individuals per km per year (multiplying by 365), assuming a constant roadkill rate across the year. Because detectability and survey intervals can influence the number of detected carcasses roadkill, we adjusted these values with correction factors proposed by Santos et al. (2011), that estimate carcasses persistence timings on roads among taxonomic groups. Then, roadkill rates were multiplied by correction factors based primarily on body size categories and survey intervals (full list of correction factors in Appendices as Table A.1).

We also compiled species-level data for 22 variables that describe diet, life-history, morphological traits and habitat preferences in birds and mammals (Table 2.1). The list of variables was initially the same for both groups, but home range was removed for birds due to low data availability, and seed diet was excluded for mammals due to low variability in the studied species which made this variable uninformative. Trait data were collected from multiple published databases, books and published papers (all trait and roadkill data are available in a public repository: https://doi.org/10.6084/m9.figshare.19236765.v1).

Table 2.1 Variables used as predictors of roadkill rates. We report the number of species with available data and in parentheses the percentage from the total (N_T) of 346 birds and 159 mammals for which roadkill estimates were available and for the subset (N_M) of 249 birds and 103 mammals from more comprehensive studies used to fit the model. Missing trait data were imputed prior to analyses (see methods). We also present *a priori* hypotheses for how each variable could influence roadkill rates. *Variables not included in the analysis

Type of variable	**Variable**	**Description**	**Hypotheses**	**Birds**		**Mammals**	
				N_T	**N_M**	**N_T**	**N_M**
Geographical	Latitude and longitude	Geographical coordinates of the centroid of every study analysed.	Roadkill rates can vary across areas due to local factors including traffic patterns, road and landscape features that could be broadly captured by coordinates.	346(100%)	249(100%)	159(100%)	103 (100%)
Morphological	Body mass	Median adult body mass in kg.	Large sized species are more visible to drivers, which decreases their probability of being hit by vehicles.	346(100%)	249(100%)	159(100%)	103 (100%)
Life-history	Age of maturity	Median age in days when individuals reach sexual maturity.	Species with shorter ages of maturity generally have higher local population abundances, which in turn can result in higher roadkill rates.	89(25.7%)	51(20.5%)	110(69.2%)	75(72.8%)
	Litter/clutch size	Median number of offspring in each reproductive event.	Species with larger litter sizes generally have higher local	330(95.3%)	236(94.8%)	146(91.8%)	93(90.3%)

Type of variable	Variable	Description	Hypotheses	Birds		Mammals	
				N_T	N_M	N_T	N_M
			population abundances, which in turn can result in higher roadkill rates.				
	Litters/clutches per year	Median number of reproductive events per year.	Species that have more litters per year generally have higher local population abundances, which in turn can result in higher roadkill rates.	109(31.5%)	68(27.3%)	111(69.8%)	71(68.9%)
	Longevity	Median lifespan in years.	Long-lived species have more opportunities to acquire experience and learn to cope with novel habitats like roads, which could result in lower roadkill rates.	119(34.3%)	76(30.5%)	133(83.6%)	92(89.3%)
	Group Size	Median size of social groups.	More individuals can be roadkilled in a single collision if animals cross roads in groups. Because group crossings are more likely to occur in social species, these species could have higher roadkill rates.	232(67.0%)	164(65.9%)	85(53.5%)	64(62.1%)

Type of variable	Variable	Description	Hypotheses	Birds		Mammals	
				N_T	N_M	N_T	N_M
	Home range	Median home range in km^2.	Species with larger home ranges are more likely to encounter and cross roads, increasing their probabilities of being roadkilled.	37(10.7%)	_*27 (10.8%)_	99(62.3%)	73(70.9%)
	Activity	Categorical descriptors of the main time of daily activity: Diurnal, Nocturnal.	Driver visibility is reduced at night, so collisions may be more common during that period resulting in higher rates for nocturnal animals.	326(94.2%)	232(93.2%)	159(100%)	103(100%)
	Population density	Median number of individuals per km^2.	Higher population densities (local abundance) can increase roadkill as more individuals may be found near and on roads.	103(29.8%)	61(24.5%)	61(38.3%)	44(42.7%)
Diet	Invertebrate diet (%)	Percentage of diet based on invertebrates.	Invertebrates may be attracted to resources near roads like verges in turn attracting animals with an invertebrate-based diet and increasing their exposure to roadkill.	343(99.1%)	246(98.8%)	157(98.7%)	102(99.0%)
	Ectotherm vertebrates' diet (%)	Percentage of diet based on ectotherm vertebrates.	Ectotherm species can use roads for thermoregulation which can	343(99.1%)	246(98.8%)	157(98.7%)	102(99.0%)

Type of variable	Variable	Description	Hypotheses	Birds		Mammals	
				N_T	N_M	N_T	N_M
			attract their predators and increase their roadkill rates.				
	Scavenger diet (%)	Percentage of diet based on scavenging.	Scavenger species can be attracted to roads to feed on dead animals increasing their roadkill rates.	343(99.1%)	246(98.8%)	157(98.7%)	102(99.0%)
	Seed diet (%)	Percentage of diet based on seeds.	Road verges can provide food sources for seed-eaters and attract them to roads increasing their roadkill rates.	343(99.1%)	246(98.8%)	NA	NA
	Plant diet (%)	Percentage of diet based on plants.	Road verges may provide food sources for herbivores and attract them to roads increasing their roadkill rates.	343(99.1%)	246(98.8%)	157(98.7%)	102(99.0%)
	Diet breadth	Number of different categories consumed (out of 10 possible diet categories).	Species with generalist diet can be attracted to diverse resources like road verge vegetation, prey feeding on verges and carrion, thus increasing their roadkill rates.	343(99.1%)	246(98.8%)	157(98.7%)	102(99.0%)
Habitat	Artificial	Whether the species occur at artificial habitats. 1=Yes, 0=No.	Roads are very common in artificial habitats so species that	346(100%)	249(100%)	158(99.4%)	103(100%)

Type of variable	Variable	Description	Hypotheses	Birds		Mammals	
				N_T	N_M	N_T	N_M
			use this habitat will be more exposed to roads.				
	Cropland	Whether the species occur at cropland habitats. 1=Yes, 0=No.	Roads are relatively common near croplands, and some species may be attracted to the resources in crops, so species that use this habitat will be more exposed to roads.	346(100%)	249(100%)	158(99.4%)	104(100%)
	Grassland	Whether the species occur at grassland habitats. 1=Yes, 0=No.	Roads can be relatively common near grasslands (particularly those in flat areas which are more suitable for infrastructure) so species that use this habitat will be more exposed to roads.	346(100%)	249(100%)	158(99.4%)	104(100%)
	Forest	Whether the species occur at forest habitats. 1=Yes, 0=No.	Species that prefer forests are more likely to be specialists and avoid novel and altered areas, decreasing their roadkill rates.	346(100%)	249(100%)	158(99.4%)	104(100%)
	Sparse vegetation	Whether the species occur at sparse vegetation habitats. 1=Yes, 0=No.	Areas with sparse vegetation can be associated to unproductive regions (with few animals)	346(100%)	249(100%)	158(99.4%)	104(100%)

Type of variable	Variable	Description	Hypotheses	Birds		Mammals	
				N_T	N_M	N_T	N_M
			where roadkill rates will be lower.				
	Water bodies	Whether the species uses aquatic habitats. 1=Yes, 0=No.	Species that use water bodies may be less likely to cross roads (unsuitable habitat) resulting in lower roadkill rates. An alternative hypothesis is that species that use water bodies may need to move if sources dry up and could cross roads while dispersing increasing their roadkill risk.	346(100%)	249(100%)	158(99.4%)	104(100%)
	Habitat breadth	Number of habitat types where a species occurs from 10 possible types.	Habitat generalists can occupy altered areas including roads or places near roads, leading to higher roadkill rates.	346(100%)	249(100%)	158(99.4%)	104(100%)

2.3.1 *Vulnerability to roadkill*

Trait data are often not available for all species but because traits are generally correlated, imputation can be a useful method for handling missing values (Johnson *et al.*, 2021). Prior to predicting roadkill rates, we imputed missing trait values using the default settings of the function “missForest” (maxiter=10, ntree=100) from the R package “missForest” (Stekhoven, 2013). This approach iteratively uses random forests to predict one trait using data from all other traits and has been shown to work well with multivariate data, outperforming other methods such as k-nearest neighbours imputation or multivariate imputation (Stekhoven & Bühlmann, 2012). To account for uncertainty in imputed values we generated and analysed 15 imputed datasets for birds and 15 for mammals. In addition, we fitted models excluding variables for which empirical data were available for <40% of species to ensure results were not affected by heavily imputed variables. Previous studies found imputation of trait data to be reliable with up to 60% of missing values (Penone *et al.*, 2014). Imputation was done using data for all species recorded as roadkill but to fit the model predicting roadkill rates we considered that studies had been conducted using a diversity of sampling methods and covered different survey areas and periods. To reduce some of the variability in estimated rates due to methodological differences we then fitted models using only studies with a minimum duration of three months, a minimum road surveyed of 5 km, and a maximum survey interval of seven days.

Following González-Suárez et al. (2018), we used machine learning Random Forest regression, which can capture non-linear relationships and interactions among predictions, to predict roadkill rates. This effective tool for prediction assembles multiple regression trees, with each constructed from a bootstrap sample of the original dataset. Approximately, two thirds of the samples form a bootstrap sample, while the other third is left out (out of bag samples – OOB samples) (Breiman, 2001). We trained models using all roadkill rates that met the methodological criteria described above as we considered splitting the data to create a single test dataset could bias our taxonomically and spatially structured data. As a result, model fit may be overestimated.

Random Forests were generated based on 2000 regression trees using the function “randomForest” from the R package “randomForest” (Liaw & Wiener, 2002). We fitted separate models for birds and mammals as different relationships could exist for each class. These models included all trait variables as well as the taxonomic order of each species to account for evolutionary relatedness and similarities across taxa not reflected in the tested traits. The models also included as predictors latitude and longitude of the central road point of the survey area of each study to broadly account for spatial variability and local factors affecting roadkill estimates.

Model performance was reported using total variance explained, which is calculated as 1 – (Mean square error (MSE)/Variance_y, where MSE is the sum of squared residuals of the OOB sample/OOB sample size and Variance_y is the variance in predicted values (Acharjee *et al.*, 2011). We used permutation feature importance to evaluate how well different features (variables), predicted roadkill. Variable importance was calculated for each feature as the difference in MSE between 2000 regression trees fitted with the original data and 2000 trees fitted after the permutation of values in that variable. The average of these differences was divided by their standard deviation and reported as variable importance (we report 15 importance estimates for each variable obtained from the analyses of the 15 imputed datasets). Permutation of important variables results in lower variance explained (poorer model performance), while permutation of less important variables does not greatly reduce the variance explained. Because of the stochastic nature of random forest, negative variable importance values may occur when by chance permuting an uninformative variable that results in slightly lower MSE than with the original data. These can be considered as effectively zero importance.

We also generated dependence plots using the function “partial” from the R package “pdp” (Greenwell, 2017). These are informative graphics to understand the relationship between the predicted response and predictor variables. These plots represented the prediction of roadkill rates for a given variable obtained by marginalizing the machine learning model output over the distribution of all other features, so the function depends only on the focal variables with interactions with other variables included.

2.3.2 *Predicting spatial roadkill risks*

We predicted roadkill rates for all Latin American birds and mammals reported as roadkill at least once in the compiled systematic surveys. We defined current distribution areas for each species using IUCN maps (IUCN, 2022) considering only those polygons with certainty of presence (i.e. possible extant, possible extinct, and extinct categories were removed). Additionally, for bird ranges for which migration classifiers are included, we selected breeding, no breeding, and passage, removing those classed as vagrant. Since distribution areas can include unsuitable habitat, the use of area of habitat has been recommended to better represent the true area used by the species (Brooks *et al.*, 2019). Therefore, to reduce overestimation of risk by including areas where a species is unlike to occur, we defined area of habitat by selecting only areas of the distribution range that matched the preferred habitats of each species. Habitats were geographically delimited using high resolution (30 arc-second², ~1sqkm) land cover datasets of 1998-2012 (Latham *et al.*, 2014).

We then defined a 1º x 1º grid of Latin America and generated a list of species present in each grid cell considering as present all whose area of habitat overlapped to any extent with the cell. Each grid was then considered as a potential study site with location defined by the geographical coordinates of the cell's centroid. Using the fitted random forest models, we then predicted roadkill rates for the species in each grid cell given trait values, taxonomy, and the cell centroid coordinates. We used median rates per species and cell calculated from the 15 imputed datasets. For each grid cell we then calculated the total roadkill rates per cell for birds and mammals by adding roadkill rates for all species in each class in that cell. Considering the length of all primary and secondary roads (Meijer *et al.*, 2018) within each grid cell, we finally calculated the total predicted number of birds and mammals roadkilled per year in each cell by multiplying the total roadkill rate by the existing kilometers of road in each species habitat.

2.4 Results

2.4.1 *Roadkilled birds and mammals in Latin America*

The full compiled roadkill dataset included 1,691 roadkill rate estimates representing 346 bird species and 159 mammal species and 85 studies from 12 Latin American countries (Argentina, Brazil, Chile, Colombia, Costa Rica, Ecuador, Guatemala, Panama, Paraguay, Peru, Mexico, and Venezuela). Most of the birds reported as roadkill are classified as Least Concern (LC) by the IUCN Red List (IUCN, 2022) with seven in higher risk categories including three listed as Near Threatened (NT): yellow-faced amazon (*Alipiopsitta xanthops*), greater rhea (*Rhea Americana*), and eastern meadowlark (*Sturnella magna*); three as Vulnerable (VU): black-masked finch (*Coryphaspiza melanotis*), sharp-tailed tyrant (*Culicivora caudacuta*), and channel-billed toucan (*Ramphastos vitellinus*); and one as Endangered (EN): violet-throated metaltail (*Metallura baroni*).

The top roadkilled birds (those with the highest corrected rates, Table 2.2) are all listed as Least Concern and include the long-tailed mockingbird (*Mimus longicaudatus,* median roadkill rate across studies: 1.56 ind./km/year), the social flycatcher (*Myiozetetes similis,* median rate 0.98 ind./km/year), and the groove-billed ani (*Crotophaga sulcirostris*, median rate 0.97 ind./km/year). Among threatened birds, the violet-throated metaltail, listed as Endangered, had a roadkill rate of 0.26 ind./km/year, which is relatively high considering its distribution is restricted to the south of Ecuador, where just a few occurrences have been reported (13 records; Tinoco et al., 2009). While potentially worrying, this roadkill rate was estimated from a single study with just two roadkilled individuals; mortality may be less frequent in other areas of the species' distribution. Relatively high rates of 0.15 ind./km/year were also reported for the eastern meadowlark which is listed as Near Threatened.

The top roadkilled mammals are listed as Least Concern (Table 2.2), but 23 species have higher IUCN risk status. Fourteen species are listed as Near Threatened; eight as Vulnerable: brown howler monkey (*Alouatta guariba*), greater naked-tailed armadillo (*Cabassous tatouay*), bristle-spined rat (*Chaetomys subspinosus*), northern tiger cat (*Leopardus tigrinus*), Brazilian dwarf brocket (*Mazama nana*), giant anteater (*Myrmecophaga tridactyla*), giant armadillo (*Priodontes maximus),* and lowland tapir (*Tapirus terrestris*); and one as Endangered: tapeti (*Sylvilagus brasiliensis*). In addition, two roadkilled mammals are listed as Data Deficient: Azara's agouti (*Dasyprocta azarae*), and red brocket (*Mazama americana*).

The Andean white-eared opossum (*Didelphis pernigra*, LC) had the highest reported rate (4.68 ind./km/year), followed by the Sechuran fox (*Lycalopex sechurae*, NT) with a rate of 3.44 ind./km/year, and the common opossum (*Didelphis marsupialis,* LC) with a median rate of 1.45 ind./km/year. While rates for the common opossum are lower, this species has been reported as roadkilled in 17 studies from six countries (Brazil, Colombia, Ecuador, Mexico, Panama, Peru), while the Andean white-eared opossum and the Sechuran fox were only reported in one study in Ecuador and Peru respectively.

Table 2.2 Top 10 roadkilled birds and mammals in Latin America in descending order. We report species name, IUCN Red List status, the number of studies in which that species was reported as roadkill and the estimated roadkill rate (when data were available for more than one study, we calculated a median rate across studies using corrected values). The number of studies and observed rates are reported considering those studies that met our methodological criteria (minimum duration of three months, a minimum road surveyed of 5 km, and a maximum survey interval of seven days) that we consider provide more reliable estimates.

Class	Order	Scientific name	IUCN status	N studies	Roadkill rate (ind./km/year)
Aves	Passeriformes	*Mimus longicaudatus*	LC	1	1.561
	Passeriformes	*Myiozetetes similis*	LC	2	0.979
	Cuculiformes	*Crotophaga sulcirostris*	LC	2	0.971
	Pelecaniformes	*Bubulcus ibis*	LC	2	0.929
	Caprimulgiformes	*Amazilia tzacatl*	LC	1	0.908
	Accipitriformes	*Rostrhamus sociabilis*	LC	1	0.888
	Passeriformes	*Geospizopsis unicolor*	LC	1	0.785
	Passeriformes	*Platyrinchus cancrominus*	LC	1	0.762
	Falconiformes	*Caracara cheriway*	LC	1	0.760
	Passeriformes	*Ochthornis littoralis*	LC	1	0.730
Mammalia	Didelphimorphia	*Didelphis pernigra*	LC	1	4.681
	Carnivora	*Lycalopex sechurae*	NT	1	3.436
	Didelphimorphia	*Didelphis marsupialis*	LC	16	1.453
	Pilosa	*Choloepus hoffmanni*	LC	3	0.859
	Rodentia	*Galea flavidens*	LC	1	0.706
	Rodentia	*Oligoryzomys flavescens*	LC	1	0.652
	Pilosa	*Tamandua mexicana*	LC	5	0.594
	Carnivora	*Conepatus chinga*	LC	5	0.532
	Carnivora	*Lycalopex gymnocercus*	LC	3	0.355
	Pilosa	*Bradypus variegatus*	LC	2	0.312

2.4.2 *Vulnerability to roadkill*

Thirty-five of the compiled studies did not meet our methodological criteria (see Methods), so models were fitted using 50 studies conducted in nine countries (Argentina, Brazil, Chile, Colombia, Ecuador, Guatemala, Mexico, Panama, and Peru) which provided 520 roadkill rate estimates for 249 bird species and 457 roadkill rates for 103 mammals. Random forest models explained on average (across models fitted for the 15 imputed datasets) 57.3 % of the observed variance in bird roadkill rates (standard deviation across the 15 datasets: 0.002), and 47.7 % for mammals (standard deviation across the 15 datasets: 0.002). Latitude and longitude (location) of the study were the most important variables for both taxonomic groups (Figure 2.1). These were expected *a priori* to be important because they are the only variables that could explain observed within-species variability in rates. In addition, several traits were also identified as relevant predictors in each taxonomic group (Figure 2.1). Among birds, larger species (>2.5 kg) with a higher population density had higher roadkill rates, as did those with a shorter lifespan, <5 years; sexual maturity ages <500 days; and at least two clutches per year (Figure 2.2). More generalist birds with wider habitat breadths (occupying more than five different habitats) and which regularly use artificial habitat, croplands, grasslands and water bodies also had higher roadkill rates (Figure 2.2). Among mammals, species with adult body mass between 2 and 35 kg, faster life-histories (lifespans < 10 years, maturity age < 500 days), higher population density, scavenger and invertebrate diets, smaller home ranges (<10 km) and generalist habits (habitat breadth > 5 categories) had higher mortality rates (Figure 2.3). Cuculiformes birds and Pilosa mammals were the taxonomic orders with the highest roadkill rates.

Models that excluded trait variables with <40% of empirical values (more imputed values) (clutches per year, longevity, maturity age, and population density for birds) produced qualitatively similar results suggesting that including highly imputed variables did not influence our inferences (Figures A.1, A.2 an A.3).

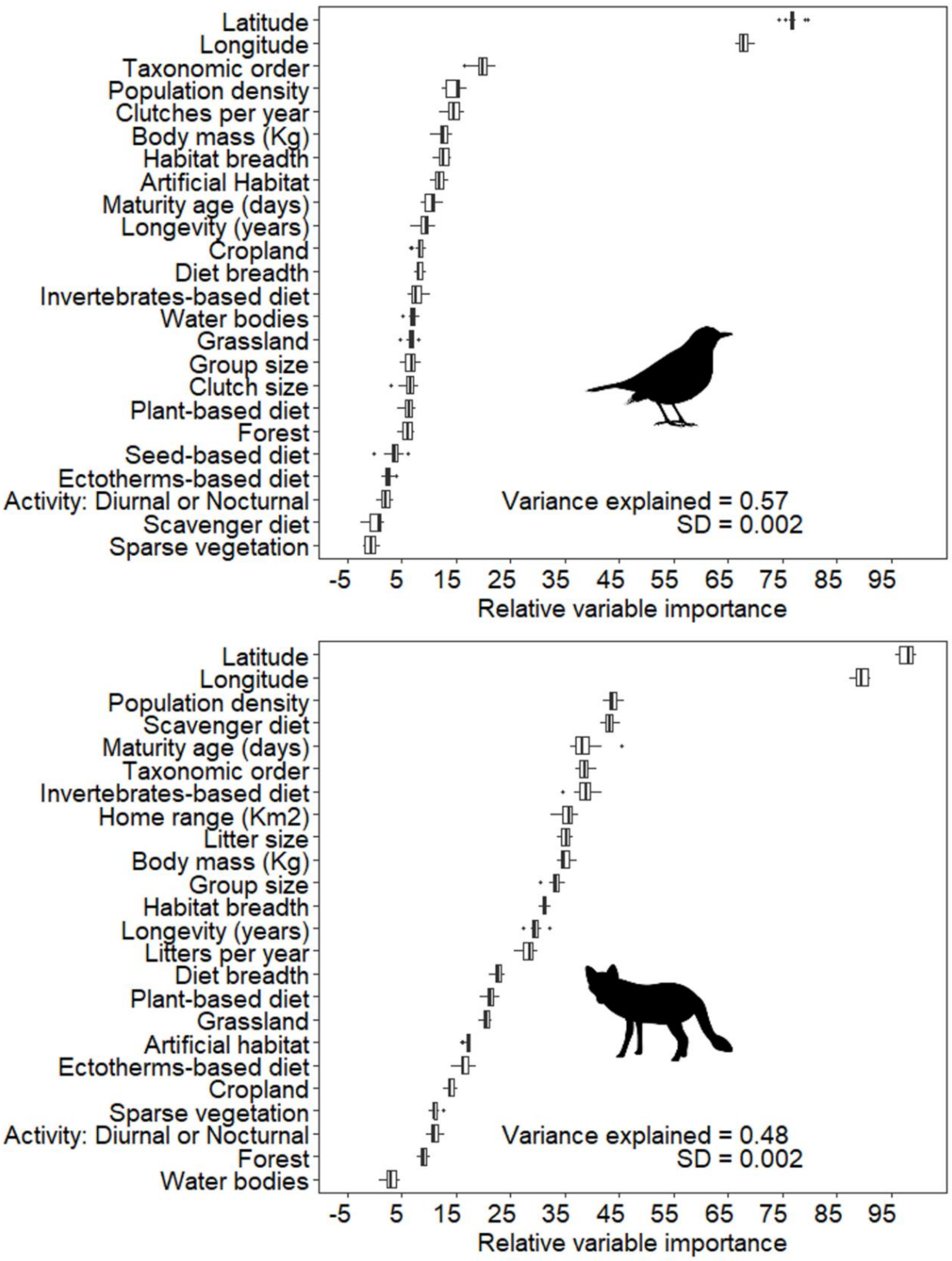


Figure 2.1 Relative importance of variables considered in random forest regression models to predict bird and mammal roadkill rates. Boxplots show results for models fitted to each of the 15 imputed datasets (trait data were not available for all species and missing values were imputed 15 times to capture uncertainty). We also report the total variance explained reported as median and standard deviation (SD) calculated from the 15 models fitted for different imputed datasets.

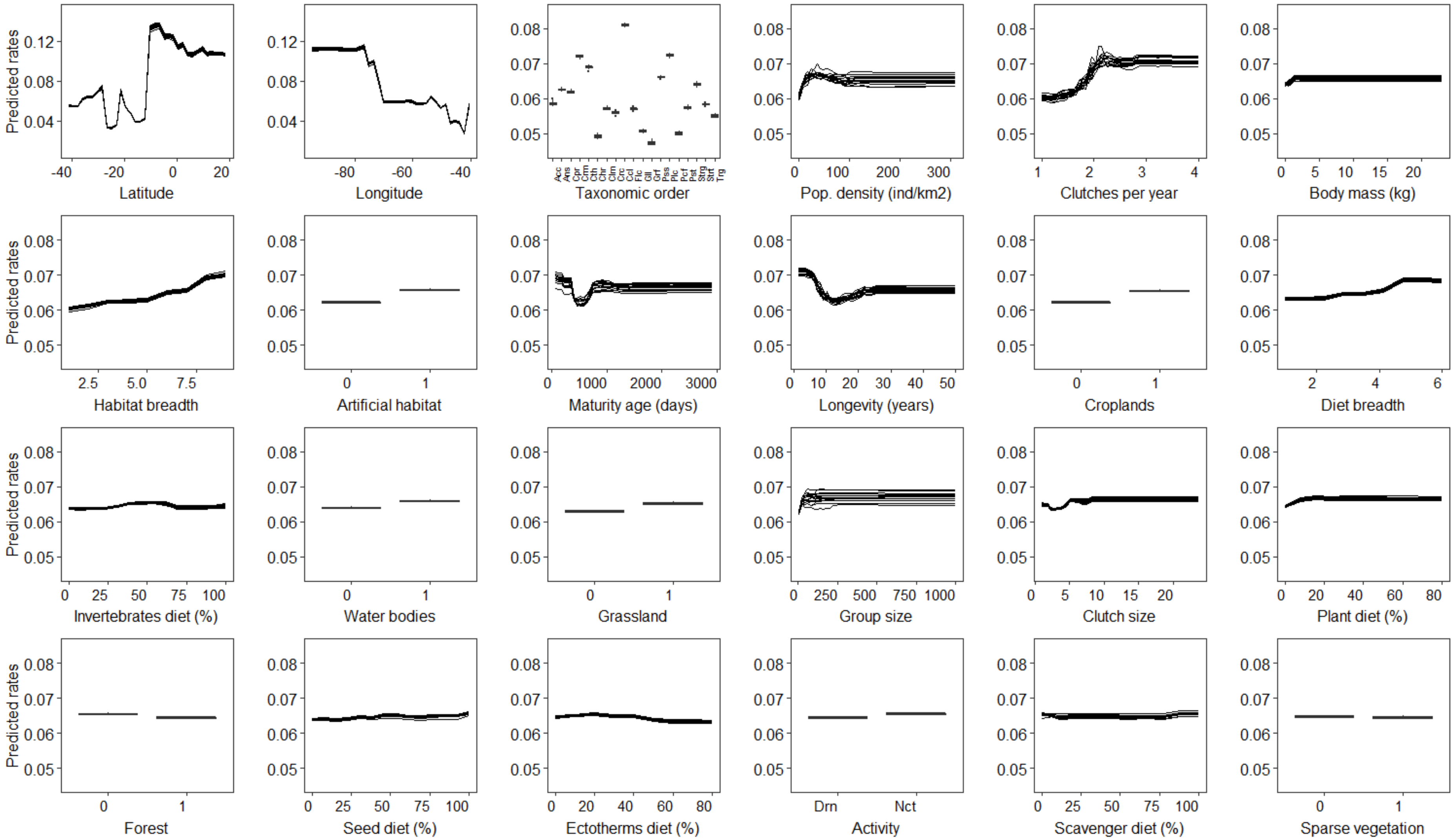


Figure 2.2 Dependence plots showing changes in predicted roadkill rates for birds for each tested predictor variable. Predictors are shown in descending order of relative variable importance (Figure 2.1). Each panel show results for the 15 models fitted for different imputed datasets

(trait data were not available for all species and missing values were imputed 15 times to capture uncertainty). For numerical predictors we show 15 lines and for categorical variables we show boxplots representing the distribution of predicted rates. For habitat preferences (Artificial habitat, Cropland, Forest, Grassland, Sparse vegetation, and Water bodies) category 1 indicates species that uses that habitat and zero indicates no use. Note that due to a wider range of predicted values, y axis scale for Latitude and Longitude is different from the rest of the variables.

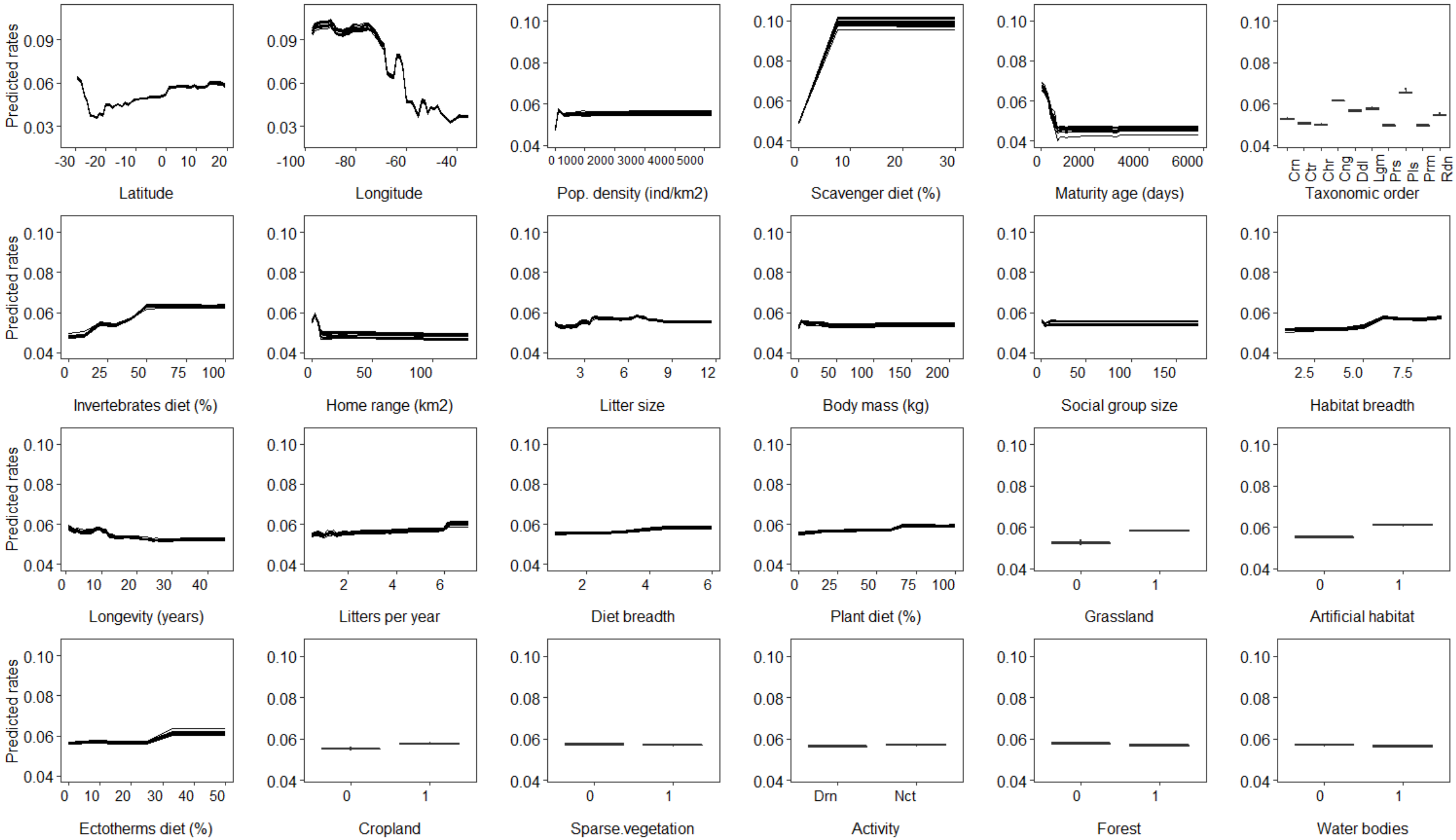


Figure 2.3 Dependence plots showing changes in predicted roadkill rates for mammals for each tested predictor variable. Predictors are shown in descending order of relative variable importance (Figure 2.1). Each panel show results for the 15 models fitted for different imputed datasets

(trait data were not available for all species and missing values were imputed 15 times to capture uncertainty). For numerical predictors we show 15 lines and for categorical variables we show boxplots representing the distribution of predicted rates. For habitat preferences (Artificial habitat, Cropland, Forest, Grassland, Sparse vegetation, and Water bodies) category 1 indicates species that uses that habitat and zero indicates no use. Note that due to a wider range of predicted values, y axis scale for Latitude and Longitude is different from the rest of the variables.

2.4.3 *Roadkill risk maps*

Our analyses predict that 12,431,670 birds, and 5,136,373 mammals can be killed on Latin American primary and secondary roads each year. These high numbers likely underestimate total mortality, because we cautiously only calculated risk for species reported in systematic studies included in our database – species known to be susceptible to roadkill. However, it is likely that many other Latin American birds and mammals are susceptible to collisions (particularly those in regions where no road surveys have been conducted). Moreover, our calculations did not consider tertiary, illegal and unofficial roads, which could dramatically increase these numbers.

Areas with higher mortality rates were mainly located across all Central America, northern Venezuela, northern and western Colombia, Ecuador, western Perú, southern Chile, central-eastern Argentina, Uruguay, and eastern Brazil. Generally, areas with high road densities resulted in high estimates of mortality per year (Figure 2.4); however, some high road density areas such as the Caribbean region, Paraguay, and western Brazil (border with Bolivia) showed low roadkill rates. High roadkill rates were not the direct result of higher richness of species known to be roadkilled (those in our dataset). Some areas with high richness such as northern Bolivia, southern Brazil, and southeast of Paraguay did not show high estimates. On the other hand, some high-risk areas in Central America, northeast and southeast Ecuador, central-eastern Argentina, and Uruguay have relatively low richness (Figure 2.5).

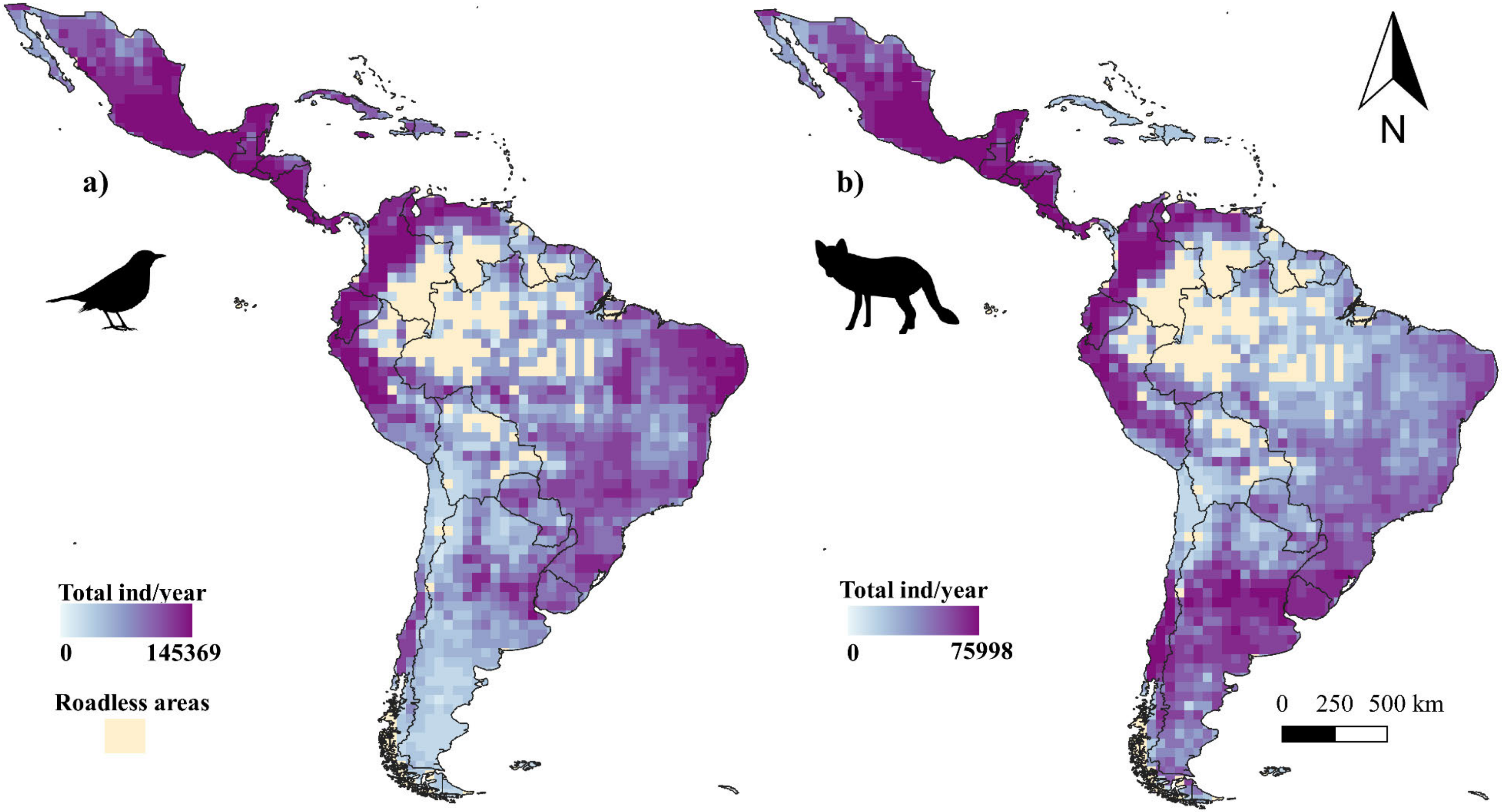


Figure 2.4 Predicted total number of birds (a) and mammals (b) to be roadkilled each year in Latin America and the Caribbean. Total numbers were calculated multiplying roadkill rates (individuals/km/year) predicted for each species and grid cell using random regression models and species traits and location data (see Table 1 for all variables) by the total length of existing main roads (km) in each grid cell. Grid cells with no predictions (cream colour) represent areas where roads are not present.

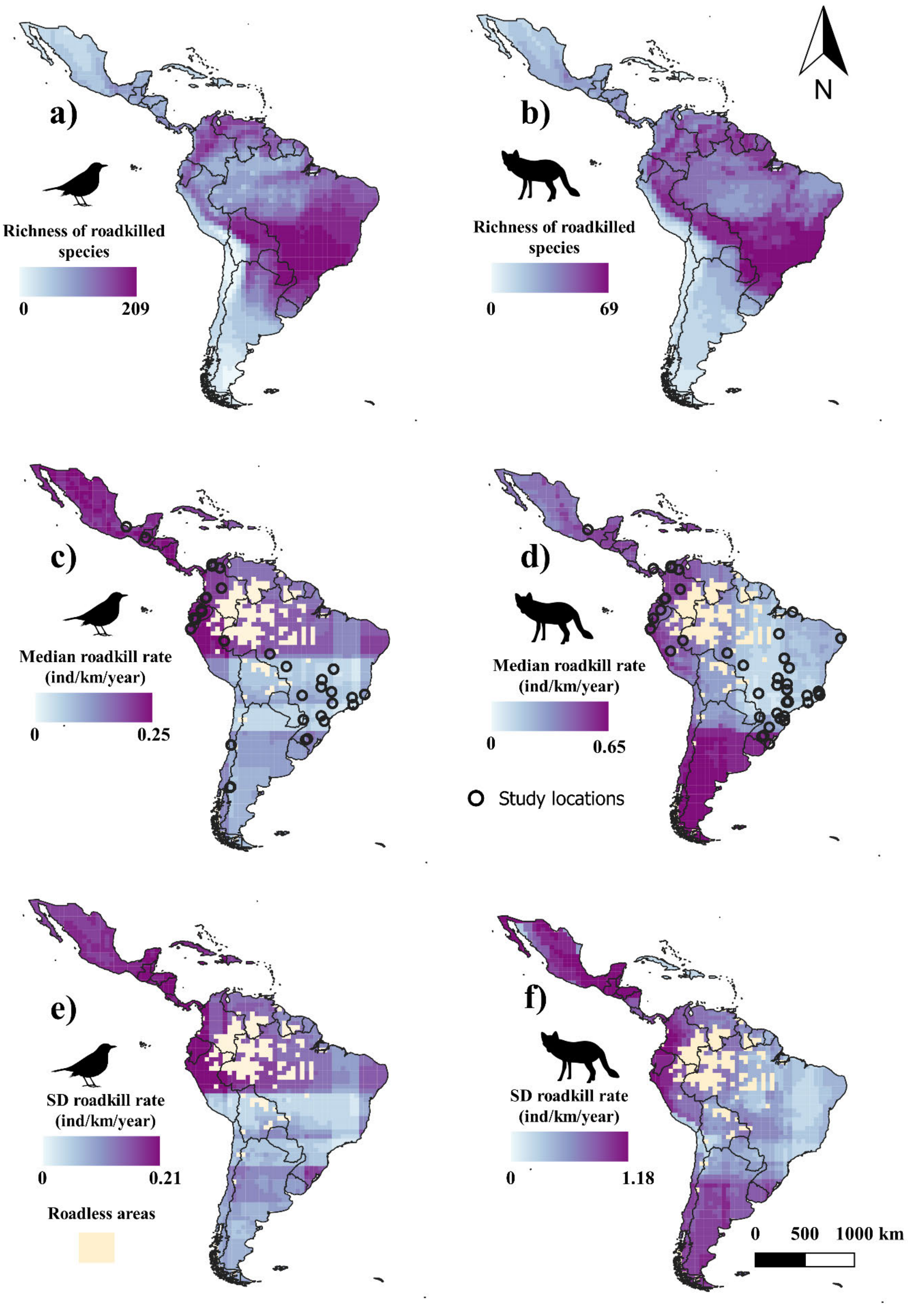


Figure 2.5. Species richness of roadkilled birds (a) and mammals (b). Predicted median roadkill rates (individuals/km/year) for birds (c) and mammals (d) obtained by averaging

species estimates obtained for each species and grid cell using random regression models and species traits and location data (see Table 1 for all variables). Standard deviation of the predicted rates values among species within each cell for birds (e) and mammals (f). In panels c and d, we show the locations of the roadkill surveys used for analyses (those that met our methodological criteria). Note that in panels c-f cream colour indicates areas without any roads.

2.5 Discussion

Our compilation revealed a diversity of birds and mammals killed on Latin American roads due to collision with vehicles. While most of these species are common and non-threatened, there are some taxa of conservation concern reported as roadkill that should be considered for future research and targeted mitigation. Observed roadkill rates were associated with several life-history, ecological, morphological traits and habitat preferences. In particular, we found higher roadkill rates in larger birds and medium-sized mammals, likewise, shorter life expectancies, wider habitat breadths, and early maturity ages were related to higher mortality for birds and mammals. Our predictive maps revealed areas of high mortality in Central America, the Northern Andean region (Venezuela, Colombia, Ecuador, and Peru), southern Chile, central-eastern Argentina, Uruguay, and eastern Brazil.

2.5.1 *Vulnerability to roadkill*

Body mass was one of the traits associated with road mortality of birds, with larger species (>2.5 kg) generally more likely to die on roads. Bird body sizes mostly varied between 3 g and 5.7 kg (median weight: 55 g) in our dataset with an extreme value of 23 kg for *Rhea americana*. On the other hand, mammalian sizes in the roadkill dataset ranged from 5 g to 207 kg (median weight: 1.15 kg), and in this group higher roadkill rates were associated with species of intermediate sizes (with a peak at ~3 kg). This pattern of higher risk in medium-sized mammals is similar to that found in Brazil by González-Suárez et al. (2018) and may be partly explained by the ability of vehicle drivers to detect and avoid collisions with larger animals. In addition, lower rates for larger animals could be associated to lower population density or local abundances; indeed larger mammals have been shown to avoid roads leading to lower population densities near roads or areas with more roads (Rytwinski & Fahrig, 2012; D'Amico *et al.*, 2016). Among Latin American mammals, large carnivores like the jaguar (*Panthera onca*) have much lower population densities near roads (Espinosa *et al.*, 2018). Indeed, we found that bird and mammal species with higher species-level population densities (expected to be on average more locally abundant) were more likely to have high roadkill rates. We note that although estimates of local abundance (or density) were not available, it is likely that some of the within species variation in roadkill rates reflect variation in local abundance.

We also found that long-lived animals are less likely to be roadkilled. As mentioned in our hypotheses, species with longer lifespans could have more opportunity to learn new skills and acquire experience with new environments like roads, reducing their risks (Street *et al.*, 2017). However, the relationship could also reflect variation in local abundance as longer lifespans are associated with larger body size and slower reproductive rates which can influence roadkill risk.

Generalists, those with wider diets and habitat breadths, were also more likely to have high roadkill rate. This likely reflects greater exposure to roads as generalists can be more likely to visit a variety of environments and be less reluctant to approach roads and take advantage of any food resource such as rubbish thrown from vehicles, carcasses, and species that use road verge vegetation. Generalist mammals have been found to use roads extensively in order to get food and move across habitats (Hill *et al.*, 2021), and other studies found also that generalist/omnivorous mammals are more vulnerable to roadkill (Cook & Blumstein, 2013). Indeed, we found that mammals with scavenger and invertebrate-based diet had higher roadkill rates. Scavengers can be directly attracted to roadkill on roads (Rød-Eriksen *et al.*, 2020), increasing their exposure to circulating vehicles and their risk of collision. Insects may also be locally abundant on roads as they can be attracted to decomposing carcasses, verge vegetation resources, and light sources near roads, and in turn, attract insectivores to roads.

Faster reproductive rates, early maturity ages and more litters or clutches per year, were related with higher roadkill mortality. These variables may be relevant due to the association with variation in local abundance or density, as species with faster reproduction rates generally have higher local abundance. In addition, previous work found an association between seasonal roadkill patterns and mating periods (Ascensão *et al.*, 2019; Canova & Balestrieri, 2019). As mating behaviour should be more frequent in species with more reproductive events per year, this can lead to more individuals of those species moving across areas looking for mates and they are more likely to encounter roads.

Previous local studies found that open areas such as grasslands and croplands are associated with higher vertebrate roadkill rates (Seo *et al.*, 2013; Freitas *et al.*, 2014; Silva *et al.*, 2019;

Medrano-Vizcaíno & Espinosa, 2021), which is supported by our large-scale results particularly for birds. These habitat types may be working as ecological traps, for instance, croplands may offer a great quantity of food resources for certain species, which could die on roads when approaching these resources, while pasturelands may act as corridors to approach crops. Although the same relation was found for mammals, habitat variables were not as important for predicting mammalian roadkill rates.

While traits were relevant predictors, as expected, much of the variation in roadkill rates was explained by location. Strong geographical patterns are common in large scale studies reflecting environmental variation and within-species variability that cannot be captured by species-level trait data (as mentioned in our hypothesis). In our case, fitting simpler models that ignored location and its interaction with trait effects (models excluding latitude and longitude) resulted in lower variance explained: 3.04% for birds (standard deviation across the 15 datasets: 0.004) and 24.9% for mammals (standard deviation across the 15 datasets: 0.001) (Figure A.4). The particularly low value for birds may reflect distinct regional trait relationships, such that traits have different effects in different regions (such interactions would be captured in the complete regression trees). For example two studies exploring how traits influence roadkill rates in European birds reported different patterns. Grilo *et al.*, (2020) found that body mass and diet were among the most important traits for predicting bird roadkill rates when using regression trees that allowed for non-linear relationships and interactions with geographical location. Meanwhile, Morelli *et al.*, (2020) used linear regression models that did not account for location or tested non-linearities and reported a weak association with body mass and no relationship with diet.

Alternative, we may have missed important traits to explain roadkill rates in birds such as mode of locomotion and movement patterns. Birds with a low-flight behaviour tend to be more roadkilled, which has been observed for owls (Kociolek *et al.*, 2015), while more mobile birds were identified as generally more susceptible to negative effects of roads (Rytwinski & Fahrig, 2012). However, for most of our studied species those data were not available, for example, concerning mobility, we were able to collect home range data for only 10% of bird species. Finally, it is possible that traits are overall poorer predictors of road impacts in birds that in mammals. Future work considering additional taxonomic groups and comparing different areas will be useful to further understand how trait-based approaches can contribute to our understanding of road mortality patterns across different taxa.

2.5.2 *Roadkill risk maps*

Our predictions offer a worrying picture with potentially more than 12 million birds and more than five million mammals dying each year across main Latin American roads. Furthermore, these estimates only reflect species with observed roadkill. The Latin America and Caribbean region is home to approximately 4,613 bird species and 1,859 mammal species (IUCN, 2022), of which our dataset included 7.50% of birds and 8.55% of mammals. Even if some species show road avoidance behaviour and are less likely to be roadkilled, road mortality risks for over 90% of the avian and mammalian biodiversity are still unknown, and thus, the total number of roadkilled birds and mammals is likely much higher than what we estimate here.

Predicted risks from the existing road network are worrying. Unfortunately, Latin America is projected to have one of the highest rates of future road development in the world (Meijer *et al.*, 2018), which is likely to cause even greater impacts on wildlife. For example, we were able to obtain data on planned roads for Ecuador (MTOP, 2016), a country with high predicted roadkill rates. Considering the existing network of 16,647.65 km of primary and secondary roads (Meijer *et al.*, 2018), our model predicts 420,861 birds and 119,599 mammals roadkilled each year. Those numbers were predicted to increase by 9.3% (39,321 additional birds and 11,174 additional mammals) with the development of 1,555.4 km of new roads planned by 2030 (MTOP, 2016). As mentioned above, the actual roadkill numbers are likely much higher as our dataset represent <20% of the avian and mammalian biodiversity of Ecuador.

Our paper provides insights into how roadkill can impact wildlife in Latin America, but there are some limitations of the data used for analyses. For instance, we assumed that taxonomic identification of roadkill was correct, but it is possible a few carcasses could have been misclassified. While it is not possible for us to correct potential taxonomic misidentification, errors are likely rare as unrecognized carcasses are generally listed as unclassified. Taxonomic disagreements or new classifications could also affect the data. For example, the common tapeti (*Sylvilagus brasiliensis*) was considered until 2018 as a widely distributed species, but in 2019 it was separated into several species. The name *S. brasiliensis* now refers to tapeti found only in Pernambuco-Brazil (Ruedas, L. & Smith, 2019). Because our data included studies conducted before 2019 when the taxon was re-evaluated, we compiled roadkill records that mention the common tapeti from 18 studies across five countries

(Argentina, Brazil, Costa Rica, Ecuador, and Mexico), none within the new restricted distribution. To map roadkill, we used current IUCN distribution data which means projected roadkill numbers for tapeti were limited to a small region potentially underrepresenting the risk for other tapeti species. While it is beyond of the scope of our study (and non-trivial) to reclassify records into new species, we expect these disparities to be minor and not bias our results.

Our work contributes to highlight the value of combining models based on traits and habitat preferences to better understand and predict threats and impacts from human activities. This approach can be applied widely to investigate other taxonomic groups and geographical areas. For instance, although very high roadkill rates for herpetofauna have been reported in several studies across the world, our understanding of which traits influence risk, and the development of predictive models remains limited. Trait data can be useful but readily available compiled sources remain relatively rare. Increased efforts to provide access to natural history description and standardized datasets will be very valuable to apply this approach and gain further insights into how roads impact wildlife. Additionally, systematic studies that report road impacts, including roadkill rates, in a clear, easy to use way, are also essential (Silva *et al.*, 2021). Some areas remain unstudied, but likely some have been surveyed yet data remain unpublished or were only presented in monitoring reports that can be difficult to obtain and do not always report raw values (e.g., providing only summary values across taxonomic groups instead of detailed roadkill observations). Easier access to roadkill survey data is essential to build improved models that lead to better decisions for wildlife conservation.

In conclusion, this first continental-scale evaluation of roadkill risk for Latin American birds and mammals offers several useful outputs identifying Latin American species and areas with higher road mortality. This approach, where trait-based models are fine-tuned with realistic spatial information (by considering habitat suitability of species), is a valuable tool for the evaluation of human impacts including roads and traffic, and we hope this will be increasingly used by ecologists and managers.

2.6 Data availability statement

All data used for analyses including roadkill rates, and associated life-history, ecological, morphological traits and habitat preferences for birds and mammals from Latin America are available in a public repository (https://doi.org/10.6084/m9.figshare.19236765.v1).

Chapter 3. Research and conservation priorities to protect wildlife from collisions with vehicles

3.1 Abstract

The rapidly expanding global road network poses threats to wildlife, including direct mortality. Given limited knowledge and resources, strategic allocation is critical. We introduce a method to identify priority areas and taxa to study and protect affected by vehicle collisions using Latin America as a case study. In this region high biodiversity and an expanding road network can result in high impacts from roads, yet emerging research expertise offers opportunities for action. To identify priority targets we combined predicted spatially-explicit roadkill rates for birds and mammals with information about the current road network and species conservation status. Priority areas for conservation (with many species susceptible to roadkill but few or inexistent roads) were largely concentrated in the Amazon; while priority areas for research (unstudied regions with many roads and many species susceptible to roadkill) occur in various areas from Southern Mexico to Chile. Priority taxa for conservation reflected studied, roadkill-susceptible groups (e.g., vultures and armadillos), while priority taxa for research were defined as either poorly studied roadkill-susceptible groups or unstudied groups of conservation concern (e.g., cuckoos and shrew opossums). Our approach offers a tool that could be applied to other areas and taxa to facilitate a more strategic allocation of resources in conservation and research in road ecology.

Keywords: Birds, Conservation, Latin America, Mammals, Roadkill, Road ecology.

3.2 Introduction

Roads are widespread features in our planet and already fragment some of the world’s last remaining wilderness areas such as the Amazon (Laurance *et al.*, 2014; Meijer *et al.*, 2018). By 2050, an additional 25 million kilometers of new roads will be constructed primarily in Africa, South and East Asia, and Latin America (Laurance *et al.*, 2014). Roads are one of the main anthropogenic causes of wildlife mortality worldwide (Hill *et al.*, 2019), and the primary cause in some regions (Taylor-Brown *et al.*, 2019). For example, an estimated 194 million birds and 29 million mammals are killed in European roads, 340 million birds in the United States, and twelve million birds and five million mammals in Latin America (Loss *et al.*, 2014; Grilo *et al.*, 2020; Medrano-Vizcaíno *et al.*, 2022c).

The relentless expansion of roads affects wildlife worldwide, but impacts can vary across different regions and habitats depending on presence of species more exposed to roads.

Research shows evidence that roadkill risk varies among taxa due to their distinct morphological and ecological characteristics. For example, higher roadkill rates are found in larger, ground-foraging birds with more diverse diets and also in medium-sized, diurnal, scavenging mammals (Caceres, 2011; González-Suárez *et al.*, 2018). Since species occur in different habitats, there are some regions with more roadkill incidence than others (Medrano-Vizcaíno *et al.*, 2022c). These generalized links between road mortality, traits and habitats suggest we could use existing information to predict wildlife mortality patterns across large geographical and taxonomic scales and thus, identify priority targets for research, infrastructure design and planning, and conservation management.

The application of prioritization tools to inform future research actions is particularly valuable in areas where risks are expected to be high (i.e., biodiverse areas with expanding road networks) but where empirical estimates of impacts are scarce. Latin America is a highly biodiverse region harboring eight biodiversity hotspots and unique endemism (Myers *et al.*, 2000) as well as ~3.5 million km of roads (Meijer *et al.*, 2018). Road impacts threaten Latin-American wildlife, but assessments are still rare with few, albeit rising, road ecology studies (Pinto *et al.*, 2020). This makes Latin America an ideal case study to test the proposed approach capitalizing on existing spatially-explicit roadkill rates predicted for bird and mammal species known to suffer mortality from collisions (obtained from Medrano-Vizcaíno *et al.*, 2022c). These data, combined with information on road network and species conservation status offer a way to identify priority areas and taxa for conservation and research.

To identify priorities, we propose that conservation efforts to reduce road impacts should focus on areas where many species susceptible to roadkill and few roads coincide (high vulnerability, low exposure). On the other hand, priority areas for research should be those currently unstudied but with many species susceptible to roadkill and many roads (high vulnerability, high exposure. Figure 3.1). Similarly, we define priority taxa for conservation considering taxonomic orders with at least one third of species recorded as roadkill and high predicted roadkill rates. We propose priority taxa for research should be currently understudied groups for which available roadkill rates are high, as well as unstudied groups in which many species are considered to be of conservation concern (Figure 3.2). This approach can be applied to other regions and different taxonomic or functional groups offering a tool to guide resource allocation in road ecology and conservation.

3.3 Methods

3.3.1 *Priority areas*

We capitalized on existing data to calculate three metrics for each 1º x 1º grid cell in Latin America (Figure 3.1). We defined local vulnerability to road mortality as the sum of predicted roadkill rates for all species occurring in that cell (see details below). Local exposure was calculated as the total length of primary and secondary roads using data from Meijer *et al.*, (2018). Finally, each cell was classified as either studied, if at least one systematic roadkill survey had been conducted in that area (centroid of the study area overlap the cell), or unstudied, if no studies were found.

Predicted roadkill rates (number of individuals killed per kilometer of road per year, ind./km/year) were available for 346 bird and 159 mammal species reported at least once as roadkill in a recent compilation of systematic road survey studies (Medrano-Vizcaíno *et al.*, 2022c). Predicted roadkill rates for each species and 1º x 1º grid cell across Latin America were obtained using trait-based machine learning Random Forest models that related observed roadkill rates from 85 systematic roadkill surveys with study location, species trait data (ecological, life-history, and morphological characteristics) and habitat preferences. Predictions were made for each cell in a species' area of habitat using current distribution ranges (IUCN, 2022) from which areas without suitable habitat were removed to better represent where species are likely to be present (Brooks *et al.*, 2019). Suitable habitats were defined based on habitat preferences of each species (described in Medrano-Vizcaíno *et al.*, (2022b) using high resolution (30 arc-second², ~1sqkm) land cover data (Latham *et al.*, 2014).

All grid cell values of local vulnerability and local exposure for birds and mammals were grouped into terciles for each metric which combined resulted in nine joint categories (Figure 3.1) and were mapped using bivariate choropleth maps in QGIS v.3.18.2-Zürich (https://qgis.org/en/site/). Conservation priority areas were proposed as those falling in the category representing the top tercile of local vulnerability and the bottom tercile of road abundance (low exposure). Top research priorities were defined as unstudied areas that fell in the top terciles of both local vulnerability and local exposure.

3.3.2 *Priority taxa*

Using predicted roadkill rates from Medrano-Vizcaíno *et al.*, (2022b) and the IUCN Red List categories (IUCN, 2022) we calculated several metrics for each taxonomic order of birds and mammals including: the total number and percentage of species reported as roadkill, the number and percentage of species classified as Threatened (Critically Endangered, Endangered, and Vulnerable), Data Deficient, and Not Threatened (Near Threatened and Least Concern) by the IUCN Red List (IUCN, 2022). We also calculated the average predicted roadkill rate for each order as the median across of all predicted roadkill rates for species in that group. We focused on taxonomic orders as priority targets to illustrate the method, but priorities could be defined at different taxonomic levels or for example considering functional groups. We propose as conservation priorities to orders with at least 1/3 of species recorded as roadkill and high predicted roadkill rates (median roadkill rate in the top tercile of values). We suggest two types of research priorities: type A for understudied but likely susceptible orders (high predicted roadkill rates – median roadkill rate in the top tercile of values, but roadkill data available for <1/3 of species), and type B for unstudied groups of conservation concern (no roadkill data available and at least 1/3 of species listed as Threatened or Data Deficient).

These proposed criteria for priority areas and taxa demonstrate the approach, but different thresholds or criteria could be used depending on the interests, needs, and resources available.

3.4 Results

3.4.1 *Priority areas*

Local vulnerability and exposure are heterogeneously distributed across Latin America, but vulnerability is generally higher in tropical areas for both birds and mammals (Figure 3.1). The bivariate maps show priority conservation areas (those with high vulnerability but low exposure) in most of the Amazon region with smaller areas in southern Argentina, northeastern Honduras, and the border of Panama with Colombia (Figure 3.1). Bird and mammal conservation areas partially overlap but are not identical, reflecting different distributions of species vulnerable to roadkill (Figure 3.1). Top research priorities (unstudied areas with high vulnerability and high exposure) occurred in most of Central America, northern regions of Venezuela and Colombia, a great part of Ecuador, western Perú, southern Chile, Uruguay, central Argentina, and some coastal areas in Brazil (particularly for birds)

(Figure 3.1). At a national scale we note that we found no reported roadkill surveys for five countries that overlap with identified top research priority areas (Belice, El Salvador, Honduras, Nicaragua, and Uruguay).

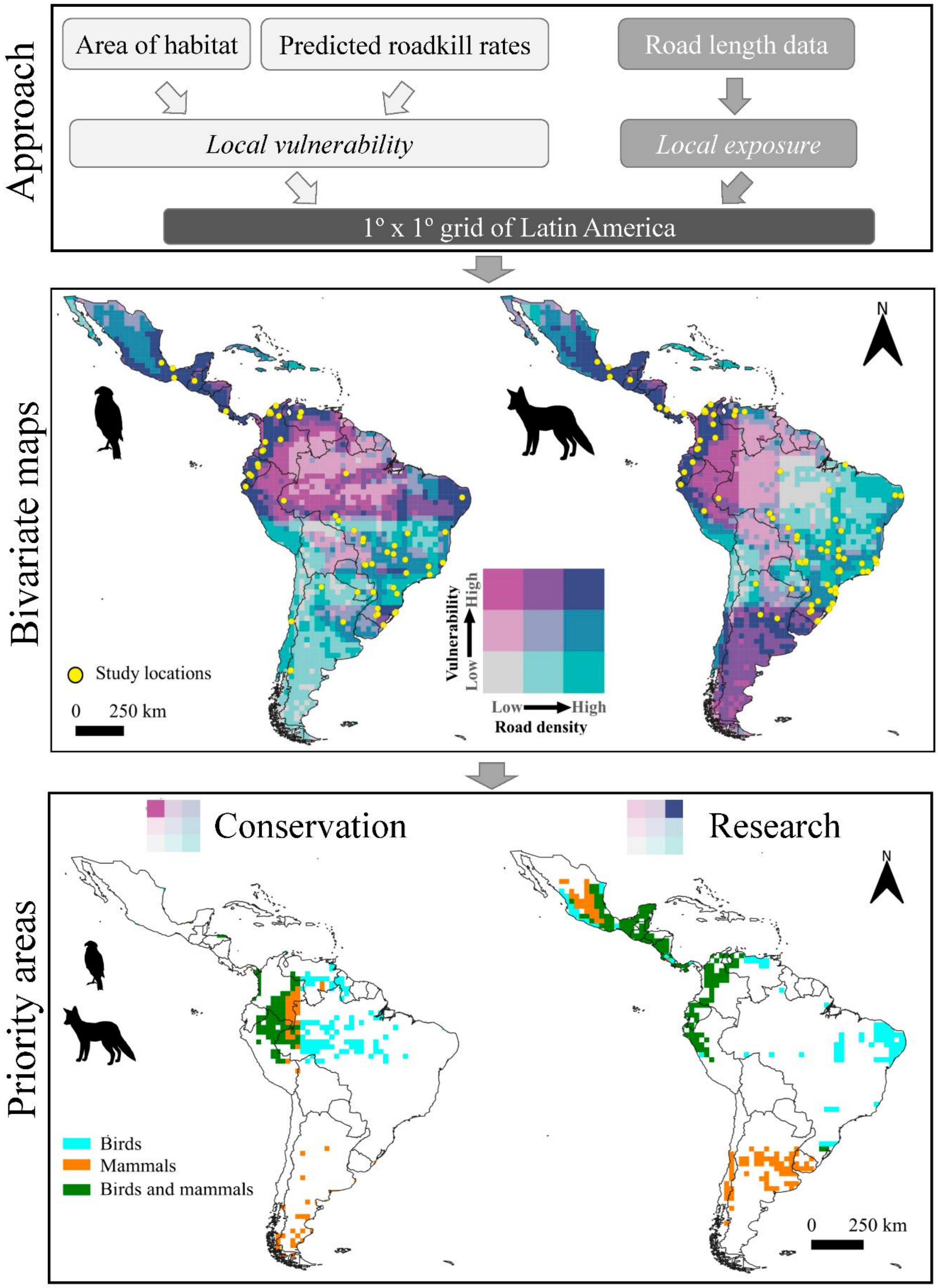


Figure 3.1 Summary of proposed approach to identify priorities (top panel) with resulting output representing maps of local vulnerability and exposure (middle panel) and the proposed priority areas for conservation and research for Latin American birds and mammals (bottom panel). Middle panel shows bivariate choropleth maps with nine categories based on terciles values for local vulnerability and exposure, with yellow symbols showing where data were collected. Bottom panel shows proposed priority areas for conservation and research based on bivariate maps (the modified legends on top of this panel highlights that we considered high vulnerability and low exposure for conservation priorities, and high vulnerability and high exposure for research priorities). Animal silhouettes were obtained from Phylopic (http://phylopic.org).

3.4.2 *Priority taxa*

Roadkill estimates were available for only 7.50% and 8.55% of Latin American birds and mammals respectively, and entire orders had no reported roadkill records (Figure 3.2). From the 29 bird orders present in Latin America, we found data for about two thirds (19) but for represented orders fewer than 50% of the species had reported roadkill estimates. Mammals were better represented with data for species in 10 of the 13 orders in Latin America and data for >50% of species for two orders, but still roadkill estimates reflected a relatively small fraction of mammalian diversity (Figure 3.2). Median predicted roadkill rates ranged from 0.019 to 0.132 ind./km/year in bird taxonomic orders (SD ranging from 0.018 to 0.129), and from 0.024 to 0.074 in mammalian orders (SD 0.018 to 0.281).

Using our suggested criteria, Cathartiformes and Cariamiformes should be considered as conservation priorities for birds, and Pilosa and Cingulata for mammals (Figure 3.2). Cathartiformes are New World vultures that as scavengers can be attracted to roads to forage on roadkill increasing their risk of collision. This group has the highest predicted rate for birds, and more than one third of species (42.86%) have been reported as roadkill. Cathartiformes includes threatened species for which estimates of roadkill are still not available but could be vulnerable to road mortality such as the Andean condor (*Vultur griphus*) and the California condor (*Gymnogyps californianus*). Cariamiformes has the second highest predicted rates for birds, with roadkill data available for one out of two existing species.

Sloths and anteaters (Pilosa) are also found frequently as roadkill (likely their slow movements increase probability of collisions when crossing roads), and more than two thirds of species (80%) have been reported as roadkill. Armadillos (Cingulata) also show vulnerability to roadkill (high rates) and is a group with an uncertain conservation status (five species classified as Data Deficient. Figure 3.2).

Cuckoos (Cuculiformes), Caprimulgiformes, Pelecaniformes, and Anseriformes were identified as research priorities type A for birds because roadkill estimates were high, yet risk for many species in the group remains unknown (Figure 3.2). Procellariiformes sea birds, and penguins (Sphenisciformes) are research priorities type B because these groups are of conservation concern, but no studies have evaluated roadkill risks. Given these priorities, road ecology research in coastal areas and islands seems particularly necessary to better understand risk for avian species in Latin America. Roadkill rates could be very low for these species, particularly those that spend most of their life at sea but, some species in these groups are known to be affected in other areas. For example, penguins have been reported as roadkill (Heber *et al.*, 2008).

Rabbits (Lagomorpha) were identified as mammalian research priorities type A with high rates combined with a high proportion of unstudied species, while the small mammals in the orders Paucituberculata and Eulipotyphla were considered research priorities type B given the current lack of knowledge and their high proportion of species listed as Threatened and Data Deficient (Figure 3.2).

We propose priority taxa for conservation and research at the level of taxonomic orders, however, vulnerability at taxonomic family and species level under different thresholds can be evaluated using data available at https://doi.org/10.6084/m9.figshare.20166359.

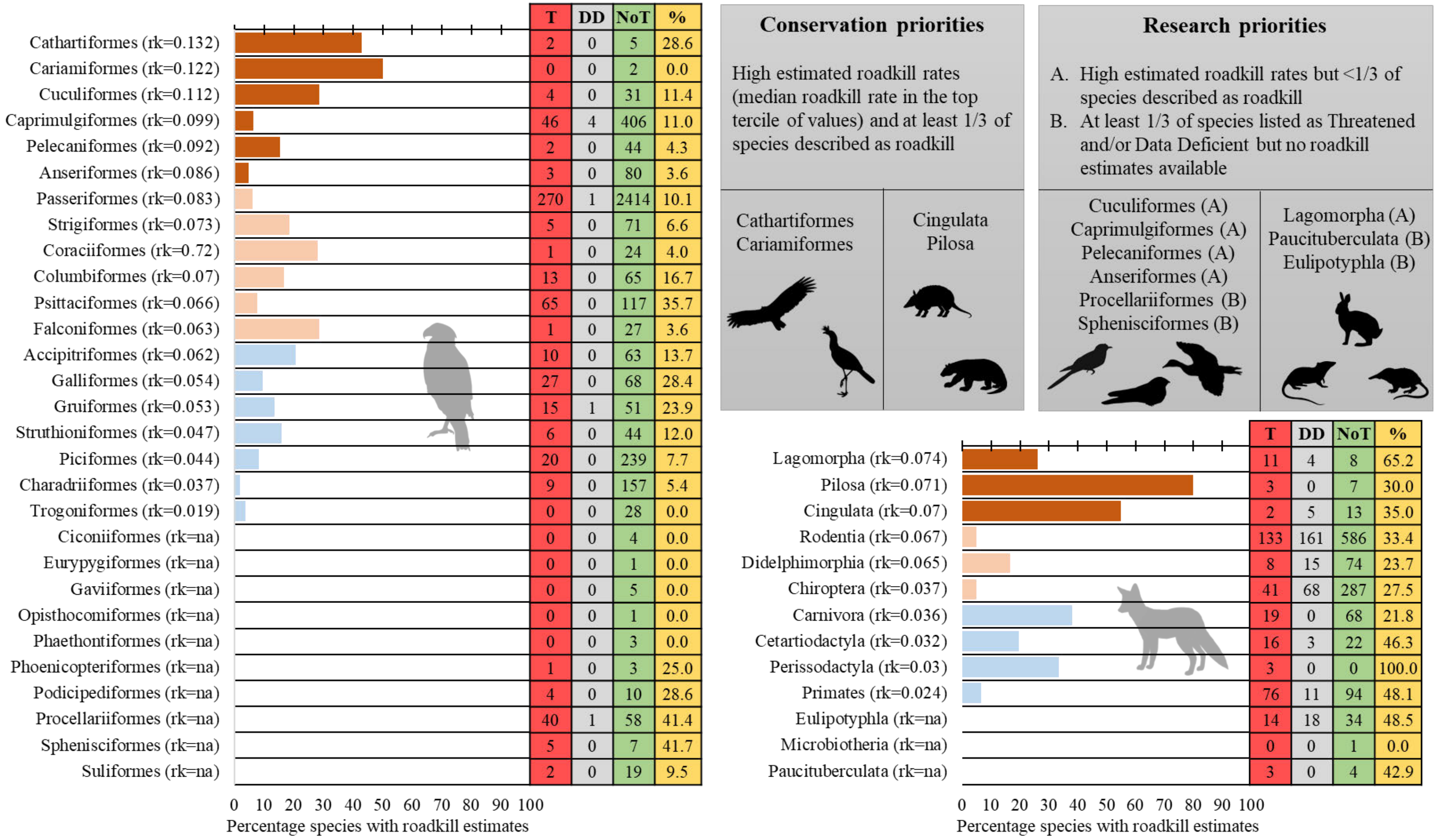


Figure 3.2 Priority conservation and research taxa and criteria values for bird and mammal taxonomic orders. Top right inset describes the proposed criteria for prioritization and the orders identified as conservation and research priorities. Plots show the percentage of species per

order for which roadkill data was available in our compilations used as criteria. Order names are followed by the estimated median roadkill rates (rk) calculated from the species rates predicted by trait-based Random Forest models (Medrano-Vizcaíno *et al.*, 2022c). Orders are listed from highest to lowest rk with bar colour indicating the terciles of the median roadkill rates used as criteria (Brown-orange bars = top tercile, peach bars = medium tercile, and light blue bars = bottom tercile). We also provide for each order the number of species classified by the IUCN as Threatened (T), Data Deficient (DD), Not Threatened (NoT) and the percentage (%) of the total listed as T and DD which was used in the criteria. Animal silhouettes were obtained from Phylopic (http://phylopic.org).

3.5 Discussion

Our approach capitalizes on existing data to generate quantitative estimates of spatial and taxonomic vulnerability that combined with data on local and taxa-specific risks (e.g., exposure to roads and conservation status) can help identify priorities for conservation and research. We apply this approach to suggest priorities for Latin America based on a set of proposed criteria. These criteria are flexible and could be adapted to reflect different needs, values, and funding trade-offs. Additional sources of risk could also be considered to further optimize conservation and research resources. This approach aims to provide a unified way to predict and map road vulnerabilities, exposure, and risks and facilitate the decision process of where and who to protect and study at a large scale. We envision this as a first step in the decision-making process that could complement existing conservation prioritization tools (Schwartz *et al.*, 2020; Zizka *et al.*, 2021) and should be followed with local and regional analyses to develop conservation and research agendas.

Application of the proposed approach to define conservation priorities in Latin America revealed the Amazon as a focal region to minimize development of infrastructure. The presence of many species with high predicted roadkill rates means that expanding the road network in this area without careful planning and adequate mitigation measures will likely have negative consequences for both wildlife and humans, as collisions can result in injuries and even fatalities (Zhao *et al.*, 2010). The Amazon is already considered a priority for conservation due to its unique forest environment, carbon sequestering potential and high biodiversity (Strassburg *et al.*, 2010). New roads would likely lead to increased wildlife mortality, but even if mitigation measures were implemented to reduce mortality, new roads facilitate further degradation and human expansion (Laurance *et al.*, 2009). Worryingly, 12,263 km of roads may be opened or improved (leading to more traffic and higher travelling speeds) in the Amazon in the next few years, and this expansion will likely be accompanied by additional illegal roads which can triple the length of official roads in some areas (Barber *et al.*, 2014; Vilela *et al.*, 2020).

Applying the proposed approach to Latin America also suggested priority areas for future studies, particularly in Central America and North-western South America. Research efforts can be driven by individual interests, but funding agencies can also define priorities and propose targeted schemes. We hope that by identifying research priorities in Latin America, where expertise is expanding but data are still limited compared to other regions (Silva *et al.*, 2021), our approach will encourage researchers and funding agencies to focus on understudied areas where species are more susceptible to road impacts and roads abound. These are areas where knowledge is most urgently needed to quantify risks and if necessary, propose mitigation measures.

Our analyses also identified taxonomic groups as conservation or research priorities. Further evaluation of species in these target groups may reveal limited risk for some due to behavioural avoidance [which can prevent roadkill but still have negative impacts by reducing gene flow (Holderegger & Di Giulio, 2010)] or preferences for local habitats where roads are absent or rare and thus, risks are few. Deeper evaluation of the proposed, admittedly coarse, priorities would help refine these targets, eliminating taxa unlikely to encounter roads or currently limited to roadless areas. We recognize that targeting taxa during roadkill assessments could present some challenges, as some species are naturally more difficult to detect even if roadkilled. For example, species-level roadkill data for smaller mammals like rodents or shrews is rare, likely because small animals are more often unrecognizable after collision – found as hairy spots on roads if at all (Cook & Blumstein, 2013). Additionally, rarer groups of small mammals (e.g., Eulipotyphla and Paucituberculata) may be wrongly classified in broad groups (like "unclassified rodents") which prevents species-specific analyses. Future research should explore ways to address these biases. Improving taxonomic identification skills or consulting with experts can be a solution. Although expensive, another solution would be identification of roadkill samples using molecular techniques.

The Latin America and Caribbean region is home to more than 4,600 birds and 1,800 mammals (IUCN, 2022), yet estimates of mortality caused by collisions with vehicles are available for <10% of species (Medrano-Vizcaíno *et al.*, 2022c). This means that the magnitude of how roads impact wildlife is likely underestimated. Finding ways to identify priority areas and taxa for research can address this limitation more efficiently to improve knowledge. At the same time, roadkill surveys can provide information about rare and poorly studied species. For example, the western mountain coati *Nasuella olivacea*, considered the least-studied carnivore of the world (Helgen *et al.*, 2009), was the second most roadkilled species in a study in Colombia (Delgado-V, 2007). Specimens collected as roadkill can contribute to understand the biology of organisms that can be difficult to study, making roadkill assessment valuable beyond the estimate of threats (Medrano-Vizcaíno & Brito-Zapata, 2021).

Latin America is not the only region where future road development and lack of road impact assessment coincide. In Asia, systematic studies focused on wildlife mortality have been conducted in only nine out of 48 countries, while in Africa there is systematic data for only five out of 54 countries (Silva *et al.*, 2021), yet rapid development of infrastructure is expected in these regions (Meijer *et al.*, 2018). While funds to assess potential risk may not always accompany road development plans, identifying regions where this research is particularly needed could aid in more directed investment of limited resources. Our approach could be applied to different taxonomic or functional groups and areas of the world to better plan research and conservation actions. Our study reveals priority areas and taxa that we hope would be valuable to ecologists, funding agencies, and decision makers in Latin America. Additionally, we provide a comprehensive dataset of predicted mortality rates of birds and mammals across all Latin America, which can be used to propose priorities for different taxonomic levels and geographic scales in this region.

3.6 Data availability statement

All data used for analyses including birds and mammals' vulnerability to roadkills and the grid for Latin America are available as appendices at:
https://doi.org/10.6084/m9.figshare.20166359.

Chapter 4. The road of >1000 corpses: landscape and road-related features that promote mortality in the Amazon

4.1 Abstract

Roads impact wildlife around the world; however, dedicated studies are lacking in many biodiverse areas such as the Amazon. Identifying which species are more often hit by vehicles and which landscape and road-related features promote roadkill is essential to guide future development and ensure adequate mitigation actions. For six months, we monitored 240 km of roads in the Ecuadorian Amazon and recorded 1,125 dead vertebrates (149 species). Reptiles were the most affected class with 380 individuals (56 species), followed by amphibians with 278 individuals (11 species), birds with 259 individuals (62 species), and mammals with 208 individuals (20 species). We used Random Forest models to explore the role of various land cover types and road sinuosity on the observed mortality. Additionally, we created heatmaps to visualize the road segments where roadkills were more frequent. For all vertebrates, mortality was more likely in straight road sections near rivers. The effect of other variables was taxa-specific. Amphibian mortality was more likely near bare soil or forest, birds and mammals died more often near herbaceous-shrubby vegetation, whereas reptile mortality occurred more often further from herbaceous-shrubby vegetation. Road segments with a high mortality (roadkill hotspots) varied across taxa. These hotspots identify areas where further research is needed to assess road impacts and where mitigation could prevent collisions. Among records, we found rare and threatened species, including some that may be new to science. Roadkill surveys not only aid in quantifying threats and informing future planning but can also provide insight into local biodiversity.

Keywords: Atractus, Caecilians, Ecuador, hotspots, roadkill, rare species.

4.2 Introduction

Roads are an important mortality source for wildlife around the world (Kioko *et al.*, 2015; Grilo *et al.*, 2020; Medrano-Vizcaíno *et al.*, 2022c); however, some regions with high biodiversity and expanding road networks are understudied (Silva *et al.*, 2021). For example, the Amazon is the most biodiverse wilderness area in the world (Sangermano *et al.*, 2012) but is threatened by growing human activities such as hunting, urbanization, agriculture, and roads (Viteri-Salazar & Toledo, 2020). The addition of over 12,000 km of new roads planned in this region for the next five years (Vilela *et al.*, 2020) may increase the risk of local extinction of several animal populations (Grilo *et al.*, 2021) and could lead to the disappearance of many undescribed species (Funk *et al.*, 2012).

Lack of local and regional information limits our understanding of how wildlife populations are affected by infrastructure and hinders sustainable development of new roads and effective mitigation plans. Conducting local studies is essential to better understand how species' traits and their habitat preferences, the natural and anthropogenic features of the landscape, the configuration of roads, and driver behaviour influence the incidence of road mortality and therefore guide future work and mitigation measures (Van Der Ree *et al.*, 2015a). For example, in Latin America, generalists, and short-lived birds and mammals with faster reproductive rates and living at higher population densities have been found to be more vulnerable to road mortality in general but distribution of these species varies regionally (Medrano-Vizcaíno *et al.*, 2022c). Road mortality has also been linked to neighboring habitat with distinct effects across vertebrate groups. For example, higher mortality occurs near water bodies in amphibians (Coelho *et al.*, 2012), near shrubs in birds (Carvalho *et al.*, 2014), near forests in mammals (Freitas *et al.*, 2015), and near grassland in reptiles (Braz & Rodrigues, 2016). Finally, the size and configuration of the road can also influence how animals use it (Mulero-Pázmány *et al.*, 2022) and their risk of collision leading to mortality (Ciocheti *et al.*, 2017).

Here we present an assessment of mortality among terrestrial vertebrates on roads in the Ecuadorian Amazon. First, we describe the species found as roadkill and calculate standardized roadkill rates. Second, we evaluated the role of distance and coverage of different land covers and road configuration on roadkill incidence and identified hotspots of mortality. We hypothesized different land covers would influence roadkill events differently based on how they affect animal abundance and movement and road traffic. In particular, we predicted a high incidence of roadkill closer to natural land types (forest and herbaceous-shrubby vegetation) where vertebrates are more abundant (Carrete *et al.*, 2009). We also predicted more roadkill of species that use human-dominated ecosystems near agriculture and urban areas where more road traffic should occur (Weisbrod *et al.*, 2003). In addition, because rivers provide water resources and individuals may approach them regularly (Seo *et al.*, 2013; Medrano-Vizcaíno, 2018), we expected higher animal movement in these areas leading to higher roadkill near rivers. We also predicted more roadkill in straighter roads particularly for species that do not show behavioural avoidance towards traffic (e.g., herpetofauna, Jacobson *et al.*, 2016). Vehicles generally circulate faster in straight (less sinuous) roads (Kang *et al.*, 2019) which may increase roadkill likelihood (Tejera *et al.*, 2018). On the other hand, sinuosity affects visibility which decreases the ability of drivers to prevent collision on the roads and therefore we may find more roadkills in sinuous road sections. To test these predictions, we collected roadkill data over a period of six months along 240 km of roads in the Napo province of Ecuador, an area of high endemism within the Amazon (Ribas *et al.*, 2022) and analysed the records at different taxonomic levels.

4.3 Methods

4.3.1 *Roadkill surveys*

Our work covered almost the entire network of primary and secondary roads in the province of Napo-Ecuador. These roads link main towns in the region and are near four protected areas: Antisana Ecological Reserve, Sumaco-Napo-Galeras National Park, Cayambe-Coca National Park, and Colonso Chalupas Biological Reserve, where the dominant ecosystems are: evergreen high montane forest of the north of the eastern Andes mountain range, evergreen montane forest of the north of the eastern Andes mountain range, evergreen low montane forest of the north of the eastern Andes mountain range, and evergreen piemontane forest of the north of the eastern Andes mountain range (MAE, 2013). The sampled areas covered an altitudinal range between 300 and 3,000 m a.s.l., with annual average temperatures ranging from 4.63 to 23.7 °C, and annual precipitation from 1100 to 3,400 mm (GADPN, 2018; FONAG -EPMAPS, 2021). All roads are paved, most of them have two lanes with a similar width and are mainly surrounded by agricultural land and forest.

From the 19th of September 2020 to 23rd March 2021 two people conducted roadkill surveys four-five days per week between 08:00 and 17:00 from a car driving at an average speed of 40 km/h (total 100 survey days). While monitoring, one member of the team was the designated driver while the passenger (always PMV) continuously scanned the road to locate carcasses on the pavement (roadkilled animals). Total study area covered 240 kms of primary and secondary paved roads. We monitored approximately half of this area in each survey day and each road section was surveyed approximately every three days. When a carcass was detected, the vehicle stopped on the side of the road on a safe area (both observers wore high-vis vests for safety). All carcasses were photographed onsite and georeferenced with a GPS unit (Garmin Etrex 22X). Then carcasses were either collected for a separate study or removed from the pavement to avoid doble counting. We aimed to identify all carcasses to species level but in some cases, the specimen was too deteriorated or differed from recognized species (potentially undescribed fauna). Taxonomic identification was based on observer knowledge, specialized guides (e.g., Ridgely, R.; Greenfield, 2006; Wallach *et al.*, 2014; Valencia *et al.*, 2016; Tirira, 2017). In some cases, further taxonomic evaluation was done in the laboratory at Universidad Regional Amazónica IKIAM.

4.3.2 *Land cover and road configuration*

We used available land cover data (Ministerio del Ambiente del Ecuador, 2018) in QGIS software v.3.18.2-Zürich (https://qgis.org/en/site/) to calculate proximity and percentage of six different land cover types (agricultural lands, bare soil, herbaceous-shrubby vegetation, human settlements, forest, rivers) for each roadkill record and control sites (geographical coordinates of random points across the surveyed road whose quantity was the same as the number of roadkill records). Control sites were at least 200 m from a recorded roadkill and a minimum of 50 m from another control site. We estimated Euclidean distances to the nearest feature (e.g., closest distance to a river) for all land covers and percentages of the dominant land covers (forest and agriculture) at two scales: circular areas centered on each roadkill and control site with radius of 100 m and 500 m.

We also evaluated how road configuration influenced roadkill, using road data from the Instituto Geográfico Militar (2017). For this, we calculated a sinuosity score for each roadkill and control site considering a section of 100 m of road with the site located in the center (as we did not know the travelling direction of the vehicle that caused the collision, we had to consider sinuosity in both directions). We then calculated the score as the actual length of the road divided by the Euclidian distance between the two ends. A straight road had a sinuosity score of 1. Other road features such as road width and number of lanes were not evaluated because surveyed roads were mostly identical.

4.3.3 *Data analyses*

First, we calculated the total number of roadkill (per class and for all identified species) and estimated standardized roadkill rates per species (ind./km/year) calculated as (total number of detected carcasses/240 km of road surveyed/185 days of survey period) *365 days. We applied machine learning Random Forest classification methods to identify features differentiating roadkill from control sites. This method ensembles multiple classification trees from a bootstrap sample of the original data, and shows higher classification accuracy than other methods such as logistic regression and linear discriminant analysis (Cutler *et al.*, 2007).

We created Random Forest models based on 1,500 classification trees using the R package "randomForest" and applying the function "randomForest" (Liaw & Wiener, 2002). We fitted a general model including data from all vertebrates, and class-specific models (i.e., amphibians, reptiles, birds, and mammals). We also fitted a model for fossorial species that included Caecilians and Amphisbaenians due to a previously known high prevalence of road mortality across this province (see Filius *et al.*, 2020; Medrano-Vizcaíno & Espinosa, 2021). In addition, for any group in which any single species or related species were overrepresented (>50% of the data within a taxonomic class), we also completed two additional analyses: 1) for the overrepresented taxa and 2) for all others in the taxonomic class. Models included 11 variables as predictors (proximity for each of the six land covers, percentage cover at 100 and 500 m for forest and agricultural land, and road sinuosity). We determined variables importance considering two measures: mean decrease accuracy and mean decrease gini index (means calculated across all fitted trees). Mean decrease accuracy is calculated as the loss of accuracy in the model when a variable (feature) is removed, while the mean decrease gini measures the impurity of nodes in split based on a variable (feature). We used the functions "important_variables" and "plot_multi_way_importance" from the R package "randomForestExplainer" (Paluszynska *et al.*, 2020) to calculate and plot these metrics. Additionally, we generated dependence plots to represent the probability of roadkill according to changes in the value of each predictor using the function "partial" from the R package "pdp" (Greenwell, 2017). This function allows visualization of the relationship between the response variable and a given feature including the interaction with the rest of features in the model.

In addition, we created heatmaps of roadkill based on kernel estimation density using QGIS software v.3.18.2-Zürich to visualize the areas where roadkills were more frequent in general (all vertebrates) and for each taxonomic class. This was done across the road network at a scale of 2 km radius and aggregated at two resolutions: 100 and 500 m. Then, we classified the heatmap data using a discrete interpolation into three classes of equal intervals, where the top tercile were identified as roadkill hotspots.

4.4 Results

We found 1,125 wildlife vertebrate carcasses representing at least 149 species (308 carcasses could not be identified to species level mainly because of their poor state). Reptiles were the most common with 380 individuals from 56 species (89.74 % of individuals and 91.5% of species were snakes), followed by amphibians with 278 individuals (11 species), birds with 259 individuals (62 species), and mammals with 208 individuals (20 species). The most roadkilled species was the cane toad *Rhinella marina* (208 carcasses: 1.71 ind./km/year), followed by the Andean opossum *Didelphis pernigra* (87 roadkills: 0.715 ind./km/year), the common opossum *Didelphis marsupialis* (48 individuals: 0.385 (ind./km/year), and the klebba's snail-eater *Dipsas klebbai* (47 individuals: 0.386 ind./km/year). The smooth-billed ani *Crotophaga ani* (23 individuals: 0.189 ind./km/year) was the most common roadkilled bird. Interestingly, we found numerous carcasses from fossorial organisms including Caecilians (40 individuals: 0.239 ind./km/year) and the speckled worm lizard *Amphisbaena bassleri* (29 individuals: 0.238 ind./km/year) (Table B.1 and Figure B.1).

Although most species found in this study (93.4%) are listed as Least Concern according to the IUCN red list (IUCN, 2022), we recorded carcasses from two species catalogued as Vulnerable: the bailey's blind snake *Trilepida anthracina* (one roadkill) and the giant armadillo *Priodontes maximus* (one roadkill); one catalogued as Near Threatened: the grey ground snake *Atractus occipitoalbus* (one roadkill); two listed as Data Deficient: the touzet's ground snake *Atractus touzeti* (two roadkills), and the Andean cottontail *Sylvilagus andinus* (one roadkill), and three not yet evaluated: *Dipsas klebbai* (47 roadkills), the giant Ecuadorian toad *Rhaebo ecuadorensis* (two roadkills), and the white-striped eyed lizard *Cercosaura oshaughnessyi* (one roadkill).

4.4.1 *How land cover and road configuration influence roadkill likelihood*

The general and class-specific models revealed consistency regarding some factors that explain road mortality but also considerable variation among groups (Figures 4.1 and 4.2). In all models, roadkill sites were more likely to be in straight roads (lower sinuosity) and near rivers than control sites. In general, distances to land cover were more important than percentages of cover to explain roadkill. However, the role of distance to land cover varied among taxa. While not always found to be a very important variable, we noted a general trend towards an increase in the likelihood of roadkill in areas with greater agricultural cover (Figure 4.1).

Class-specific models revealed that amphibians were more likely to be roadkilled on straight roads located near rivers, bare soil, or forest, and at intermediate distances from herbaceous-shrubby vegetation. Reptile mortality was higher on straight roads away from herbaceous-shrubby vegetation, and near rivers. Roadkilled birds were more often found across straight roads near herbaceous-shrubby vegetation, with less clear effects for other variables. Mammals were more likely to die from collisions on straight roads at low to intermediate distances from herbaceous-shrubby vegetation (Figures 4.1 and 4.2).

For amphibians we separately analysed data from the common cane toad (very common species) and other taxa. Distance to river was the most important variable in both models, and cane toads were more likely to be found as roadkill away from herbaceous-shrubby vegetation (Figures B.2 and B.3). Because of the high abundance of opossums (*Didelphis pernigra* and *Didelphis marsupialis*), we also analysed these separately from other mammals, but relevant factors were largely consistent in both models (Figs. B.2 and B.4).

Fossorial species that included caecilians and amphisbaenians were more likely roadkilled on straight roads located near herbaceous-shrubby vegetation, and rivers. Additionally, mortality likelihood was higher on roads near and with high cover of agricultural land and forest (Figure B.5).

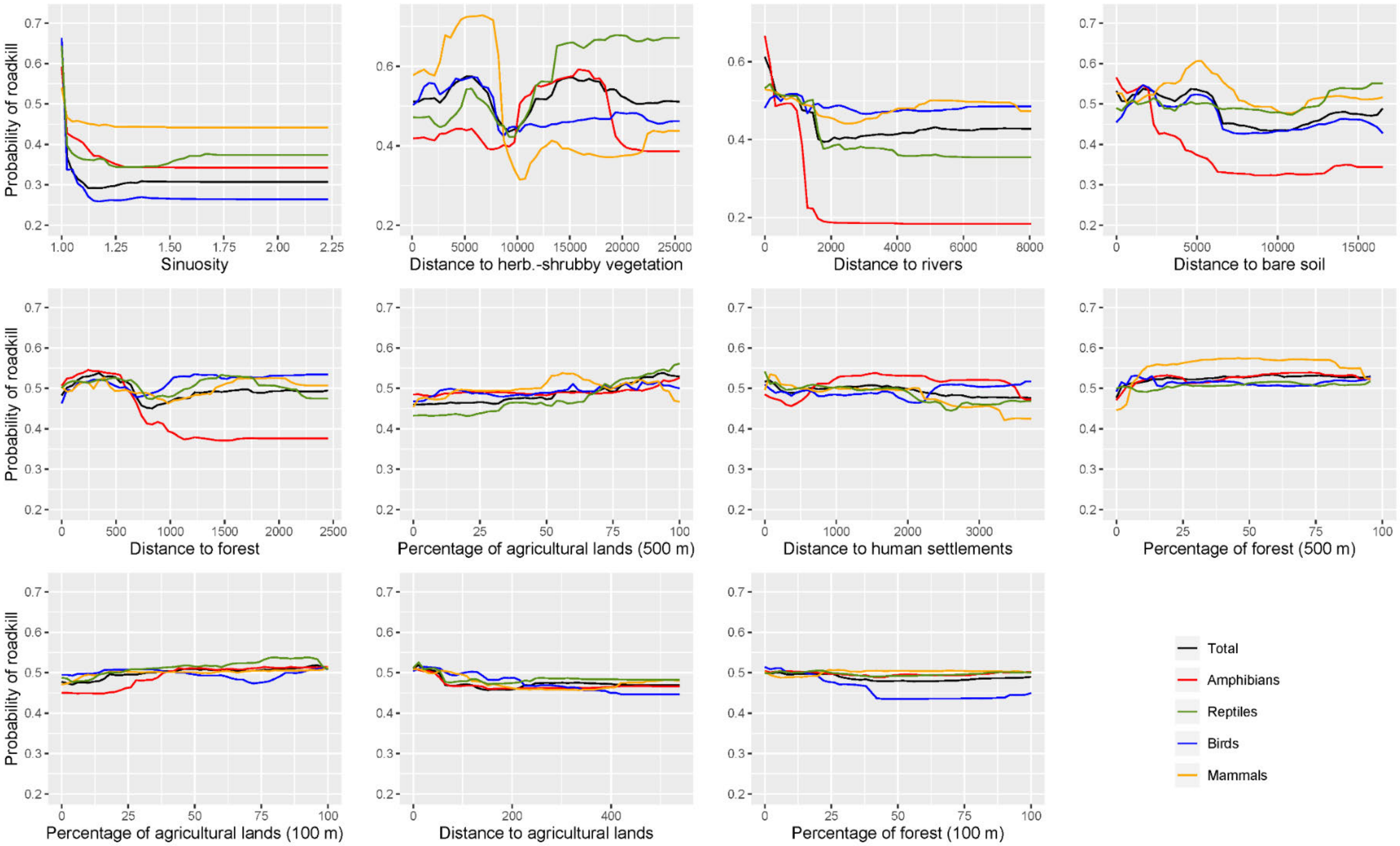


Figure 4.1 Dependence plots showing how land cover and road configuration influence probabilty of vertebrate roadkill in the Napo region of Ecuador. Plots are shown in descending order of variable importance for models fitted for all vertebrates (Total) and the separate taxonomic classes.

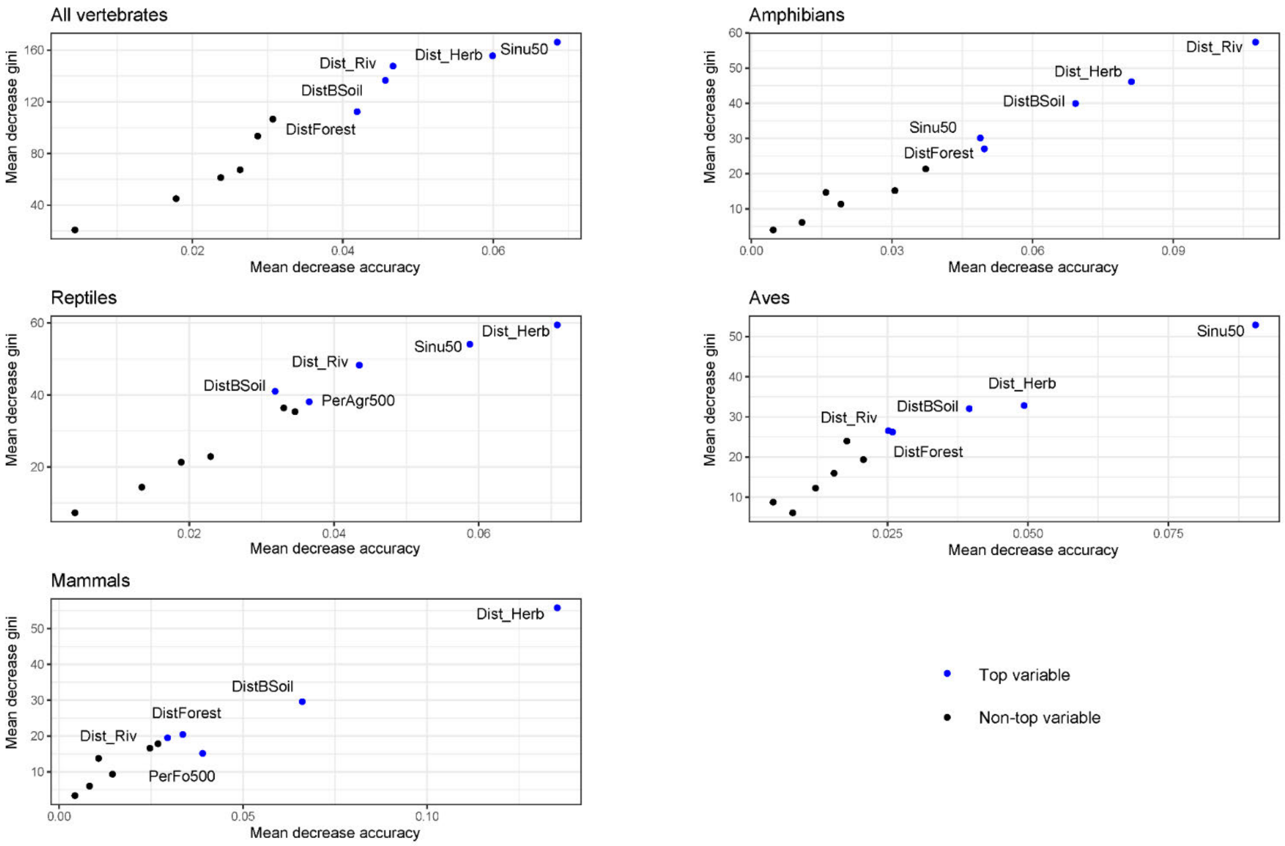


Figure 4.2 Variable importance from Random Forest models evaluating the effect of land cover and road configuration on vertebrate roadkill in the Napo region of Ecuador. Results are presented for models fitted for all vertebrates combined and for each taxonomic group. Variable importance is based on mean gini decrease and mean accuracy decrease. Labels indicate the five most important variables in blue dots (higher accuracy and gini decrease values). Sinu50=sinuosity (50 m), Dist_Herb=distance to herbaceous-shrubby vegetation, Dist_Riv=distance to rivers, DistForest=distance to forest, DistBsoil=Distance to bare soil, PerAgr500=Percentage of agricultural lands (500 m buffer), PerFo500=Percentage of forest (500 m buffer).

4.4.2 *Roadkill hotspots*

We identified several general roadkill hotspots (data from all vertebrates) particularly located around urban areas in Sala honda, Baeza, Oritoyacu, Puerto Napo, El Ansu, Santa Rosa and Buena Esperanza (Figure 4.3). At the lowest scale these areas had more than 3.7 detected carcasses in 100 m, with the larger scale reflecting over 25 vertebrates killed in the 2 km stretch over a 6-month period (~1 vertebrate killed per week). Some of these areas were also detected as hotspots when analysing data for each taxonomic class separately, but also new taxa-specific hotspots were detected. For amphibians (excluding *R. marina*), hotspots were near Oritoyacu, Urcusiqui, and Sarayacu towns (≥ 1.92 roadkills/100 m, ≥ 2.81 roadkills/500 m, and ≥ 4.45 roadkills/2 km. Figure B.6). Hotspots for *R. marina* were distinct and found at low altitude (between 373 and 569 m a.s.l.) near Puerto Napo, Sindy, Pucaurcu, Buena Esperanza, and Santa Rosa towns (≥ 2.37 roadkills/100 m, ≥ 5.58 roadkills/500 m, and ≥ 10.24 roadkills/2 km. Figure B.7). We identified three reptile hotspots near Sala honda, Baeza, and Santa Rosa (≥ 3.21 roadkills/100 m, ≥ 7.46 roadkills/500 m, and ≥ 12 roadkills/2 km. Figure B.8). There were four hotspots of bird roadkill, one near San Francisco de Borja and Baeza and the other three near Las Cavernas, Calmituyacu, El Ansu, Buena Esperanza, and Santa Rosa (≥ 2.23 roadkills/100 m, ≥ 2.83 roadkills/500 m, and ≥ 6.38 roadkills/2 km. Figure B.9). We found one hotspot of mammal roadkill (excluding opossums) near Baeza (≥ 1.29 roadkills/100 m, ≥ 1.81 roadkills/500 m, and ≥ 4.19 roadkills/2 km) which was near one of the four hotspots identified for opossums (the other three hotspots for opossums were near Sardinas, Sala honda, Oritoyacu, and Cuyuja. Figures B.10 and B.11).

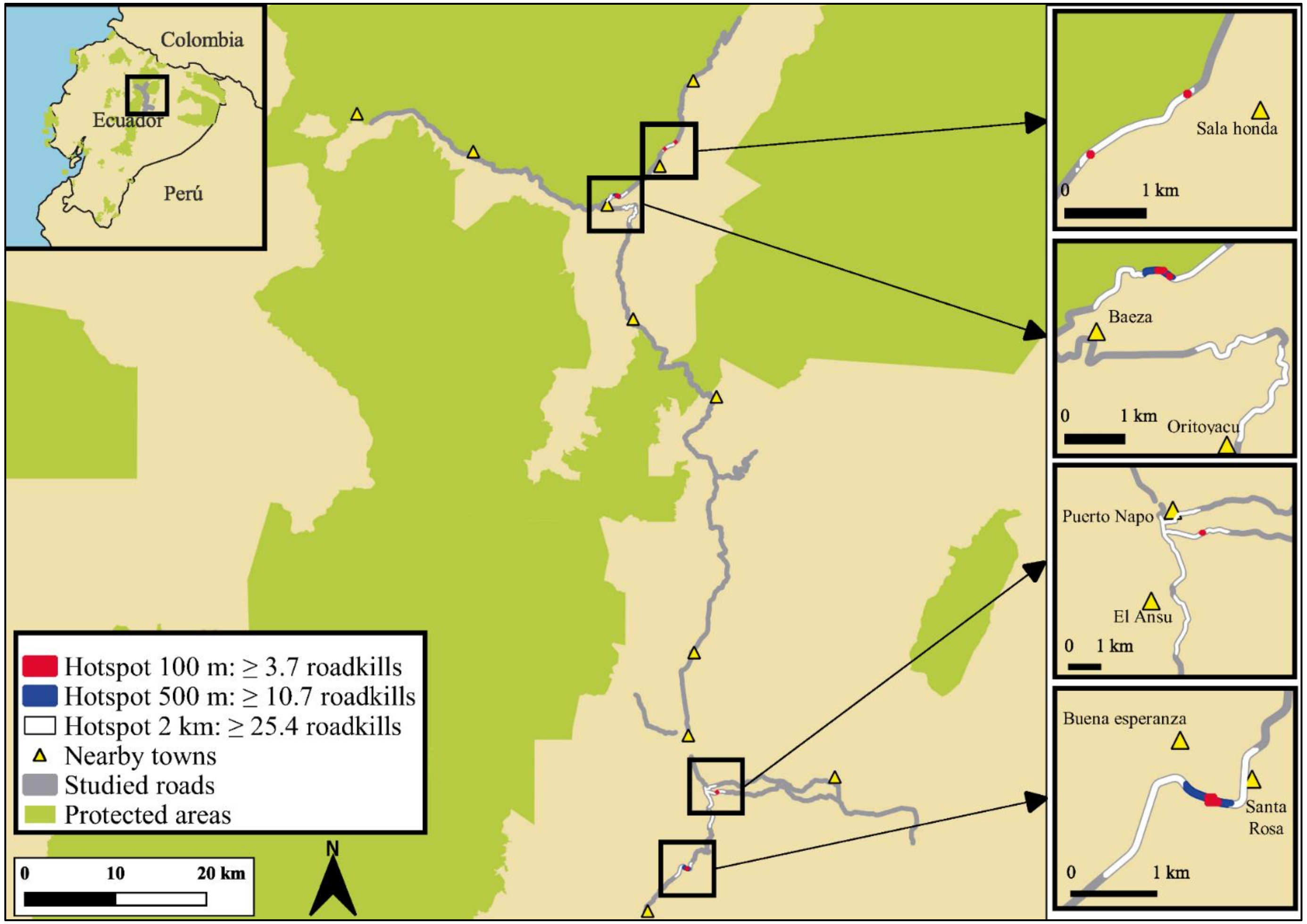


Figure 4.3 Roadkill hotspots across the 240 km of our study area in Napo when data for all vertebrates were combined (amphibians, reptiles, birds, and mammals).

4.5 Discussion

Our study in the Ecuadorian Amazon revealed a great diversity of wild tetrapods being killed on roads (149 species from 1,125 carcasses). This mortality followed some general spatial patterns for all vertebrates but also showed variation between classes and in some cases within classes. Consistently roadkill occurred more often on straight roads (lower sinuosity) near rivers for all vertebrates. Distances to herbaceous-shrubby vegetation, forest, and bare soil were also important predictors but their association with mortality were taxa-dependent. We additionally identified that the location of mortality hotspots varied among taxa but were mainly located near urban areas.

4.5.1 *Wildlife mortality*

We estimate approximately 9.25 vertebrates/km die on roads in this province each year. This number is likely an underestimate because individuals may be hit by vehicles but died away from the road and not all carcasses are detected during road surveys (Ogletree & Mead, 2020). Moreover, factors such as weather conditions, survey method and vehicle speed, and the destruction or removal of carcasses by predators, scavengers, and circulating vehicles influence detectability (Santos *et al.*, 2011).

Our roadkill list includes a great proportion of the species distributed in Napo province. The area hosts 85 amphibian species, 48 reptiles, 587 birds, and 134 mammals (Calles López, 2008). Roadkill affected 12.94% of these amphibians, all reptiles (in fact, we found more species than those previously described as present in Napo), 10.56% of birds, and 14.9% of mammals. Although it is evident that Napo roads are a noticeable source of mortality for many vertebrates, the fact that we recorded more reptiles' species than those already reported in the literature reflects the great biological richness of this area where the description of new species and distribution ranges is frequent (e.g., Medrano-Vizcaíno & Brito-Zapata, 2021; Melo-Sampaio *et al.*, 2021). However, it also reflects the major impact of roads on reptiles. Indeed, reptiles were the most commonly found in our study. This may relate to higher local abundance but also could reflect driver behaviour. Snakes (89.74 % of roadkilled reptiles in our study) can be intentionally killed by drivers (de Resende Assis *et al.*, 2020).

The mortality of threatened species was low, with single records for the giant armadillo and the bailey's blind snake, both catalogued as Vulnerable (IUCN, 2022). However, *Atractus* spp snakes were the most roadkilled genus of reptiles. This is a poorly studied genus of neotropical herpetofauna (Cisneros-Heredia, 2005), with 23% of the species distributed in Ecuador listed as threatened, and 47% as Data Deficient or remaining unassessed (Torres-Carvajal *et al.*, 2022). Additionally, caecilians, considered as the least known terrestrial vertebrates (Jared *et al.*, 2019), with 56% of the species distributed in Ecuador classified as threatened, and 28% as Data Deficient or remaining unassessed (Ron *et al.*, 2021), had the second highest mortality in amphibians. While detailed population assessments are needed to understand the impacts of road mortality, for threatened species even low roadkill rates can have major impacts on their populations (Grilo *et al.*, 2021). Our study suggests that roads in Napo could be affecting the vertebrate community, including threatened, poorly studied, and even undescribed species. Research and conservation actions should focus on threatened and poorly-known species, but a special effort to quantify risk for snakes seems a priority as road mortality of even few individuals can lead to local extinctions (Row *et al.*, 2007).

4.5.2 *How land cover and road configuration influence roadkill*

Road configuration was an important predictor in all cases, supporting our hypothesis that because vehicles circulate faster on straight roads, drivers are likely be less able to detect and avoid collision on these areas leading to a higher wildlife mortality. Our alternative hypothesis, that sinuous roads could prevent early detection was not clearly supported, although in some groups roadkill increased slightly with higher sinuosity. This could reflect a trade-off between speed and visibility: mortality is low in areas where speed needs to be reduced due to curves and visibility is still enough to detect and avoid some animals crossing roads. Also as hypothesized, generally for all vertebrates roadkill occurred more often in areas closer to rivers (see also Bastos *et al.*, 2019; Lala *et al.*, 2021). The need for individuals to access this vital resource can increase movement near water sources leading to greater mortality (Newmark *et al.*, 1996). Proximity to rivers was especially relevant for amphibians, which is not surprising given how many of these species breed and live in water (Ficetola & De Bernardi, 2004). This association has been described in other studies in temperate regions (e.g., Seo *et al.*, 2013; D’Amico *et al.*, 2015) and has led to suggestions that the construction of artificial water bodies near roads is avoided as it can particularly affect amphibian populations (Coelho *et al.*, 2012).

The effect of other land cover types was taxa specific. Proximity to bare soil or forest predicted amphibian roadkill, potentially linked to higher abundance and movement within suitable habitats (forests) and to increased movement when crossing largely unsuitable areas to access other resources (bare soil). Bird and mammal mortality was greater near herbaceous-shrubby vegetation (see also Plante *et al.*, 2019; Ferreguetti *et al.*, 2020). For birds, collision risk may increase with low or medium high vegetation as birds that fly from low vegetation to low vegetation likely stay closer to the ground and thus in the path of circulating vehicles increasing collision risk (Santos *et al.*, 2016). Likewise, shrubs near roads are used as nestling sites for some birds, and as refuge and foraging sites for birds and mammals, increasing their risk of mortality due to a close interaction with roads (Gunson *et al.*, 2011; Bravo-Naranjo *et al.*, 2019). Shrubby vegetation near road edges can also reduce visibility preventing drivers from noticing approaching birds or mammals in time to avoid collision and preventing animals from detecting travelling vehicles (Lala *et al.*, 2021). Additionally, areas with herbaceous-shrubby vegetation may attract animals, leading to greater local abundance and movement and thus, higher roadkill likelihood. These effects would increase when vehicles circulate faster (as expected on straight roads). Reptiles roadkill occurred more often close to human settlements and in areas of higher agricultural land cover, potentially because these areas often hosts high prey abundances (e.g., rodents, Stenseth et al., 2003). Finally, a higher mortality of fossorial species (caecilians and amphisbaenians) associated to herbaceous-shrubby vegetation, rivers, agricultural land and forest could be due to soils rich in organic matter across these land covers. These soils offer shelter, and harbor a great diversity of invertebrates, which can be attractive for these fossorial species whose diet is mainly composed by earthworms and insects (Amorim *et al.*, 2019; Jared *et al.*, 2019; Kouete & Blackburn, 2020). Nevertheless, soil compaction near roads could limit underground movements forcing fossorial animals to emerge to the surface, and consequently expose them to vehicular traffic (Maschio *et al.*, 2016).

4.5.3 *Roadkill hotspots*

Identified hotspots were generally in the vicinity of towns, likely due to increased traffic. When hotspots were defined for different classes (and even for different taxa within class) we found that locations were not consistent. This can reflect differences in animal behaviour, movement and abundance, as well as traffic patterns and driver behaviour, factors that can lead to different hotspots for various taxa (Teixeira *et al.*, 2013; Silveira Miranda *et al.*, 2020). Two roadkill hotspots were consistent with hotspots identified in a study conducted in 2014 (see Medrano-Vizcaíno & Espinosa, 2021). These areas were located near Baeza and Sala honda towns and could be potential sites for further research to assess how roadkill impacts long term population viability.

Road ecology studies not only provide information on wildlife mortality but can also expand our knowledge of local biodiversity. Records from our survey have already contributed to extend the geographic distribution ranges for six snake species rarely observed in the wild (*Anilius scytale, Drymarchon corais, Erythrolamprus breviceps, Micrurus lemniscatus, Oxyrhopus vanidicus, Trilepida anthracina*. Medrano-Vizcaíno & Brito-Zapata, 2021). Additionally, ongoing research could identify new species and extended geographic areas among roadkill specimens of caecilians and *Atractus* snakes, which are poorly-studied vertebrates with taxonomic uncertainties (Cisneros-Heredia, 2005; Wilkinson, 2012). A roadkilled specimen collected in a previous study in this area (Medrano-Vizcaíno & Espinosa, 2021) was described as a new snake species by Melo-Sampaio et al., (2021).

Our work, besides revealing how certain landscape and road features can drive to a higher mortality across the biodiverse Amazonian province of Napo, also provides a relevant insight into taxonomic groups and areas that need special attention and dedicated research to assess the impact of road mortality on their populations. The replication of our approach across other biodiverse areas could be considered as a first step of a framework of evaluation of the impact of roadkill on local extinction risk. We hope that this work can be useful to guide future research and conservation initiatives to favor wildlife populations.

4.6 Data availability

Datasets that include distances to landscape features, percentage of cover, and road sinuosity are available at https://figshare.com/s/b8ddfa99cef4a22698ee.

Chapter 5. First national assessment of wildlife mortality in Ecuador: an effort from citizens and academia to collect roadkill data at country scale.

5.1 Abstract

Ecuador has both high richness and high endemism of species which are increasingly threatened by anthropic pressures, including roads. However, research evaluating the effects of roads remains scarce, making it difficult to develop mitigation plans. Here, we present the first national assessment of wildlife mortality that allow us to 1) identify species and areas where mortality occurs due to collision with vehicles and 2) reveal knowledge gaps. We bring together data from systematic surveys and citizen science efforts in Ecuador to present a dataset with >5,000 wildlife roadkill records from 392 species. Systematic surveys were reported by ten studies conducted in five out of the 24 Ecuadorian provinces. Collectively they revealed 242 species with mortality rates ranging from 0.008 to 95.56 ind./km/year. The highest rates were for the yellow warbler *Setophaga petechia* in Galapagos (95.56 ind./km/year), the cane toad *Rhinella marina* in Napo (16.91 ind./km/year), and the small ground-finch *Geospiza fuliginosa* in Galapagos (14.11 ind./km/year). Citizen science and other no systematic monitoring provided 1,705 roadkill records representing all the 24 provinces of Ecuador and 262 identified species. The common opossum *Didelphis marsupialis,* the Andean white-eared opossum *Didelphis pernigra*, and the yellow warbler *Setophaga petechia* were more commonly reported (250, 104, and 81 individuals respectively). Across all sources we found 15 species listed as Threatened and six as Data Deficient by the IUCN. We suggest stronger research efforts on areas where mortality of endemic or threatened species could be critical for populations, such as in Galapagos. This first assessment of wildlife mortality on Ecuadorian roads represents contributions from several sectors including academia, members of the public, and government underlining the value of wider engagement and collaboration. We hope these findings and the compiled dataset will guide sustainable planning of infrastructure in Ecuador and ultimately, contribute to reduce wildlife mortality on roads.

Keywords: Biodiversity hotspot, citizen science, Galapagos, road ecology, threatened species, vertebrates.

5.2 Introduction

Ecuador is a small (283,561 km²) but highly biodiverse country hosting two biodiversity hotspots: Choco/Darien/Western Ecuador and Tropical Andes (considered to have the highest richness and endemism of vertebrates species of the planet; Myers et al., 2000). Approximately 659 amphibians species (Ron *et al.*, 2021), 500 reptiles species (Torres-Carvajal *et al.*, 2022), 1,699 birds species (Freile & Poveda, 2019), and 466 mammals species (Brito *et al.*, 2021) have been described in Ecuador. Likely, these numbers are highly underestimated as new species are still regularly identified (e.g., Leonan et al., 2022; Guayasamin et al., 2022; Brito et al., 2022) and cryptic species are probably common (Funk *et al.*, 2012). Ecuador is also a highly anthropized country transected by more than 16,647 km of primary and secondary roads (Meijer *et al.*, 2018) and a recent study estimated 420,861 birds and 119,599 mammals are roadkilled in Ecuador each year (Medrano-Vizcaíno *et al.*, 2022c). Additionally, with a further 1,555 km of new roads planned by 2030 (MTOP, 2016), roadkill numbers are predicted to increase by 9.3% (Medrano-Vizcaíno *et al.*, 2022c). High road mortality, high biodiversity, and limited information have contributed to propose this territory as a priority for road ecology research (Medrano-Vizcaíno *et al.*, 2023b). Indeed, systematic research (i.e., theses, and scientific papers) focused on wildlife mortality on roads is rare in this country. To our knowledge, the first study of this type was conducted in 2007, in the Galapagos Islands, but focused on a single species, the Galapagos lava lizard (*Microlophus albemarlensis*) (Tanner & Perry, 2007). The first study in continental Ecuador was conducted in 2014 (covering all tetrapods) (Medrano-Vizcaíno, 2015). Although a few additional studies have been published since then (González, 2018; Aguilar *et al.*, 2019; Filius *et al.*, 2020; García-Carrasco *et al.*, 2020; Zavala, 2020; Gaón & Valdez, 2021; Armendáriz, 2022), research is still limited. This paucity of road impact assessments is common to all Latin America (Pinto *et al.*, 2020), and poses a great challenge to assess potential threats for biodiversity.

Understanding the effects of roads on wildlife populations is key for conservation plans that include mitigation of current impacts, and to assess risk and inform the planning of future roads. However, conducting systematic roadkill surveys is costly and requires funding for fieldwork, which can be scarce or unavailable in developing countries. The continuous advancement of technology and internet access has provided the opportunity for new ways to gather data with citizens being involved in science projects as active collaborators. Citizen science is a valuable approach that engages diverse people, and offers a way to obtain field data without high costs (e.g., Mueller *et al.*, 2019; Medrano-Vizcaíno *et al.*, 2020). Data on road impacts obtained from citizen science projects can complement systematic surveys. Indeed, several citizen science projects have collected roadkill data and contributed to inform about the magnitude of road impacts on wildlife in different parts of the world (see Périquet *et al.*, 2018; Chyn *et al.*, 2019; Raymond *et al.*, 2021; Valerio *et al.*, 2021; Swinnen *et al.*, 2022). Moreover, involving the public with the collection of roadkill data offers an opportunity to provide environmental education and awareness in local communities (Vercayie & Herremans, 2015), which could significantly contribute to reduce wildlife mortality on roads.

The scarce and disperse information on road impacts in Ecuador has limited the potential to identify the areas and species most affected and likely has prevented the development of environmental policies to require consideration of road impacts during the planning of new infrastructures. As a first step to address the knowledge gap, we present a national database consisting of >5,000 roadkill records from systematic studies and non-systematic methods representing 392 wild species. Analysing this database, we identify species with high mortality that may require protection measures and reveal unstudied areas (research gaps) where citizen and scientific efforts are needed to best understand the impacts of roads on Ecuadorian wildlife. Compiling this data required collaboration among citizens and academics. We hope to raise awareness of the issue of road impacts on wildlife in Ecuador and encourage policy makers and researchers to work together to collect needed data to guide conservation plans and sustainable roads.

5.3 Methods

Data from systematic studies control and report sampling effort allowing to estimate standardized roadkill rates (number of individuals killed per area and time span) for different species and areas. However, these studies, and thus their data, are often limited to particular road sections and periods of time. On the other hand, citizen science data can cover wider spatial and temporal windows, but sampling is often not regulated or quantified making the definition of standardized estimates complicated. To avoid methodological issues, data obtained from these two approaches are presented separately.

5.3.1 *Records from systematic studies*

We searched for roadkill surveys conducted in Ecuador and described in peer-reviewed publications and theses using four search engines: Google Scholar, PubMed, Web of Science, and Scopus. This search was performed in English and Spanish (official language of Ecuador) using the keywords “roadkill Ecuador”, “wildlife mortality Ecuador”, “amphibians roadkills Ecuador”, “birds roadkills Ecuador”, “mammals roadkills Ecuador”, “reptiles roadkills Ecuador”, “snakes roadkills Ecuador”, “frogs roadkills Ecuador”, “atropellamiento fauna Ecuador”, “atropellamiento anfibios Ecuador”, “atropellamiento aves Ecuador”, “atropellamiento mamíferos Ecuador”, “atropellamiento reptiles Ecuador”, “atropellamiento serpientes Ecuador”, “atropellamiento sapos Ecuador”, “atropellamiento ranas Ecuador”. When a study was located, but the roadkill records were not available, we contacted authors asking to provide their data.

From each identified study we collected: taxonomic information of roadkilled organisms, number of roadkills per species or lowest identified taxonomic group, length of road surveyed, survey method (car, motorcycle, bicycle, or walking), time interval between surveys, total sampling period, and geographic coordinates of each roadkill record if available. As original records could include synonyms or obsolete species names, we additionally provided taxonomic information standardized using the IUCN nomenclature. We removed any records of domestic and farm animals. For each study we then calculated roadkill rates per species (only specimens identified to species were considered) by dividing the total number of records by the total length in kilometers of the surveyed road(s) and the total sampling period (days) (number of days between the first day until the last day of fieldwork). This value was converted to number of individuals per km per year (ind./km/year) multiplying by 365. Standardized rates allow comparison of road mortality among species and areas where systematic studies have been conducted.

5.3.2 *Citizen science and other records*

Our main source of data was our citizen science project: REMFA (https://remfa.webnode.co.uk/). This project started in September 2020 and is ongoing, for this study we capture records reported in the first 24 months (September 2020-2022). REMFA is an initiative that in addition to capture roadkill data seeks to promote environmental education on road ecology to citizens. Using word of mouth, traditional and social media we invited people to share photos and geographic location of roadkill events in Ecuador. Communications included emails, social networks (Facebook, Instagram and Twitter), messaging platforms (Whatsapp), and the mobile App Epicollect5 (dedicated project “Animales atropellados Ecuador”). We also compiled roadkill records from iNaturalist (https://www.inaturalist.org/), an open global online system where naturalists and citizen scientists share observations of biodiversity. Additionally, we included sporadic roadkill records found in the scientific literature but that had not been gathered via systematic surveys.

All records were checked (confirming species identification when photographs were available and location when GPS data were provided) and combined into a standardized electronic database. Without standardized methodologies and no information on monitoring effort, estimating roadkill rates, as done for systematic studies, was not possible. Instead, we summarize these data by reporting total number of records for identified taxonomic classes and species (when identified), and total for each Ecuadorian province.

For systematic and non-systematic data, when geographical coordinates of roadkill events were not available, we defined coordinates as the central point of the geographical reference provided by citizens or the published source. For these cases, we included an uncertainty value to reflect the potential error in the estimated coordinates based on the described area or road. This was given in km when we had information on the location of the road where the roadkill was found, or in km^2 when the road was not described but had information on the administrative area.

5.4 Results

In total we compiled 5,010 roadkill events from both systematic surveys and non-systematic studies (citizen science and other studies). Most of these (4,244) had accurate geographic information with GPS coordinates taken at the site where roadkill was found. In total, roadkill records represented at least 392 wildlife species. Most were non-threatened fauna, but two are listed as Critically Endangered, four as Endangered, nine as Vulnerable, and six as Data Deficient by the IUCN (IUCN, 2022). Nearly all records were tetrapods with relatively similar proportions of records in the four classes but marked differences in the diversity of species identified. Birds had the highest number of roadkills: 1,428 (28.50% of the total) representing 200 species, followed by reptiles with 1,356 records (27.06%) from 123 species, 1,326 records (26.47%) for 94 mammalian species, and 895 records (17.86%) for 36 amphibian species. We obtained five records via citizen science (0.1%) for two invertebrates classes, Malacostraca and Clitellata. This vertebrate bias does not reflect lack of roadkill among invertebrates but detectability and the most common target groups of systematic surveys and citizen science efforts.

Although amphibians had the lowest number of records, the cane toad *Rhinella marina* was the most roadkilled species with 532 records (more than half of the records for amphibians). The second and third most recorded species were two marsupials: the common opossum *Didelphis marsupialis* (n=454) and the Andean white-eared opossum *Didelphis pernigra* (n=336). A bird, the yellow warbler *Setophaga petechia* (n=193), and a reptile, the common green iguana *Iguana iguana* (n=126) were the fourth and fifth most roadkilled species.

5.4.1 *Systematic studies*

We compiled 3,305 wildlife roadkill records from ten systematic surveys conducted on Ecuadorian roads. These corresponded to five published papers, four theses, and data collected for Chapter 4 of this thesis. Georeferenced data were available for eight of these studies and comprised 2,744 roadkill records.

Systematic survey studies were not only rare, but also geographically biased (Figure 5.1), with three studies conducted in Napo province (Filius *et al.*, 2020; Medrano-Vizcaíno & Espinosa, 2021; Medrano-Vizcaíno *et al.*, 2022a), two in Galapagos (Tanner & Perry, 2007; García-Carrasco *et al.*, 2020), two in Guayas (González, 2018; Armendáriz, 2022), two in Manabi (Zavala, 2020; Gaón & Valdez, 2021) and one in Azuay (Aguilar *et al.*, 2019).

Collectively systematic studies surveyed only 2.7 % (454.5 km) of the 16,647.65 km of primary and secondary roads in Ecuador (Meijer *et al.*, 2018) and yet reported roadkill events for at least 242 species (i.e., not all individuals had been identified). Seven out of the ten studies reported data for all tetrapods, while two studies focused on birds, and one on a single species: the lava lizard *Microlophus albemarlensis.* Most records were for reptiles (965 from 76 species; 80 unidentified individuals) and birds (943 from 130 species; 224 unidentified individuals), followed by amphibians (717 from 24 species; 71 unidentified individuals), and mammals (680 from 52 species; 86 unidentified individuals). Roads were monitored using different methods: driving a car, a bicycle, a motorcycle and walking. Survey intervals varied between 1.4 and 7 days with the total survey period ranging from 27 to 425 days (Table 5.1).

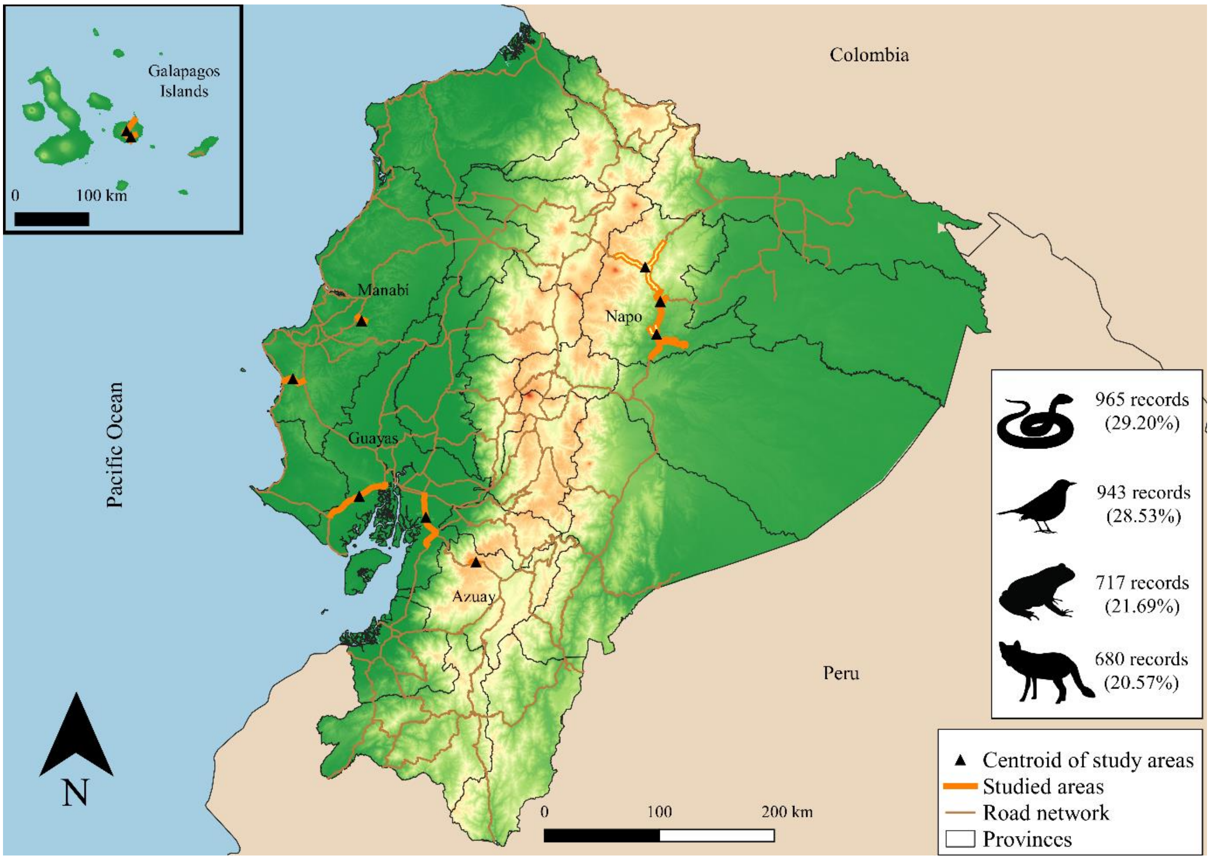


Figure 5.1. Ecuadorian road network (primary and secondary roads) highlighting the sites and roads where systematic studies were conducted and the total number of roadkill records per taxonomic class across all studies. For Napo province, Medrano-Vizcaíno *et al.*, (2022a) study area covers almost the whole province of Napo overlapping with the areas studied by Filius *et al.*, (2020) and Medrano-Vizcaíno & Espinosa, (2021), both shown in a clearer colour.

Table 5.1. Systematic roadkill surveys conducted in Ecuador. For each study we list: the province where data were collected, citation, studied taxonomic group, total number of roadkill records, length of road surveyed, survey period, survey interval, and survey method.

Province	**Study**	**Studied taxa**	**Roadkills (n)**	**Road length (km)**	**Survey period (days)**	**Survey interval (days)**	**Survey method**
Azuay	Aguilar et al., 2019	Birds	60	15	365	7	Walking
Galapagos	Tanner and Perry, 2007	*Microlophus albemarlensis*	71	40	27	7	Motorcycle
Galapagos	García-Carrasco et al., 2020	Birds	168	13.8	30	2	Bicycle
Guayas	González, 2018	Tetrapods	224	51	425	3.5	Car
Guayas	Armendáriz, 2022	Tetrapods	246	58.1	273	7	Car
Manabi	Zavala, 2020	Tetrapods	52	26.6	119	3.5	Car
Manabi	Gaón and Valdez, 2021	Tetrapods	321	10	91	1.4	Walking
Napo	Filius et al., 2020	Tetrapods	590	15.88	121	1.4	Bicycle
Napo	Medrano-Vizcaíno & Espinosa, 2021	Tetrapods	445	99	168	2.3	Car
Napo	Medrano-Vizcaíno *et al.* 2022a	Tetrapods	1,125	240	185	1.4	Car

Estimates of roadkill rates varied greatly across species (median=0.05 ind./km/year, SD=5.89 ind./km/year), with several taxa, particularly birds and reptiles, estimated to suffer high mortality rates in Galapagos and Napo (Table 5.2; Appendix 1). Although most roadkilled species were categorized as Least Concern by the IUCN Red List (IUCN, 2022), six species were found in a category of conservation concern, and two as Data Deficient (Table 5.3). While roadkill rates for these threatened species are not very high, due to their vulnerability, road mortality could pose a threat to their persistence. Although the tapeti (*Sylvilagus brasiliensis*), considered as Endangered, was reported in four studies with a median roadkill rate of 0.028 ind./km/year (range=0.008-0.79), it is possible that these records correspond to other *Sylvilagus* species, as the IUCN restricts the distribution of *S. brasiliensis* to Pernambuco-Brazil (IUCN, 2022).

Table 5.2. Top ten most roadkilled wildlife in Ecuador as reported in systematic surveys. We report taxonomic information (class, order and species name), IUCN Red List status, the number of studies in which that species was recorded, the estimated standardized roadkill rate (if a species was detected in more than one study, we report its highest rate), and the province for the reported roadkill rate.

Class	Order	Species	IUCN status	No. studies	Roadkill rate (ind./km/year)	Province
Aves	Passeriformes	*Setophaga petechia*	LC	1	95.56	Galapagos
Amphibia	Anura	*Rhinella marina*	LC	5	63.88	Manabi
Reptilia	Squamata	*Microlophus albemarlensis*	LC	1	24.00	Galapagos
Aves	Passeriformes	*Geospiza fuliginosa*	LC	1	13.65	Galapagos
Reptilia	Squamata	*Amphisbaena fuliginosa*	LC	2	11.68	Napo
Reptilia	Squamata	*Atractus collaris*	LC	1	8.67	Napo
Aves	Cuculiformes	*Crotophaga ani*	LC	6	6.83	Galapagos
Aves	Passeriformes	*Mimus parvulus*	LC	2	6.83	Galapagos
Reptilia	Squamata	*Atractus elaps*	LC	2	6.78	Napo
Reptilia	Squamata	*Atractus major*	LC	2	6.41	Napo

Table 5.3. Species listed as Threatened or Data Deficient by the IUCN Red List or not yet unassessed that were reported as roadkill by systematic surveys in Ecuador. We provide taxonomic information (class, order and species name), IUCN Red List status, the number of studies in which that species was recorded, the estimated roadkill rate (if a species was detected in more than one study, we report its highest rate), and the province for the reported roadkill.

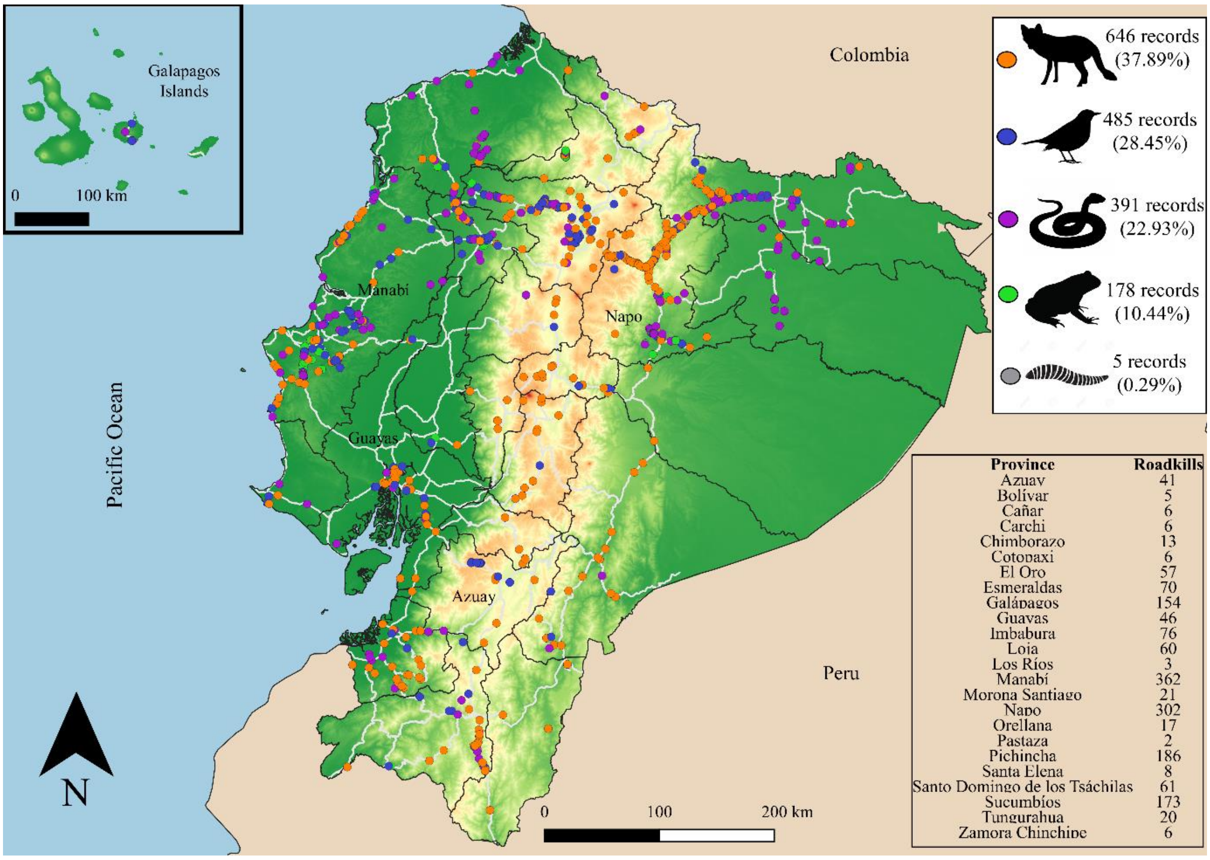
Class	Order	Species	IUCN status	No. studies	Roadkill rate (ind./km/year)	Province
Aves	Apodiformes	*Metallura baroni*	EN	1	0.13	Azuay
Mammalia	Lagomorpha	*Sylvilagus brasiliensis*	EN	3	0.79	Manabi
Reptilia	Squamata	*Coniophanes dromiciformis*	VU	1	1.19	Manabi
Mammalia	Carnivora	*Leopardus tigrinus*	VU	1	0.02	Napo
Mammalia	Cingulata	*Priodontes maximus*	VU	1	0.008	Napo
Mammalia	Squamata	*Trilepida anthracina*	VU	1	0.008	Napo
Mammalia	Rodentia	*Ichthyomys tweedii*	DD	1	3.17	Manabi
Reptilia	Squamata	*Atractus touzeti*	DD	1	0.02	Napo
Mammalia	Lagomorpha	*Sylvilagus andinus*	DD	1	0.008	Napo
Reptilia	Squamata	*Dipsas georgejetti*	NE	1	0.02	Guayas

5.4.2 *Citizen science and other records*

Citizen science and other non-systematic records provided a smaller sample of 1,705 roadkill records but offered a much wider geographical coverage than the systematic data with records from all 24 provinces of Ecuador (Figure 5.2). Most records were from Manabi province (362), Napo (302), and Pichincha (186). Records were mainly obtained from the REMFA citizen science project (698 records), iNaturalist (556 records), and from 7 scientific studies that reported 154 roadkill records collected sporadically, not in a systematic survey. Our initiative REMFA offers different ways to report roadkill and we found difference in frequency of use, with most data reported via Whatsapp (457 records), followed by the Epicollect App (165 records), Social networks (Facebook, Twitter, and Instagram; 73 records), and Email (3 records).

Among all non-systematic records, mammals were the most registered group (646 records, 63 species), followed by birds (485 records, 127 species), reptiles (391 records, 86 species) and amphibians (178 records, 22 species). Two marsupial mammals (*D. marsupialis and D. pernigra*) and the yellow warbler (*S. petechia*) were the most frequently reported species (250, 104, and 81 respectively. Table 5.4). Several species were reported from multiple locations and provinces, with *D. marsupialis* roadkill reports from 16 provinces, *D. pernigra* from eleven, *Tamandua mexicana* and *Conepatus semistriatus* from nine, and *Coragyps atratus* from eight provinces.

As with the systematic data, most records represented species classified as Least Concern by the IUCN Red List (IUCN, 2022) but there were twelve species of conservation concern, four currently listed as Data Deficient, and one not yet assessed by the IUCN (Table 5.5).



Province	Roadkills
Azuay	41
Bolívar	5
Cañar	6
Carchi	6
Chimborazo	13
Cotopaxi	6
El Oro	57
Esmeraldas	70
Galápagos	154
Guayas	46
Imbabura	76
Loja	60
Los Ríos	3
Manabí	362
Morona Santiago	21
Napo	302
Orellana	17
Pastaza	2
Pichincha	186
Santa Elena	8
Santo Domingo de los Tsáchilas	61
Sucumbíos	173
Tungurahua	20
Zamora Chinchipe	6

Figure 5.2. Location of all roadkill records compiled from citizen science and other sources and total number of records for each taxonomic Class and province.

Table 5.4. Top ten roadkilled wildlife in Ecuador based on citizen science and other sources (non-systematic studies). We report taxonomic information (Class, Order and Species names), IUCN Red List status, and the total number of roadkill records.

Class	Order	Species	IUCN status	N roadkills
Mammalia	Didelphimorphia	*Didelphis marsupialis*	LC	250
Mammalia	Didelphimorphia	*Didelphis pernigra*	LC	104
Aves	Passeriformes	*Setophaga petechia*	LC	81
Amphibia	Anura	*Rhinella marina*	LC	53
Reptilia	Squamata	*Atractus dunni*	NT	37
Aves	Cathartiformes	*Coragyps atratus*	LC	37
Mammalia	Carnivora	*Conepatus semistriatus*	LC	25
Reptilia	Squamata	*Iguana iguana*	LC	23
Reptilia	Squamata	*Boa imperator*	LC	23
Reptilia	Squamata	*Epicrates cenchria*	LC	20

Table 5.5 Species listed as Threatened or Data Deficient by the IUCN Red List or not yet unassessed that were reported as roadkill by citizen scientists and in non-systematic studies in Ecuador. We provide species name, IUCN Red List status, and the number of roadkill records.

Species	IUCN status	N roadkills
Chelonoidis porteri	CR	1
Pterodroma phaeopygia	CR	1
Porthidium arcosae	EN	3
Metallura baroni	EN	1
Tapirus pinchaque	EN	1
Ceratophrys stolzmanni	VU	7
Coniophanes dromiciformis	VU	4
Dipsas elegans	VU	2
Leopardus tigrinus	VU	2
Mazama rufina	VU	2
Alouatta palliata	VU	2
Chelonoidis denticulata	VU	1
Sylvilagus andinus	DD	7
Chironius flavopictus	DD	4
Coendou quichua	DD	2
Synophis calamitus	DD	1
Dipsas georgejetti	NE	13

5.5 Discussion

We compiled a large dataset that describes mortality due to wildlife-vehicle collision in Ecuador based on both systematic surveys and non-systematic records that came from citizen science and opportunistic observations reported in the scientific literature. Collectively, these data reveal that 13.66% of described vertebrate species from Ecuador are susceptible to die on roads, a number that likely underestimates the true impact of roads as not all areas are well sampled and smaller and cryptic species may be underreported. We see a need for additional systematic surveys, which can provide more comparable estimates, and have been few and limited to some areas. Likewise, citizen science reports are overrepresenting certain areas, with gaps in other regions.

We found that marsupials (*D. marsupialis*, and *D. pernigra*) represented more than a half of mammalian records (systematic and non-systematic) reported in 21 out of the 24 provinces. Their generalist habits (diet and habitat), together with increased abundance and intermediate body masses could be important factors for their high mortality (Medrano-Vizcaíno *et al.*, 2022c). Likewise, species with a scavenger or omnivorous diet such as the American black vulture *Coragyps atratus,* the smooth-billed ani *Crotophaga ani* and the groove-billed ani *Crotophaga sulcirostris* comprised a great part of the avian records. The common green iguana *Iguana iguana* was the most common roadkilled reptile. While this is mainly a herbivore, iguanas have been recorded feeding on carcasses of deer and opossums (Anderson & Enge, 2012). Roadkilled animals and invertebrates that get attracted to these carcasses can be a food source for scavengers, which attracted to road then have a higher probability of being hit by a car.

Among the systematic studies we estimated particularly high standardized mortality rates in some cases. Studies in Galapagos (García-Carrasco *et al.*, 2020), Napo (Filius *et al.*, 2020), and Manabi (Gaón & Valdez, 2021) where roadkill rates were disproportionally high in comparison with other studies were all conducted in small areas (13.8, 15.8, and 10 km, respectively). These areas could be hotspots of mortality that do not represent rates across wider areas (overestimate risk). On the other hand, roads in these studies were surveyed by bicycle. Slower survey methods like bicycle or walking can result in higher detectability, 8.4 times higher than using a car (Wang *et al.*, 2022). Therefore, these studies may be better capturing the true mortality, at least for those smaller areas.

How road impacts wildlife in areas of biological importance should be further studied. For example high road mortality for endemics of the Galapagos Islands could threaten population persistence (Wiedenfeld, 2006; Tejera *et al.*, 2018). In this territory, threatened species comprise 20 out of 43 birds species, 18 out of 42 reptiles species, and 6 out of 9 non-cetacean mammals species (IUCN, 2022), for which the impacts of roads on their populations remain unexplored. But also non-threatened species may be impacted. For example, the yellow warbler *Setophaga petechia*, that was commonly reported as roadkill, has experienced a dramatic population decline, and although low insect abundance due to intense use of herbicides has been associated with this decline (Dvorak *et al.*, 2012), road mortality could contribute to further declines (or already be a factor). While there have been two systematic studies in Galapagos these offer limited insight as one focused on a single species focus (Tanner & Perry, 2007) and the other represented a single one-month survey (García-Carrasco *et al.*, 2020). The real impact of roads on wildlife populations in Galapagos remains unknown and considering the high endemism and quantity of threatened species, we think that this area deserves special attention for research.

We found roadkill records for 15 Threatened and six Data Deficient species, with some of them showing repeatedly in different regions of Ecuador. The Peters' running snake *Coniophanes dromiciformis,* is a Vulnerable and little known species recorded in only nine locations of Ecuador (Cisneros-Heredia, 2021). This range-restricted species showed a roadkill rate of 1.19 ind./km/year in Manabi, with an additional four non-systematic records. Another worrying case is the Violet-throated Metaltail *Metallura baroni*. This bird, catalogued as Endangered and endemic to Azuay and Cañar provinces, is known from five locations (BirdLife International, 2016), and we estimated roadkill mortality of 0.13 ind./km/year in Azuay, with an additional non-systematic record. In addition, we gathered seven non-systematic records of the pacific horned frog *Ceratophrys stolzmanni* in Manabi, a Vulnerable and rare species whose entire population is distributed in less than eight subpopulations (IUCN SSC Amphibian Specialist Group, 2018). Directing research efforts on roads within the distribution areas of these range-restricted species could be important to determine the impact of roads on their populations.

Additionally, poorly studied organisms such as Caecilians and *Atractus* sp. snakes were also regularly reported as roadkill. These animals are among the least known vertebrates, with several taxonomic uncertainties and unknown conservation status for many of them (Cisneros-Heredia, 2005; Wilkinson, 2012; IUCN, 2022). Both groups were frequently detected across three studies in Napo (Filius *et al.*, 2020; Medrano-Vizcaíno & Espinosa, 2021; Medrano-Vizcaíno *et al.*, 2022a), and non-systematic data included 41 records of *Atractus* sp. snakes. In a survey conducted in 2014, Medrano-Vizcaíno & Espinosa (2021), found two roadkilled individuals attributed to the genus *Atractus* in Napo that were latter described as new species (described by Melo-Sampaio *et al*., 2021; Arteaga *et al*., 2022). Road ecology research in areas with poorly-known and undescribed species would be valuable, both to quantify impacts but also to potential expand our understanding of these species.

Our study offers an overview of wildlife mortality on Ecuadorian roads, but likely underestimates the impact. We obtained roadkill records from all the 24 provinces in Ecuador, but systematic studies were only available for five, and non-systematic data was disproportionally distributed in provinces like Manabi, and Napo (more than 300 records for each province). While in Bolivar, Cañar, Carchi, Cotopaxi, Galapagos, Los Rios, Pastaza, Santa Elena, and Zamora Chinchipe, we gathered less than ten records per province. Our results also show that citizen science data can complement systematic studies and identify top roadkill species (like *D. marsupialis, D. pernigra, S. petechia,* and *R. marina*) that were both commonly reported in non-systematic and systematic data from Galapagos, Manabi, Guayas and Napo (González, 2018; Filius *et al.*, 2020; García-Carrasco *et al.*, 2020; Zavala, 2020; Gaón & Valdez, 2021; Medrano-Vizcaíno & Espinosa, 2021). We hope by expanding our network of citizen scientists in REMFA we will be able to fill geographical gaps of information, and gain insights into wildlife mortality in areas where systematic studies are lacking.

A limitation in out compiled dataset is the assumption of correct taxonomic identification in systematic studies (and our own correct identification in non-systematic records). For example, some species were reported outside their known distribution ranges in Ecuador: *Neacomys amoneus, Mesoclemmys heliostemma, Myrmochanes hemileucus, Pseudocolopteryx acutipennis, Rhogeessa io, Scinax ruber,* and *Xenoxybelis argenteus,* and even one species not distributed in Ecuador was reported (*Rhinella arenarum*). These may be misidentifications (likely for *R. arenarum*), but previous roadkilled specimens have revealed new distribution areas for certain species (Medrano-Vizcaíno & Brito-Zapata, 2021). Roadkill records can provide valuable information about the biology and ecology of species and with correct taxonomic identification contribute to our understanding of biodiversity.

Road ecology research in Ecuador is gaining interest but is still relatively limited. Promoting and guiding additional research and public engagement is important. Through our citizen science project REMFA we have given a special relevance to science communication, which has been vital to reach citizens to join our work, and we are now engaging with policy makers. The active involvement of government ministries such as the Ministry of Environment, Water and Ecological Transition, together with the Ministry of Public Works is necessary for the inclusion of adequate policies to reduce wildlife mortality across existing roads, and to plan sustainable roads for the future. We hope that this work can be an initial step towards these national aims for wildlife conservation.

5.6 Data availability

We provide 333 roadkill rates calculated for 242 species found in systematic studies and a database of 5,010 roadkill records (4,244 with accurate geographic coordinates taken at the roadkill site) from 392 species at: https://doi.org/10.6084/m9.figshare.21313650.v1

Chapter 6. Discussion

Mortality on roads negatively impacts many Latin American and Caribbean wildlife species; however, some organisms and areas harbour particularly high mortality rates. This thesis shows that the analysis of wildlife traits, habitat preferences, and landscape and road-related features can be valuable to explain mortality patterns, evaluate the impacts of roads on wildlife, and guide future research and protection actions. Additional efforts that included the involvement of citizens and government institutions showed to be complementary factors for evaluation, and essential for raising awareness about wildlife mortality on roads.

6.1 Thesis overview

In this thesis, a large compilation of wildlife roadkill rates from studies conducted in Latin America and the Caribbean, allowed the identification of intrinsic characteristics of species such as diet, life-history, habitat preferences, and morphological traits, as important drivers of road mortality for wild vertebrates (chapter 2). Further analyses of these data (chapter 3) allowed recognizing areas in Latin America that should be prioritised for research (unstudied zones with many roads and species susceptible to roadkill), such as Central America and North-western South America, and priority areas for conservation (zones with many species susceptible to roadkill but a low road density) such as the Amazon. We were also able to propose groups, such as vultures and armadillos, as conservation priorities, and cuckoos and shrew opossums as research priorities (chapter 3). Chapter 4 focused on a smaller scale within an identified conservation priority area (the Amazon) and presents data results from a fieldwork campaign that found 1,125 roadkilled animals over a 100 days-survey across 240 km of roads. Roadkilled animals included rare and threatened species. The analysis of the spatial distribution of these roadkill records together with land cover and road feature data revealed key drivers of mortality including road sinuosity and the proximity of rivers, forest, and herbaceous-shrubby vegetation. Although there was considerable variation among studied taxa. Finally, chapter 5 presents a unique dataset of roadkill records for the country of Ecuador (which included data from a citizen science project that I founded and lead in this country). Evaluation of these data allow us to identify species with a high reported mortality, and spatial and taxa information gaps. The work conducted in this chapter highlights the importance of joint efforts from academia, citizens and government institutions for road ecology projects (chapter 5).

6.2 A widely applicable approach

In 2018, spatial and species level predictions of road mortality risk for Brazilian wildlife were developed using species traits and their distribution areas (González-Suárez *et al.*, 2018). This methodology was later replicated for European wildlife (Grilo *et al.*, 2020), demonstrating the wide applicability of this approach. Our predictive models of mortality rates for Latin American birds and mammals (chapter 2) used the same approach; however, we included additional traits variables, and considered the habitat preferences of species (which has not been included in the previous research for Brazil and Europe). The inclusion of habitat preferences was especially important to refine spatial predictions, offering a more accurate and realistic approach that considers habitat suitability of species. We also refined on the proposed approach limiting predictions to species known to be involved in wildlife-vehicle collisions, while previous studies have predicted mortality rates for all present species. Even with this conservative approach, we predicted millions of birds and mammals dying on Latin American roads each year emphasizing the considerable impact of roads on wildlife.

Taking a step further, we then used the predictions from these models to inform on areas and species that need special attention for research and protection (chapter 3). This approach could be applied to other regions and be valuable especially for understudied and potentially impacted regions of the planet. For example, this approach could be applied in Asia and Africa where future road development is also likely to be substantial, yet road ecology research in these continents is limited. Recent summaries suggests data from systematic road surveys are only available for nine out of 48 countries in Asia and in five out of 54 countries in Africa (Meijer *et al.*, 2018; Silva *et al.*, 2021). Predictive models of wildlife mortality risk can offer a tool to characterize large scale mortality patterns, which combined with information on roads and conservation status of species could be used to determine research and conservation priorities (as done in chapter 3). As in Latin America, the limited resources and funding in many countries in Africa and Asia can be a challenge to conduct research. Identifying focal priorities for research could contribute to a more strategic allocation of resources. Additionally, the identification of areas where the development of roads could be critical for wildlife (priority areas for conservation) can be important to limit roads construction in these areas, which can potentially avoid catastrophic losses of biodiversity.

Our analyses for Latin America and Caribbean also allowed us to identify certain taxonomic groups of birds and mammals for which research and conservation efforts should be reinforced. This type of knowledge is also urgent for other taxonomic groups. For example, in Africa, amphibians are the least studied taxonomic group in road ecology, followed by reptiles and insects (Collinson *et al.*, 2019). In Taiwan (Asia), snakes were the most reported animals (Chyn *et al.*, 2019), while in Latin America, although amphibians and reptiles are less studied than birds and mammals, they seem to suffer a higher mortality (Pinto *et al.*, 2020). Road mortality can be a great threat for many organisms, but reptiles seem to be especially vulnerable, as even low mortality rates can lead to a higher risk of local extinction (Row *et al.*, 2007). On the other hand, to our knowledge, there are no published studies evaluating road mortality of insects in Latin America, which is not a surprise as this topic is practically unexplored. Although just a few studies have been conducted in the planet (e.g., Shyama Prasad Rao & Saptha Girish, 2007; Baxter-Gilbert *et al.*, 2015), they have shown extremely high mortality rates. Road ecology studies are urgently needed for amphibians, reptiles, and insects, nevertheless, lack of data on biological, and ecological aspects of some organisms may limit the development of evaluation tools.

6.3 Constructing more informative models

Trait-based approaches can be useful to identify general patterns but often can be limited by data availability (González-Suárez *et al.*, 2012). The development of these comparative models can be challenging when data availability for predictor variables is limited. Availability of trait data, for example, can be incomplete even for well-studied organisms (Kissling *et al.*, 2014), and although the use of imputed data can be an alternative to fill these gaps (when incompleteness is not so high) (Johnson *et al.*, 2021), large scale analyses can include several poorly known species, for which, evidently, trait data can be widely unavailable.

We acknowledge that our analyses missed potentially important traits related to roadkill risk that could have enhanced our models in chapters 2 and 3. Variables related to mobility patterns are expected to be important drivers of mortality (Rytwinski & Fahrig, 2012). For example, home range was among the top predictors for mammals mortality in Brazil (González-Suárez *et al.*, 2018), Europe (Grilo *et al.*, 2020), and Latin America (this thesis), where, species with smaller home ranges showed higher mortality rates. Nevertheless, insufficient home range data did not allow this evaluation for birds, likewise, similar limitations could be found when developing models for amphibians, reptiles and insects.

The analysis of road avoidance behaviour is another relevant aspect to consider. The different responses that species exhibit in relation to the presence of roads and traffic can influence on their patterns of movement, distribution and habitat occupancy (Paterson *et al.*, 2019; Duffett *et al.*, 2020). For example, while impalas *Aepyceros melampus* show an apparent habituation to roads presence (Mulero-Pázmány *et al.*, 2016), other organisms such as the red deer *Cervus elaphus*, the wild boar *Sus scrofa,* and jaguars *Panthera onca* prefer areas away from roads (D'Amico *et al.*, 2016; Espinosa *et al.*, 2018). Other species can even contract their home ranges to avoid these infrastructures, such as Florida panthers (*Puma concolor coryi*), and bobcats (*Lynx rufus*) (Cramer & Portier, 2001). Therefore, the analysis of road avoidance could be key for the development of more accurate models that identify species and areas vulnerable to roadkill. Unfortunately, the limited knowledge on this topic for all taxonomic groups represents a great barrier to considering this likely important information.

Additionally, models could be improved with better information on species distribution and habitat requirements. The high cryptic diversity in biodiverse areas can result in inaccurate information about species distribution, habitat selection, and conservation status (Funk *et al.*, 2012). Locality data are increasingly available via online repositories of observations such as the Global Biodiversity Information Facility (GBIF) (http://www.gbif.org/). However, information is still variable across regions and sometimes inaccurate. While roadkill is not desirable, particularly for rare or threatened species, roadkill data can also fill these information gaps, even for poorly known species (Schwartz *et al.*, 2020; Medrano-Vizcaíno & Brito-Zapata, 2021).

6.4 Road ecology studies as a source for new information on species

Data collected during road ecology research can provide important information about the biology, ecology, and other aspects of wildlife. For example, while conducting road surveys I observed a laughing falcon *Herpetotheres cachinnans* decapitating a venomous snake (*Bothrops atrox*) before ingesting the rest of the body; showing a possible learnt behaviour to avoid injury from venomous prey (Medrano-Vizcaíno, 2019). During the surveys I conducted in Napo-Ecuador I also noticed that the yellow-headed caracara *Milvago chimachima* was frequently placed at tree branches on roadsides, and a couple of times this bird was hunting caecilians that were trying to cross roads. It is possible that this species has learned an efficient way to obtain food. Interestingly, despite of their close interaction with roads, we did not find any roadkilled individuals, which may also reflect learnt behaviour to avoid incoming vehicles. To my knowledge, there are no previous reports of caecilians being preyed by this falcon, therefore this observation enriches our knowledge on the natural enemies of caecilians and the diet of the yellow-headed caracara. Other studies also show that data collected on roads can be useful to learn about diets. Roadkilled individuals have been used to describe the diet of species such as the European badgers (*Meles meles*) in southeast and central regions of Norway (Gomes *et al.*, 2020), the endangered snakes: Western Rattlesnake (*Crotalus oreganus*) and Gophersnake (*Pituophis catenifer*) in British Columbia-Canada (McAllister *et al.*, 2016), and the crab-eating fox *Cerdocyon thous* in Brazil (Dutra-Vieira *et al.*, 2021).

Roadkill records can also provide information on wildlife distribution ranges. In Romania, new distribution areas were described for the javelin sand boa *Eryx jaculus* using a roadkilled individual (Covaciu-Marcov *et al.*, 2012). In Colombia, a roadkilled individual provided new information about the distribution of the rarest carnivore of the neotropics, the Colombian weasel *Mustela felipei* (only eight records are known from Colombia and Ecuador) (Pisso-Florez *et al.*, 2022). Roadkill records collected for this thesis in Napo-Ecuador were used to report new distribution areas for six snake species for which natural history information is scarce (*Anilius scytale, Drymarchon corais, Erythrolamprus breviceps, Micrurus lemniscatus, Oxyrhopus vanidicus, Trilepida anthracina*) (Medrano-Vizcaíno & Brito-Zapata, 2021).

Additionally, samples collected on roads can be used for genetic and parasitological studies without incurring in unethical procedures that could harm live animals. For example, in Texas-USA, and Tamalipas-Mexico, the population status and genetic diversity of the hog-nosed skuns *Conepatus leuconotus* was studied using samples from roadkilled individuals (Holbrook *et al.*, 2012), and a similar research was conducted in Sao Paulo-Brazil for pumas (*Puma concolor*) (Miotto *et al.*, 2011). Roadkilled individuals have been used also to determine parasite load of wild species. For example, for the jaguarundi *Herpailurus yagouarundi* in Santa Catarina-Brazil (de Quadros *et al.*, 2014), the giant anteater *Myrmecophaga tridactyla* in Minas Grais-Brazil (Frank *et al.*, 2012) and the crested porcupine *Hystrix cristata* in Tuscani and Latium-Italy (Mori *et al.*, 2015). As part of my research I also started a collaboration for which I collected 592 roadkilled individuals from 102 species that currently comprise a biobank of 820 samples of muscle, blood, bones, and organs (brain, intestine, heart, liver, lung and spleen). We obtained funds in Ecuador to study parasites and microbiome from these samples (https://www.cedia.edu.ec/financiamiento-a-proyectos-cepra/estudio-de-parasitos-y-microbioma-de-fauna-silvestre-en-dos-de-las-zonas-mas-biodiversas-del-planeta-los-andes-tropicales-y-choco-darien-en-ecuador), which also provides the opportunity to conduct future genetic analyses.

Roadkilled animals have been also used to discover new species. This was the case of the nightjar *Caprimulgus solala* in Ethiopia, (Safford *et al.*, 1995), and two snakes in Ecuador: *Atractus ukupacha* and *Atractus zgap*. These last two species were described by Melo-Sampaio *et al.*, (2021), and Arteaga *et al.*, (2022) based on roadkilled individuals that I found in 2014 in Napo-Ecuador (Medrano-Vizcaíno, 2015). While conducting fieldwork for chapter 4, we found some specimens in Napo-Ecuador that correspond to caecilians and snakes from the genus *Atractus* and are currently being studied as possible new species.

As evidenced, road ecology provides many opportunities to develop parallel studies that could provide a better knowledge on wild species. Data obtained during road ecology fieldwork such as geographical coordinates of roadkills, number of roadkills per species, morphological measures from individuals, or even organs weight can be valuable data for further research. Therefore, the compilation of these data and their publication as datasets could facilitate new analyses that allow understanding ecological processes in a better way.

6.5 Availability of data and future research

The consolidation and publication of large databases offer the possibility to have diverse analyses from researchers with different backgrounds and perspectives, which can contribute to a better understanding of nature. For example, the availability of compiled trait datasets of birds and mammals (e.g., Jones *et al.*, 2009; Wilman *et al.*, 2014; Myhrvold *et al.*, 2015) facilitated analyses for chapters 2 and 3 of this thesis, and in other studies these compilations were also used to predict roadkill risk for birds and mammals in Brazil (González-Suárez *et al.*, 2018) and Europe (Grilo *et al.*, 2020). The analyses of these trait datasets have been also important to understand other ecological processes such as the movement ecology of mammals (Tucker *et al.*, 2018), wildlife nocturnality (Gaynor *et al.*, 2018), and extinction risk (Yusefi *et al.*, 2022). Likewise, roadkill datasets have been valuable to expand our knowledge on road ecology. For example, a large roadkill dataset of Brazilian wildlife published by Grilo *et al.*, (2018) has so far been used to: estimate the mortality of mammals at a national scale in Brazil (Pinto *et al.*, 2022), analyse the location of roadkill hotspots over time (Dávila-Orozco *et al.*, 2022), and identify potential areas for mitigation for felids (Cerqueira *et al.*, 2021). These works generated relevant information to inform potential actions to protect wildlife, highlighting the importance of publishing and making data freely available.

As part of this thesis, we generated eight datasets that have been made fully available to facilitate Open Research. These could be used by other researchers and aid in developing conservation actions. From chapter 2, we generated a dataset of 1,691 roadkill rate estimates that represents 346 bird species and 159 mammal species that were obtained from 85 studies representing 12 Latin American countries (Medrano-Vizcaíno *et al.*, 2022b). Chapter 3 resulted in four datasets: two with estimations of predicted vulnerability for 500 species of birds and mammals across 1,989 1° x 1° grid cells covering Latin America (one dataset for birds and another for mammals), and two more with predicted vulnerability of each bird and mammal species per cell (one dataset with 201,319 estimates for birds, and another with 71,839 estimates for mammals) (Medrano-Vizcaíno *et al.*, 2023c). Future research could involve the analysis of these data at different spatial and taxonomic levels. For example, for chapter 2, mortality rates were predicted for 1° x 1° grid cells, but if a regional or country-scale level was preferable, different scales could be used. Similarly, in chapter 3, we identified priority taxa for research and conservation at taxonomic order level; however, additional analyses could identify priorities at any other taxonomic level such as family, genus, or even species level, depending on research aims.

From chapter 4, we generated detailed data for 1,125 roadkill records of birds, mammals, amphibians, and reptiles, which have been made available with other detailed data in the large dataset compiled for chapter 5. Records from chapter 4 include information on proximity distances from each roadkill site to different land covers, the percentage of land covers around each roadkill site and the sinuosity of road segments where roadkills occurred. The analyses of this chapter focused on how different land covers drive road mortality patterns of vertebrates at taxonomic class level (with some analyses at lower taxonomic levels). Additional analyses could consider effects for species with sufficient records, or these data could be combined with future surveys to identify temporal changes. Detailed roadkill records can inform conservation actions for particular species, as done for the Florida panther *Puma concolor coryi* (Cramer & Portier, 2001), the capybara *Hydrochoerus hydrochaeris* (Bueno *et al.*, 2013), the crab-eating fox *Cerdocyon thous*, the maned wolf *Chrysocyon* *brachyurus*, the ocelot *Leopardus pardalis* and the jaguarundi *Herpailurus yagouaroundi* (Costa *et al.*, 2022).

Finally, in chapter 5 we produced two datasets: one with 333 roadkill rates calculated for 242 species found in systematic studies and another database of 5,010 georeferenced records gathered from systematic and non-systematic surveys, representing roadkill data for 392 birds, mammals, amphibians, and reptiles in Ecuador (Medrano-Vizcaíno *et al.*, 2023a). Both datasets provide opportunities for further research. For example, the compilation of roadkill rates across countries and continents have been used to compare mortality rates across taxa (Pinto *et al.*, 2020), to estimate extinction risk for mammals (Grilo *et al.*, 2021), and to evaluate wildlife roadkill risk at country and continent level (González-Suárez *et al.*, 2018; Grilo *et al.*, 2020). On the other hand, datasets with georeferenced roadkills offer the opportunity to conduct studies such as species distribution modelling (Chyn *et al.*, 2021), or analyses of spatial and temporal patterns of wildlife mortality (Valerio *et al.*, 2021).

While these data offer many opportunities, it is important to acknowledge that compilations and datasets can present inaccuracies or biases. For example, methodologies for data collection can be different across systematic surveys, which are also conducted in diverse study areas with different wildlife and landscape composition, leading to some differences that need to be considered by researchers. Regarding non-systematic surveys, the absence of a standardized methodology for data collection can lead to biases and inaccuracy. From these work road ecology effort in Ecuador would benefit from: 1) more standardized roadkill surveys representing diverse habitats and areas across the country, and 2) a greater involvement from non-scientists reporting roadkill incidents via citizen science initiatives. The gathering of more data that allow consolidating more taxonomically, spatial and temporally representative datasets can be important to reduce biases, facilitate research and applied use, and improve inferences.

As a good example of a strong citizen science project, in the United Kingdom the project "The Road Lab" (https://www.theroadlab.co.uk/) has been active since 2013 and has received over 89,000 roadkill reports. This large quantity of data collected throughout the UK has allowed different studies such as the identification of spatial and temporal patterns of mortality for the pheasant *Phasianus colchicus* (Madden & Perkins, 2017), the evaluation of the mortality risk on British roads for the European hedgehog *Eurinaceus europaeus* (Wright *et al.*, 2020), and the identification of temporal patterns of mortality for the 19 most commonly reported taxa in the UK (Raymond *et al.*, 2021). Likewise, the use of roadkill data from citizen science allowed the study of seasonal and taxonomic patterns of mortality in Taiwan (Chyn *et al.*, 2019), and the recognition of roadkill hotspots in South Africa (Périquet *et al.*, 2018). In Italy, the use of these data allowed locating areas with a higher probability of collision for the endangered Eurasian otter *Lutra lutra* (Fabrizio *et al.*, 2019). These studies exemplify that citizen science can be valuable for conservation science. In particular, in developing countries, where economic resources for research are scarce, these efforts can be essential.

6.6 Citizen science and road ecology in Ecuador

In Ecuador, REMFA is the only citizen science project focused on road ecology. This is an ongoing initiative that I founded two years ago, and that now includes the collaboration of biologists, members of the public, and people from the government. We also maintain a partnership with the Latin American Transport Working Group (https://latinamericatransportationecology.org/countries/ecuador/), which is supported by the IUCN and joins road ecologists from Latin America working towards the reduction of wildlife mortality on roads and the planification of transport systems that ease animal movement.

REMFA, besides allowing the consolidation of a national dataset of roadkills in Ecuador, has disseminated environmental education related to road ecology through social networks such as Facebook (https://www.facebook.com/profile.php?id=100063511063333), Instagram (https://instagram.com/remfa_fauna_silv_atrop?igshid=YmMyMTA2M2Y=), and Twitter (https://twitter.com/RAtropellada). Also through interviews to national newspapers (https://www.elcomercio.com/actualidad/ecuador/zarigueyas-animales-atropellamiento-fauna-silvestre.html; https://www.elcomercio.com/tendencias/ambiente/vehiculos-amenaza-fauna-atropellamientos-especies.html), radio, and digital media (https://www.facebook.com/watch/live/?ref=watch_permalink&v=822723335002001). All these activities together with the organization of the first Ecuadorian Symposium of Road Ecology (held in Guayas-Ecuador as part of the III National Congress of Wildlife Management: https://aem.mamiferosdelecuador.com/congresosyeventosm/congresos-2021.html), have helped to gain a higher visibility of REMFA among citizens, therefore receiving more roadkill reports across the country.

These important advances for road ecology research in Ecuador have been essential to increase awareness of how roads affect wildlife, and to generate interest by researchers to study related topics. We aim to maintain this project over time, which will hopefully gather large quantities of data that will be freely available at REMFA’s webpage (https://remfa.webnode.co.uk/). This will surely be useful for researchers to develop studies that inform on protection actions, and for decision makers to plan future roads minimizing impacts on biodiversity.

6.7 Final remarks

The rapid expansion of road network across the world, and specially in biodiverse areas requires urgent actions to avoid potentially catastrophic losses of wildlife. The research presented in this thesis shows different approaches that can be efficient tools for road mortality risk assessment. However, gaining knowledge on the impacts of roads on wildlife is not sufficient to reduce their mortality. Conservation science work must be accompanied by a close interaction with members of the public and decision makers in order to communicate the effects on wildlife and to inform the decision process leading to management actions to mitigate impacts in the present and to prevent or limit future negative impacts. I have been working on this matter in Ecuador and although the involvement of decision makers is just emerging (i.e., now they have a better understanding of the situation and have expressed interest in joint work to reduce this impact), research conducted throughout this thesis can be considered as a reference for future actions related to conservation regarding road impacts in this country. More broadly, approaches applied in this thesis are also relevant for conservation and research efforts that could take place in other countries, or at continental and global efforts. Overall, this thesis aims to contribute towards reaching a balance between the wellbeing of people and wildlife, promoting respect to all living beings.

Appendices

6.7.1 *A. Supplementary material for chapter 2*

Table A.1 Correction factors for calculating roadkill rates

Correction factor used for calculating corrected roadkill rates considering groups of fauna, body mass, and survey intervals of each study. When survey interval was less than one day, no correction factor was applied. These correction factors were based on the estimates published by Santos et al., (2011).

Group	Body mass (kg)	Survey interval (days)	Correction factor
Birds	0.003-0.2	1.0-1.4	1.634
		1.5-3	1.797
		>3	1. 968
	0.201-23	1.0-1.4	1.283
		1.5-3	1.391
		>3	1.717
Birds of prey	1.75-1.6	1.0-1.4	1.255
		1.5-3	1.327
		>3	1.555
Non volant mammals	0.004-0.3	1.0-1.4	1.611
		1.5-3	1.759
		>3	1.970
	0.301-1.1	1.0-1.4	1.403
		1.5-3	1.527
		>3	1.714
	1.101- 210	1.0-1.4	1.196
		1.5-3	1.294
		>3	1.457
Volant mammals: Chiroptera	0.499-8.721	1.0-1.4	1.854
		1.5-3	1.963
		>3	2.000

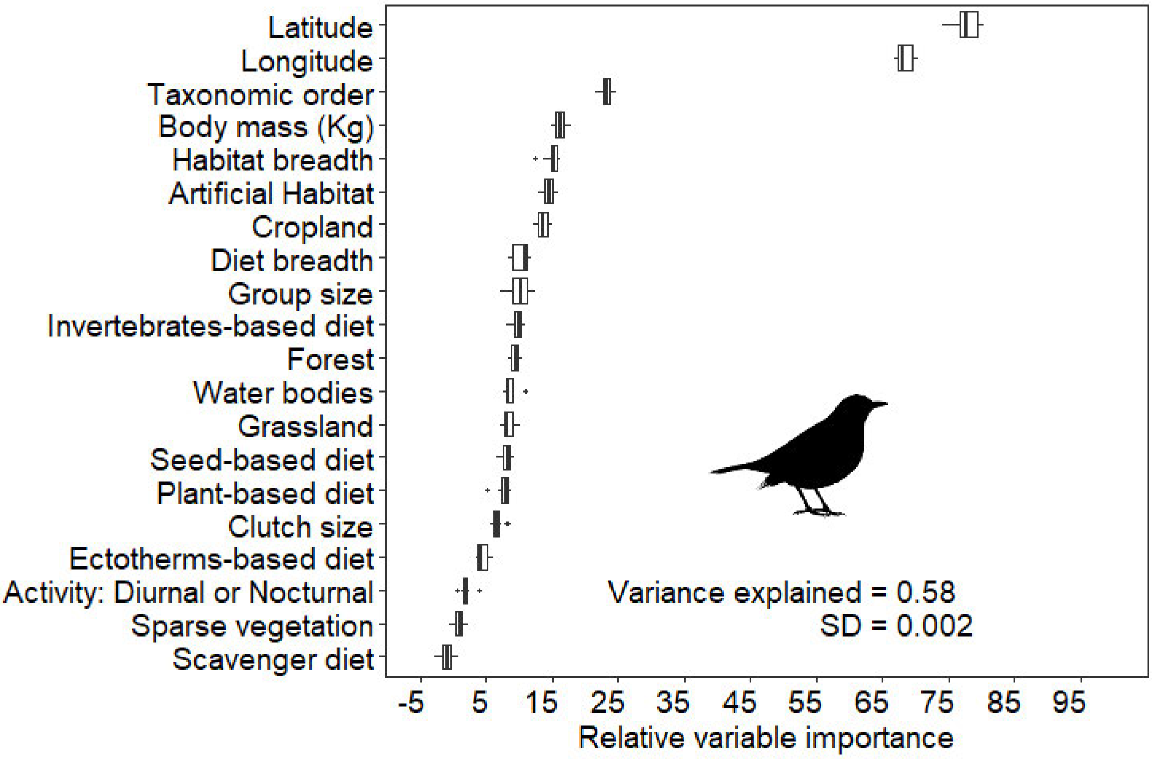


Figure A.1 Relative importance of variables (excluding trait variables with <40% of empirical values) considered in random forest regression models to predict bird roadkill rates.

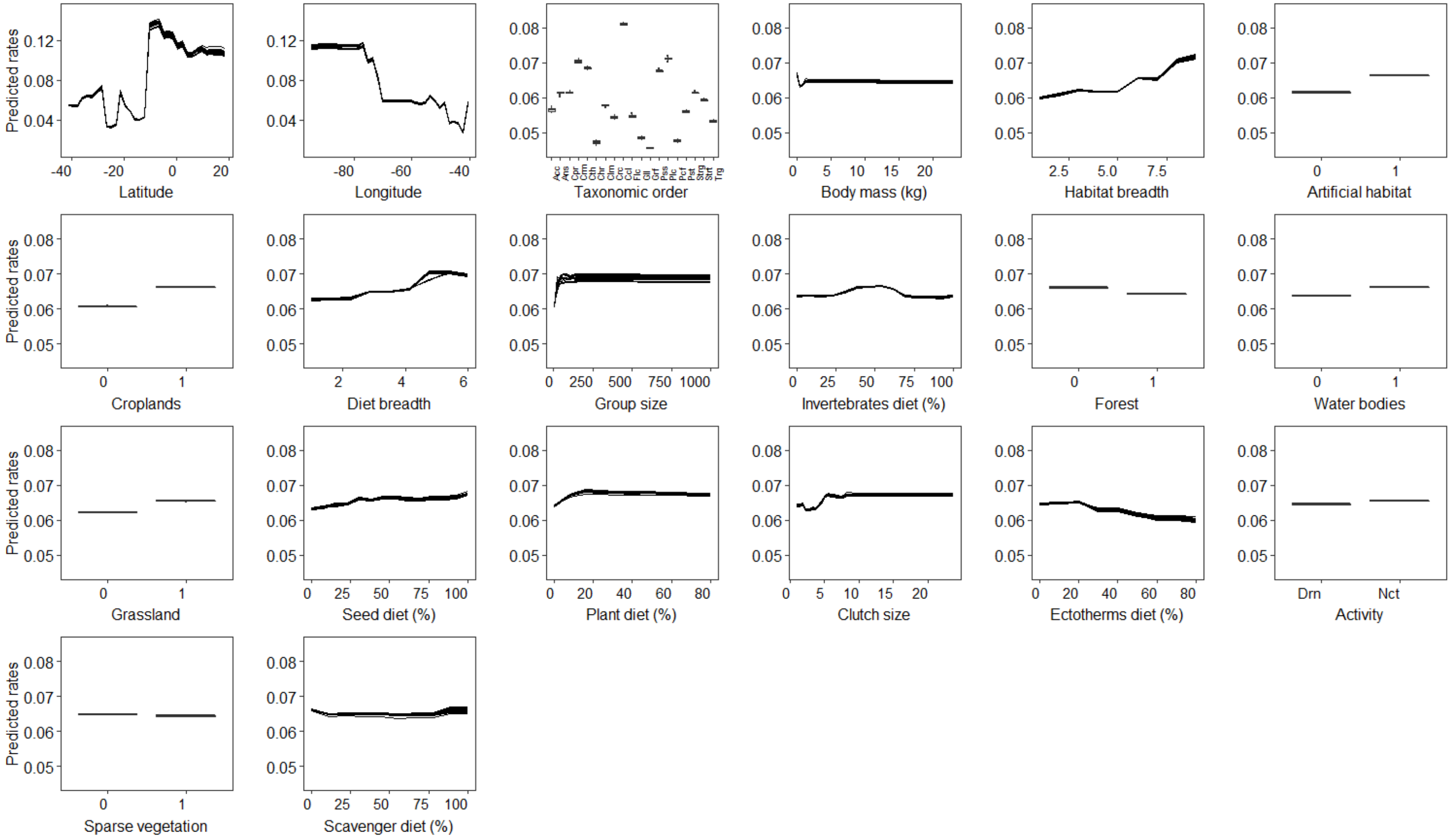


Figure A.2 Dependence plots showing changes in predicted roadkill rates for birds for each tested predictor variable (excluding trait variables with <40% of empirical values). Predictors are shown in descending order of relative variable importance (Figure A.1). Each panel show results for the 15 models fitted for different imputed datasets (trait data were not available for all species and missing values were imputed 15 times to capture uncertainty). For numerical predictors we show 15 lines and for categorical variables we show boxplots representing the distribution of predicted rates. For habitat preferences (Artificial habitat, Cropland, Forest, Grassland, Sparse vegetation, and Water bodies) category 1 indicates species that uses that habitat and zero indicates no use.

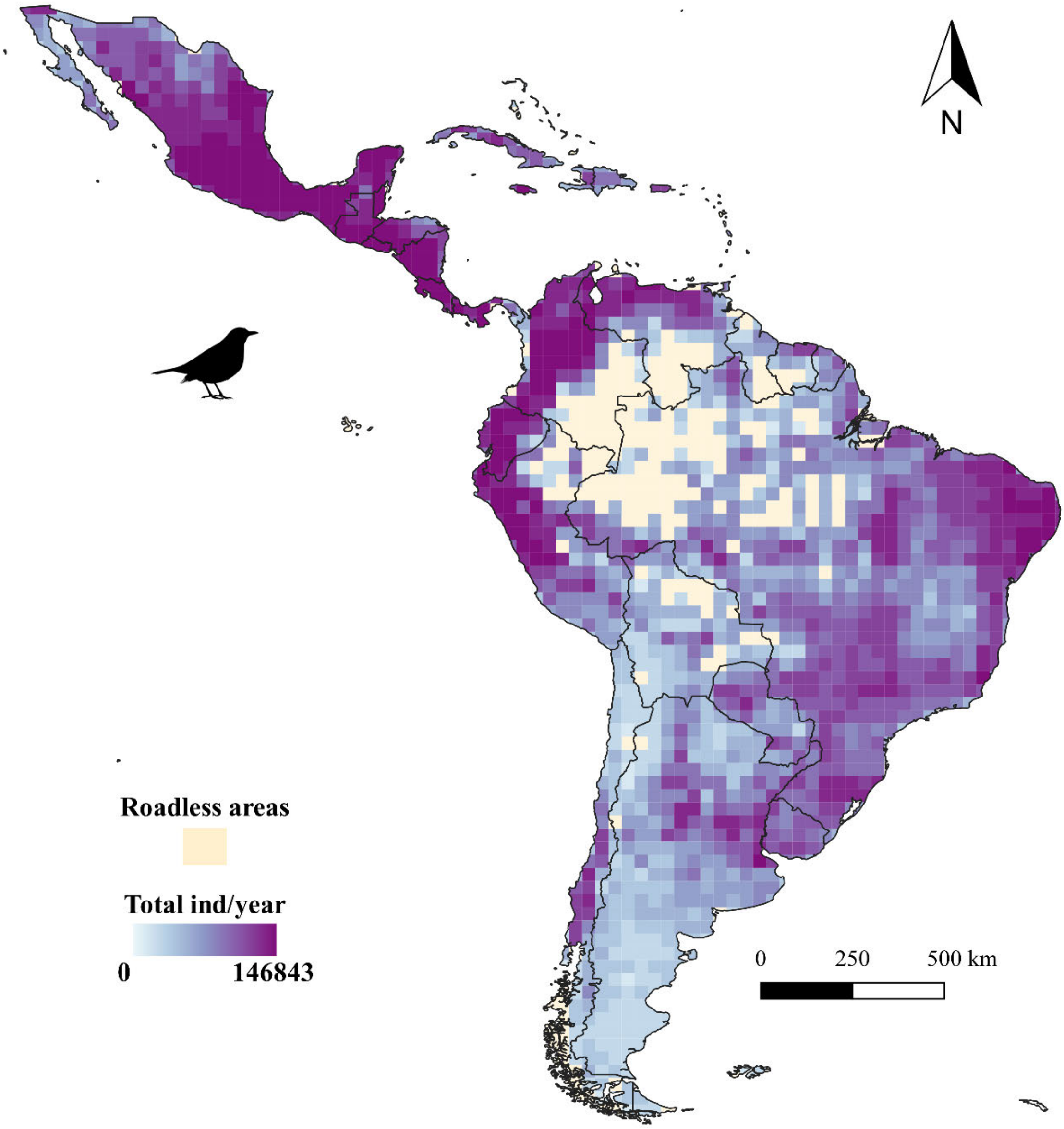


Figure A.3 Predicted total number of birds to be roadkilled each year in Latin America and the Caribbean excluding trait variables with <40% of empirical values. Total numbers were calculated multiplying roadkill rates (individuals/km/year) predicted for each species and grid cell using random regression models and species traits and location data by the total length of existing main roads (km) in each grid cell. Grid cells with no predictions (cream colour) represent areas where roads are not present.

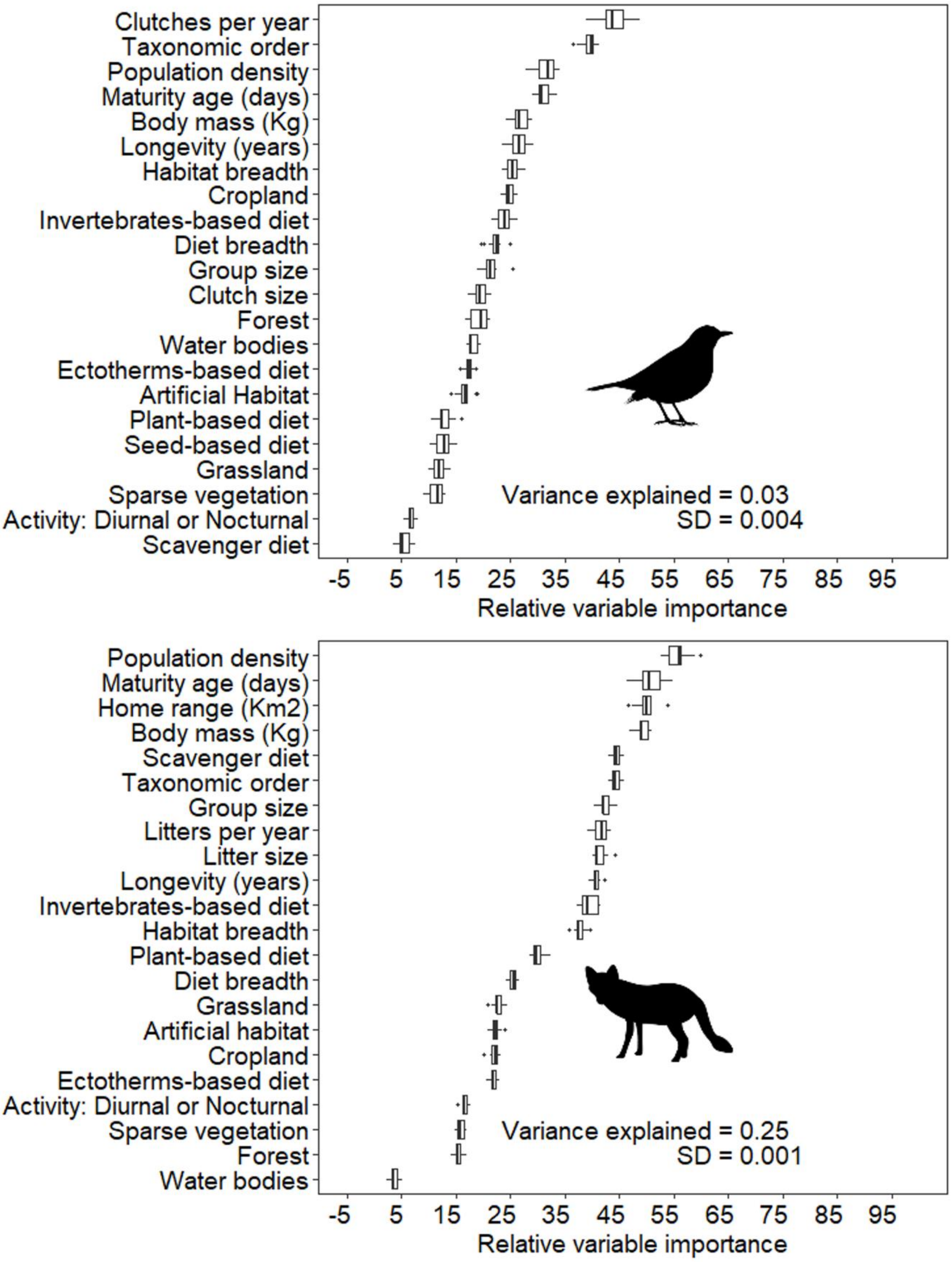


Figure A.4 Relative importance of variables (excluding latitude and longitude) considered in random forest regression models to predict birds and mammals roadkill rates.

6.7.2 *B. Supplementary material for chapter 4*

Table B.1 List of roadkilled vertebrates detected during a six-month systematic survey of 240 km of paved roads in the Napo region of Ecuador. We list the lowest taxonomic level identified, the total number of carcasses detected over the 6-month period (N), and standardized roadkill rates calculated by dividing the total number of records (N) by the total length of our study area (240 km) and the survey period (number of days between the first day until the last day of fieldwork, i.e., 185 days). This value was multiplied by 365 to return a rate in number of individuals per km per year.

Class	Species	N	Roadkill rate (ind./km/year)
Amphibians	*Rhinella marina*	208	1.710
	Caecilia sp.	40	0.329
	Unidentified Hylidae	6	0.049
	Boana boans	4	0.033
	Phyllomedusa vaillantii	4	0.033
	Boana lanciformis	3	0.025
	Unidentified Anura	3	0.025
	Boana sp.	2	0.016
	Phyllomedusa sp.	2	0.016
	Rhaebo ecuadorensis	2	0.016
	Rhaebo sp.	2	0.016
	Leptodactylus sp.	1	0.008
	Pristimantis sp.	1	0.008
Reptiles	*Atractus sp.*	49	0.403
	Dipsas klebbai	47	0.386
	Amphisbaena bassleri	29	0.238
	Unidentified Colubridae	26	0.214
	Dipsas indica	17	0.140
	Dipsas sp.	17	0.140
	Clelia clelia	16	0.132
	Atractus orcesi	13	0.107
	Atractus elaps	12	0.099
	Micrurus lemniscatus	12	0.099
	Dipsas catesbyi	10	0.082
	Erythrolamprus aesculapii	10	0.082
	Siphlophis compressus	10	0.082
	Unidentified Serpentes	9	0.074
	Ameiva ameiva	7	0.058

Class	Species	N	Roadkill rate (ind./km/year)
	Chironius scurrulus	7	0.058
	Siphlophis ayauma	7	0.058
	Epicrates cenchria	6	0.049
	Micrurus ornatissimus	6	0.049
	Chironius monticola	5	0.041
	Oxyrhopus petolarius	5	0.041
	Boa constrictor	4	0.033
	Bothrops atrox	4	0.033
	Imantodes cenchoa	4	0.033
	Micrurus sp.	4	0.033
	Chironius multiventris	3	0.025
	Chironius sp.	3	0.025
	Atractus touzeti	2	0.016
	Dipsas vermiculata	2	0.016
	Erythrolamprus sp.	2	0.016
	Oxyrhopus vanidicus	2	0.016
	Tantilla melanocephala	2	0.016
	Xenodon rabdocephalus	2	0.016
	Anilius scytale	1	0.008
	Anolis sp.	1	0.008
	Atractus major	1	0.008
	Atractus occipitoalbus	1	0.008
	Atractus snethlageae	1	0.008
	Cercosaura oshaughnessyi	1	0.008
	Corallus hortulana	1	0.008
	Dendrophidium sp.	1	0.008
	Drymarchon corais	1	0.008
	Drymoluber dichrous	1	0.008
	Erythrolamprus breviceps	1	0.008
	Erythrolamprus guentheri	1	0.008
	Erythrolamprus reginae	1	0.008
	Helicops angulatus	1	0.008
	Mastigodryas sp.	1	0.008
	Micrurus narduccii	1	0.008
	Micrurus steindachneri	1	0.008
	Ninia hudsoni	1	0.008
	Ninia sp.	1	0.008
	Oxybelis aeneus	1	0.008
	Oxybelis fulgidus	1	0.008

Class	**Species**	**N**	**Roadkill rate (ind./km/year)**
	Oxyrhopus melanogenys	1	0.008
	Spilotes pullatus	1	0.008
	Trilepida anthracina	1	0.008
	Tupinambis cuzcoensis	1	0.008
	Unidentified Viperidae	1	0.008
Birds	Unidentified bird	64	0.526
	Crotophaga ani	23	0.189
	Megascops choliba	16	0.132
	Notiochelidon cyanoleuca	16	0.132
	Ramphocelus carbo	12	0.099
	Zonotrichia capensis	11	0.090
	Turdus ignobilis	10	0.082
	Mionectes striaticollis	9	0.074
	Cyanocorax yncas	8	0.066
	Coragyps atratus	5	0.041
	Phaethornis hispidus	5	0.041
	Porphyrio martinica	5	0.041
	Myadestes ralloides	3	0.025
	Thraupis episcopus	3	0.025
	Turdus serranus	3	0.025
	Unidentified Emberizidae	3	0.025
	Unidentified Furnariidae	3	0.025
	Unidentified Passeriformes	3	0.025
	Volatinia jacarina	3	0.025
	Maschalethraupis surinama	2	0.016
	Spinus magellanicus	2	0.016
	Sporophila sp.	2	0.016
	Stilpnia cyanicollis	2	0.016
	Unidentified Apodiformes	2	0.016
	Amaurolimnas concolor	1	0.008
	Anisognathus lacrymosus	1	0.008
	Anisognathus somptuosus	1	0.008
	Automolus ochrolaemus	1	0.008
	Cacicus sp.	1	0.008
	Celeus elegans	1	0.008
	Cercomacroides nigriscens	1	0.008
	Chamaepetes goudotii	1	0.008
	Coereba flaveola	1	0.008
	Columba livia	1	0.008

Class	Species	N	Roadkill rate (ind./km/year)
	Daptrius ater	1	0.008
	Diglossa albilatera	1	0.008
	Drymophila striatta	1	0.008
	Glaucidium brasilianum	1	0.008
	Islerothraupis luctuosa	1	0.008
	Laterallus exilis	1	0.008
	Laterallus melanophaius	1	0.008
	Mimus gilvus	1	0.008
	Mionectes sp.	1	0.008
	Myiothlypis coronata	1	0.008
	Passer domesticus	1	0.008
	Pipraeidea melanonota	1	0.008
	Psarocolius angustifrons	1	0.008
	Pteroglossus inscriptus	1	0.008
	Setophaga fusca	1	0.008
	Sicalis flaveola	1	0.008
	Spinus olivaceus	1	0.008
	Sporophila angolensis	1	0.008
	Stelgidopteryx ruficollis	1	0.008
	Synallaxis azarae	1	0.008
	Tangara arthus	1	0.008
	Tangara mexicana	1	0.008
	Tangara parzudakii	1	0.008
	Tangara sp.	1	0.008
	Tangara xanthocephala	1	0.008
	Thraupis palmarum	1	0.008
	Troglodytes aedon	1	0.008
	Trogon collaris	1	0.008
	Trogon sp.	1	0.008
	Turdus fuscater	1	0.008
	Tyrannus melancholicus	1	0.008
	Unidentified Cathartidae	1	0.008
	Unidentified Thraupidae	1	0.008
	Uropsalis lyra	1	0.008
Mammals	*Didelphis pernigra*	87	0.715
	Didelphis marsupialis	48	0.395
	Unidentified Rodentia	27	0.222
	Marmosa sp.	8	0.066
	Sciurus granatensis	6	0.049

Class	Species	N	Roadkill rate (ind./km/year)
	Unidentified Chiroptera	6	0.049
	Carollia sp.	3	0.025
	Conepatus semistriatus	2	0.016
	Mustela frenata	2	0.016
	Nephelomys sp.	2	0.016
	Potos flavus	2	0.016
	Thomasomys aureus	2	0.016
	Unidentified Muridae	2	0.016
	Coendou sp.	1	0.008
	Didelphis sp.	1	0.008
	Herpailurus yagouaroundi	1	0.008
	Leopardus sp.	1	0.008
	Metachirus nudicaudatus	1	0.008
	Myoprocta pratti	1	0.008
	Notosciurus sp.	1	0.008
	Philander andersoni	1	0.008
	Priodontes maximus	1	0.008
	Sylvilagus andinus	1	0.008
	Unidentified Mammalia	1	0.008
Total		**1,125**	

Figure B.1 a) Amphisbaenians (*Amphisbaenia bassleri*), b) Atractus snakes (unknown species), c) caecilians (unknown species), and d) opposums *Didelphis marsupialis* found during fieldwork.

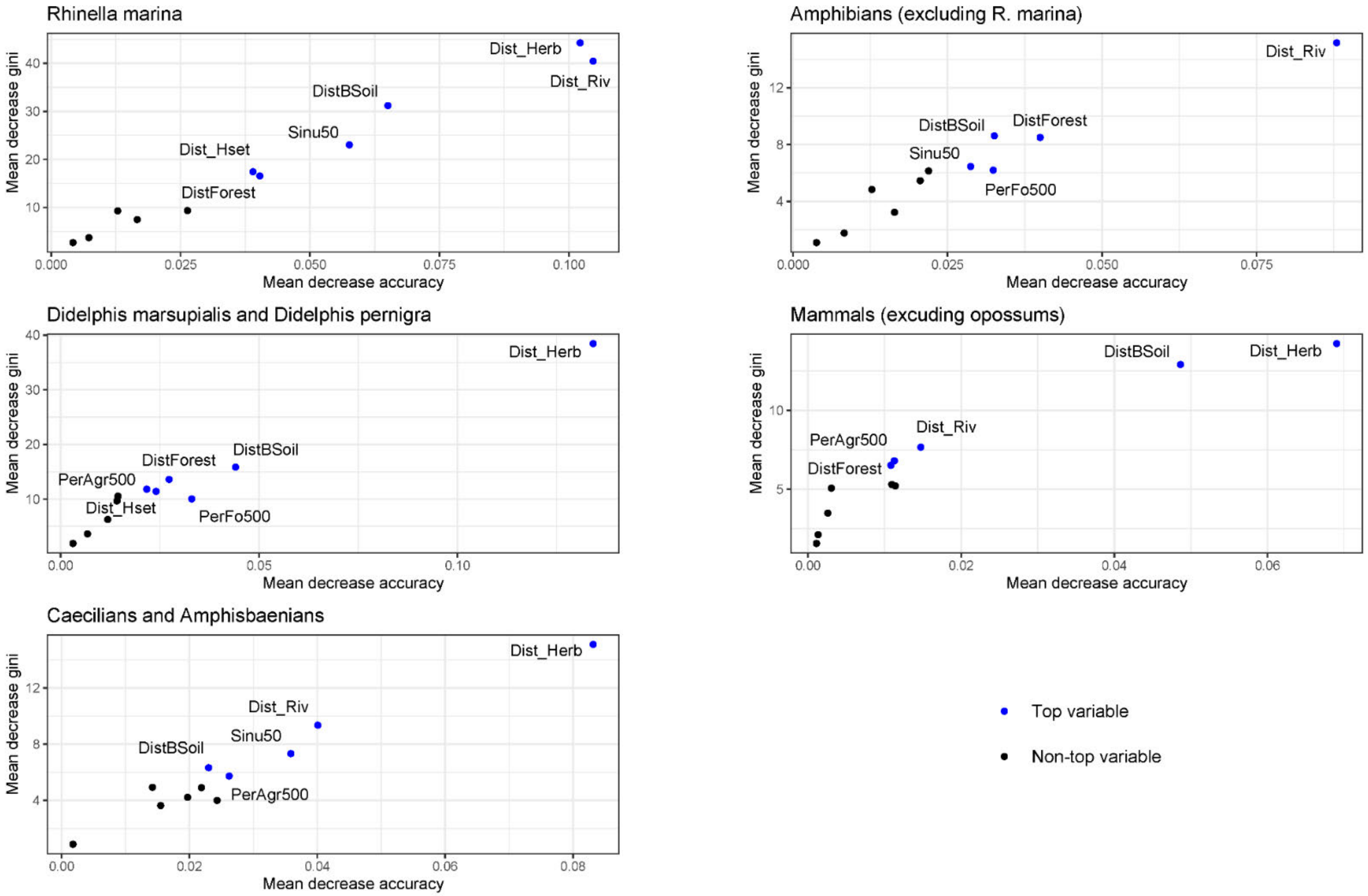


Figure B.2 Variables importance for *R. marina*, amphibians excluding *R. marina*, Opossums, Mammals excluding opossums, and fossorial organisms (Caecilians and Amphisbanians) based on the gini decrease and the mean accuracy decrease. Sinu50=sinuosity (50 m), Dist_Herb=distance to herbaceous-shrubby vegetation, Dist_Riv=distance to rivers, DistForest=distance to forest, DistBsoil=Distance to bare soil, DistHset=Distance to human settlements, PerAgr500=Percentage of agricultural lands (500 m buffer), PerFo500=Percentage of forest (500 m buffer).

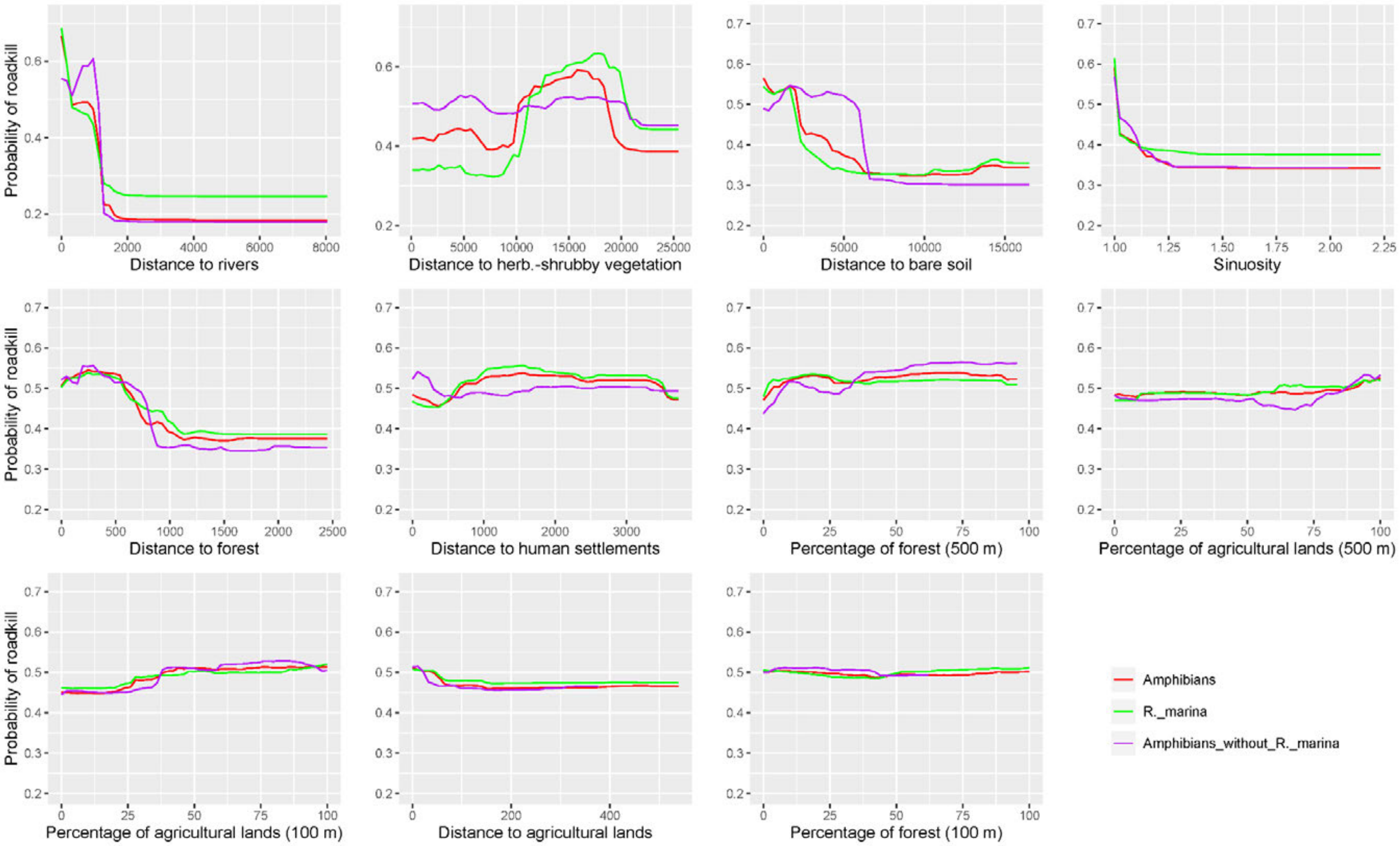


Figure B.3 Dependence plots showing how land cover and road configuration influence probabilty of roadkill of amphibians, *R. marina*, and amphibians without *R. marina* in the Napo region of Ecuador. Plots are shown in descending order of variable importance for amphibians.

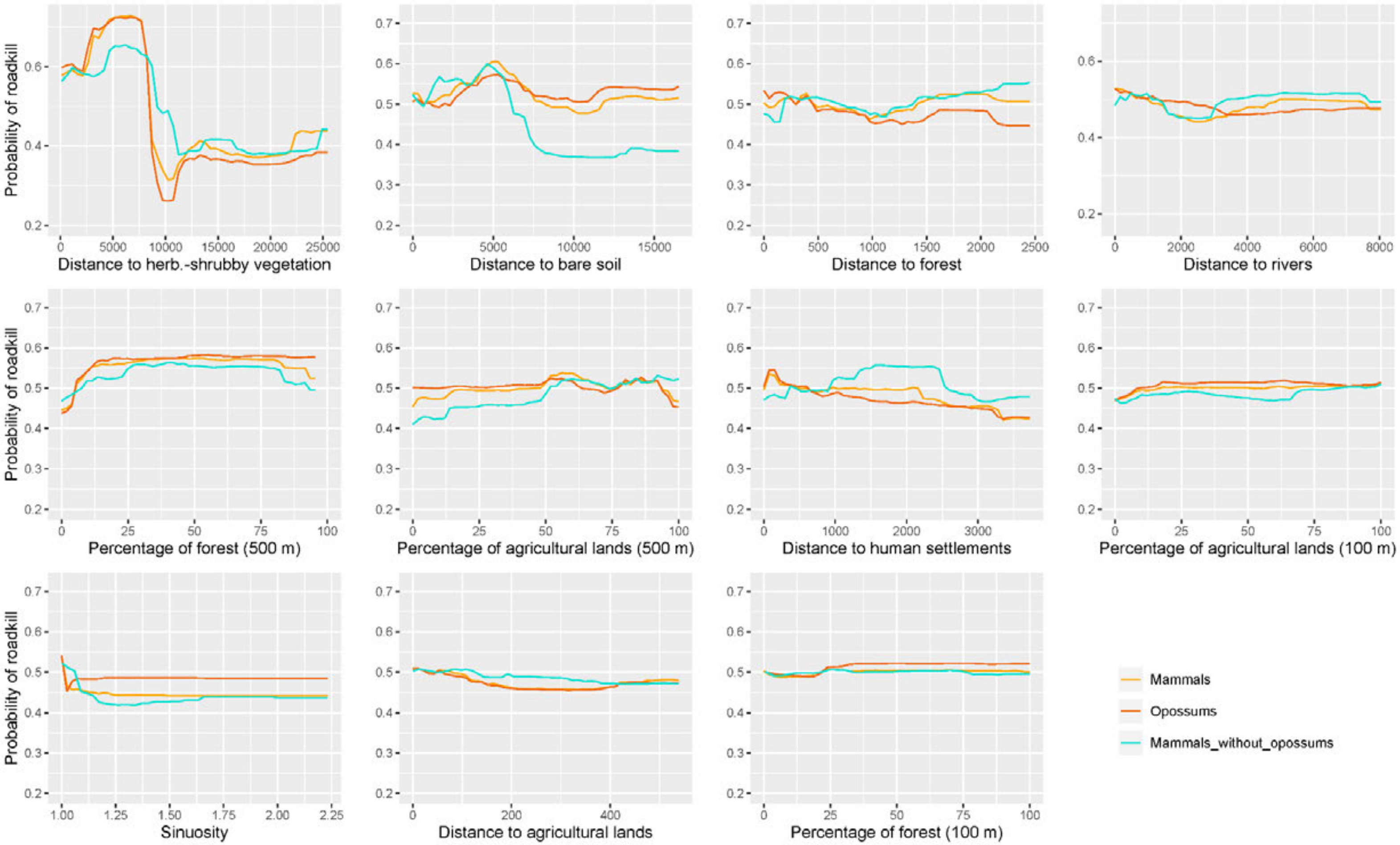


Figure B.4 Dependence plots showing how land cover and road configuration influence probabilty of roadkill of mammals, opossums, and mammals without opossums in the Napo region of Ecuador. Plots are shown in descending order of variable importance for mammals.

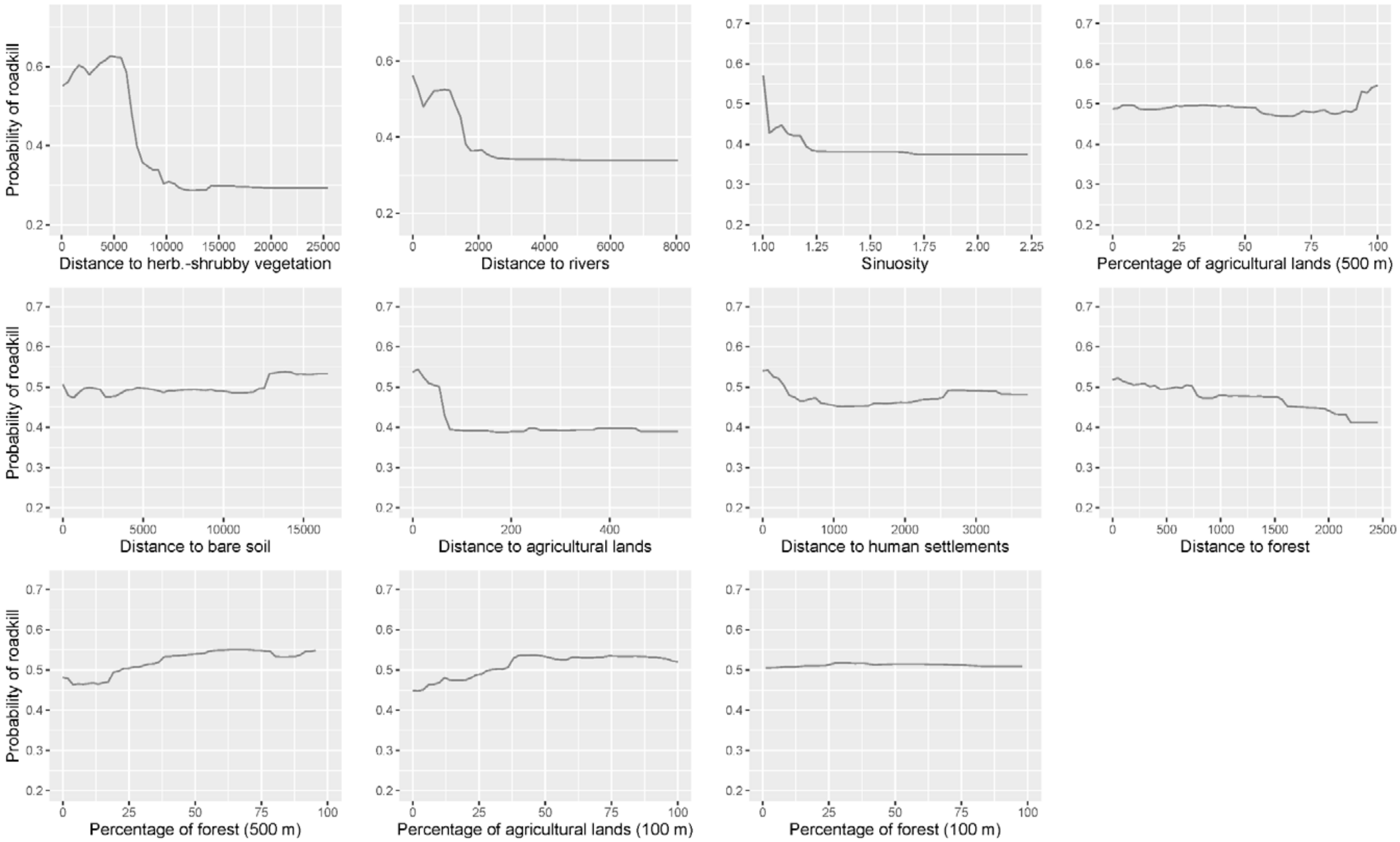


Figure B.5 Dependence plots showing how land cover and road configuration influence probabilty of roadkill of amphisbaenians and caecilians in the Napo region of Ecuador. Plots are shown in descending order of variable importance for fossorial organisms.

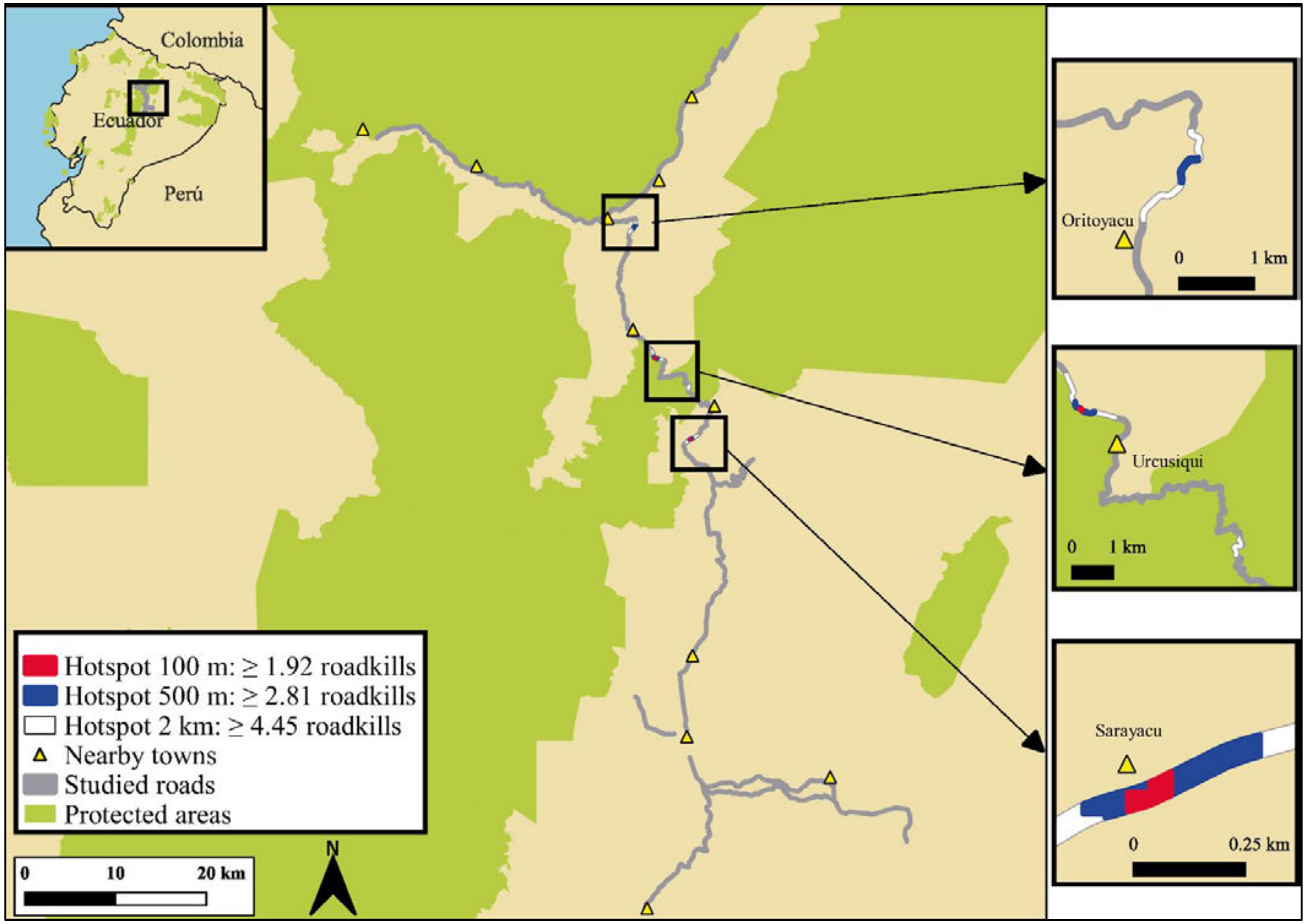


Figure B.6 Roadkill hotspots for amphibians (excluding *R. marina*) across 240 km of the primary and secondary road network of the Napo region of Ecuador.

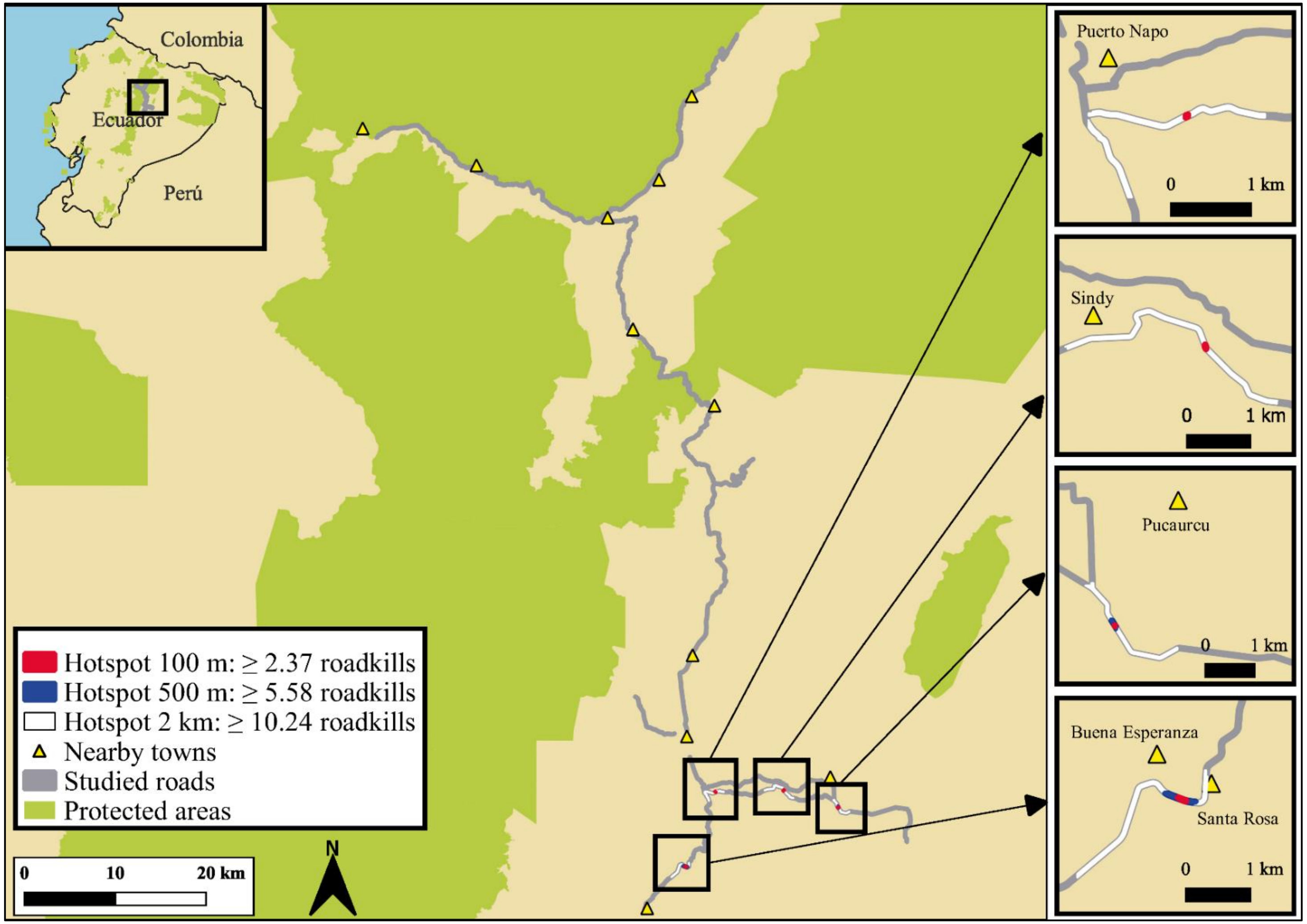


Figure B.7 Roadkill hotspots for *R. marina* across 240 km of the primary and secondary road network of the Napo region of Ecuador.

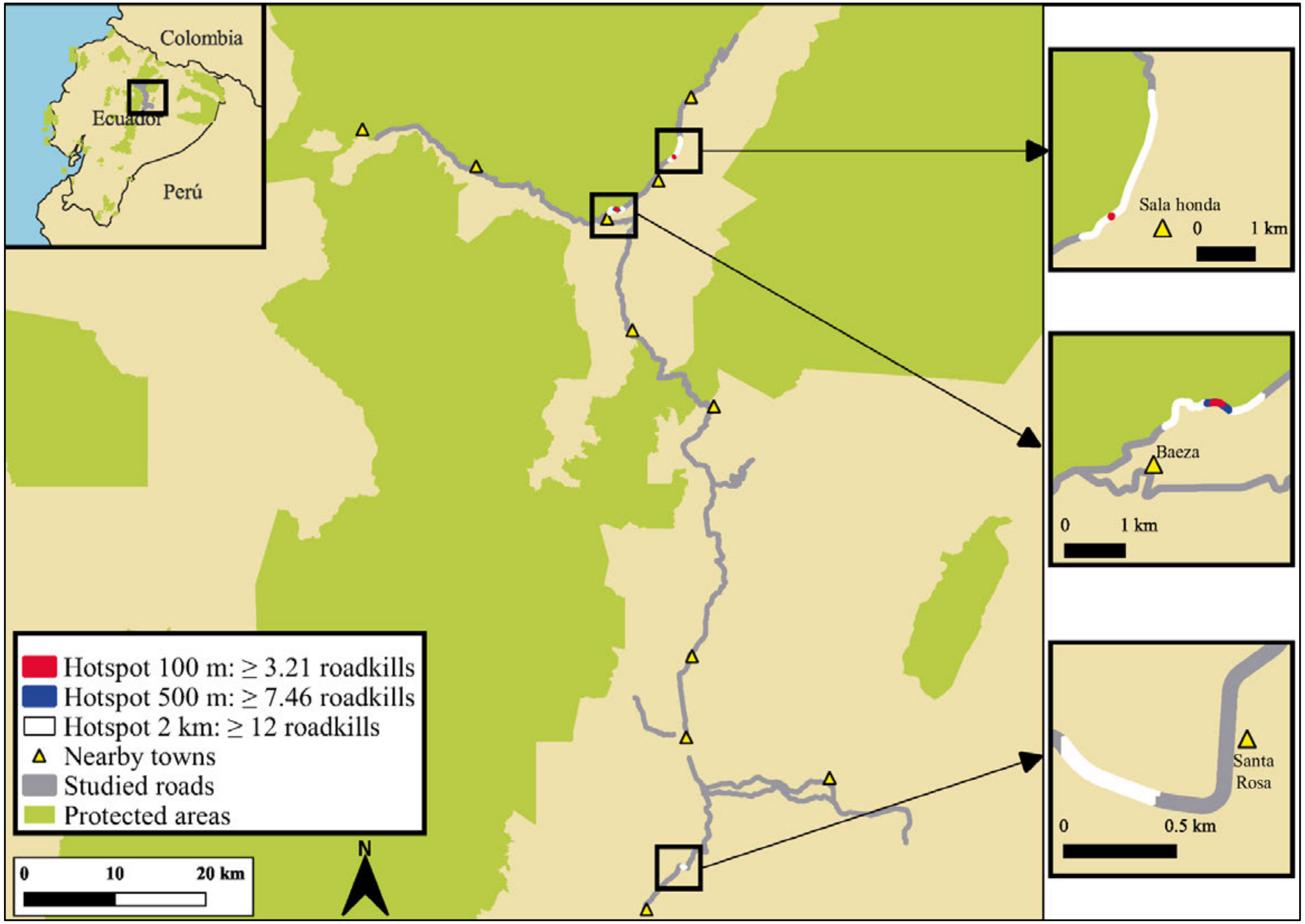


Figure B.8 Roadkill hotspots for reptiles across 240 km of the primary and secondary road network of the Napo region of Ecuador.

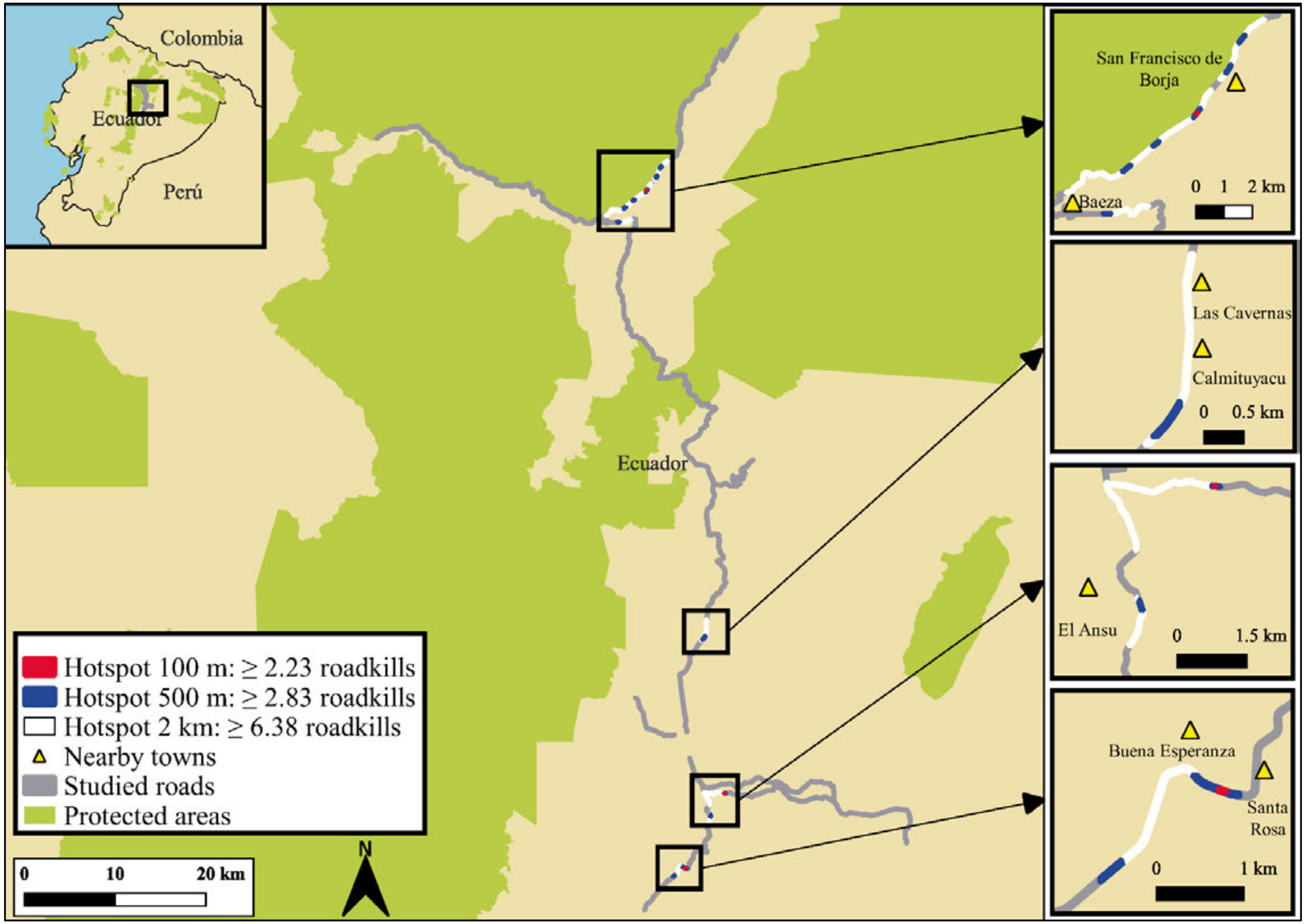


Figure B.9 Roadkill hotspots for birds across the 240 km of primary and secondary road network of the Napo region of Ecuador.

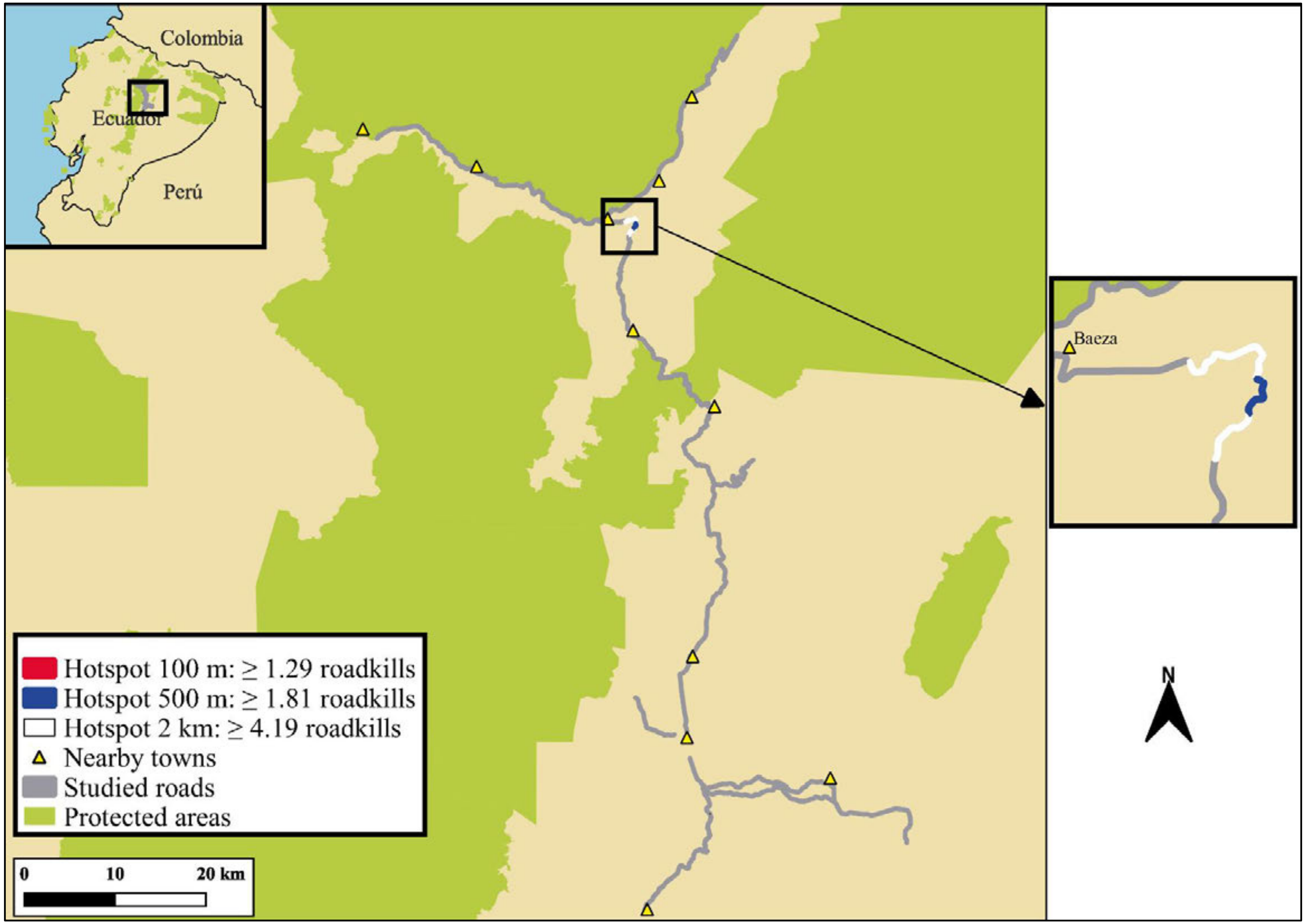


Figure B.10 Roadkill hotspots for Mammals (excluding opossums) across the 240 km of primary and secondary road network of the Napo region of Ecuador.

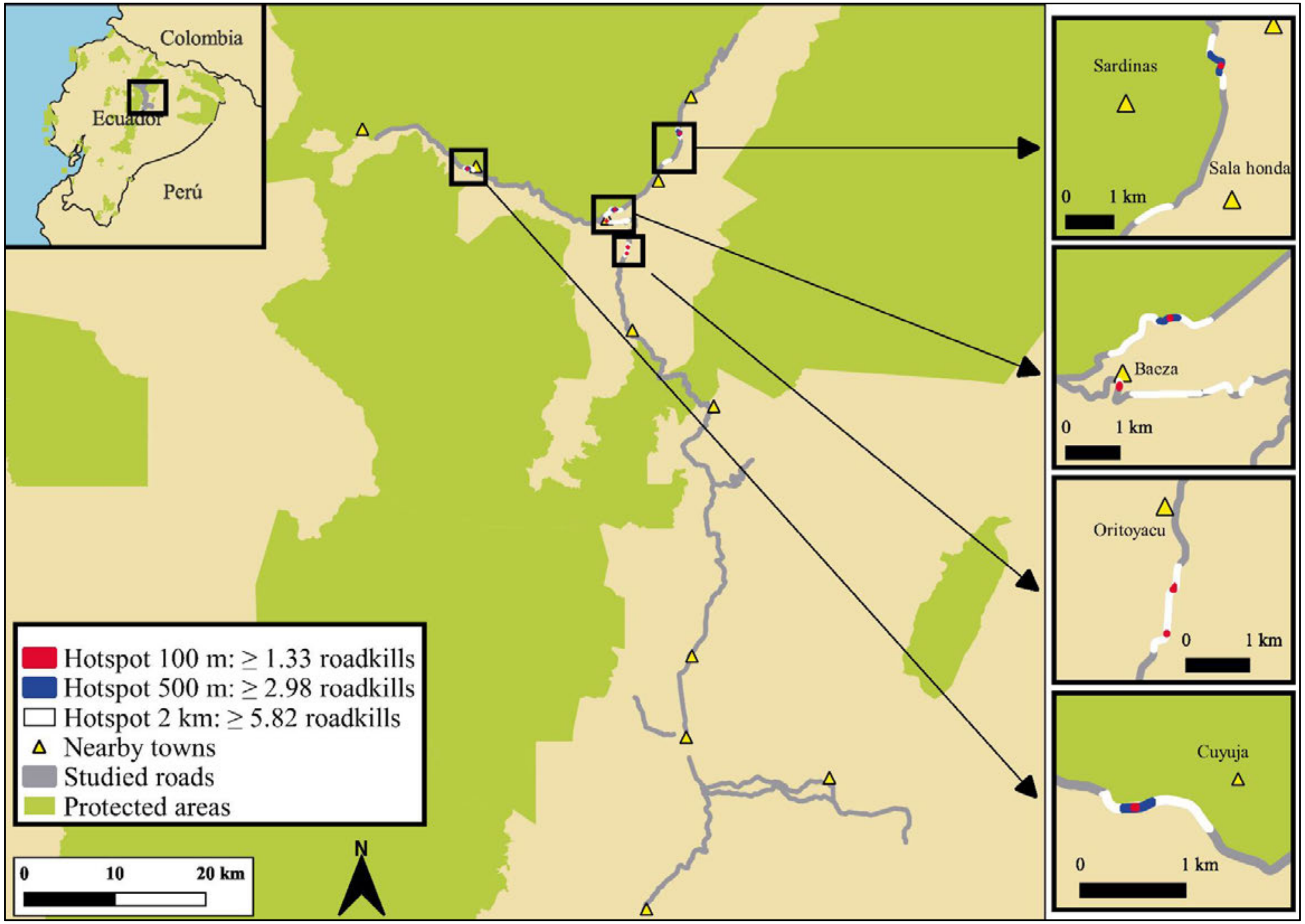


Figure B.11 Roadkill hotspots for opossums across 240 km of the primary and secondary road network of the Napo region of Ecuador.

References

Acharjee, A., Kloosterman, B., de Vos, R.C.H., Werij, J.S., Bachem, C.W.B., Visser, R.G.F. & Maliepaard, C. (2011) Data integration and network reconstruction with ~omics data using Random Forest regression in potato. *Analytica Chimica Acta*, **705**, 56–63.

Aguilar, J.M., Nieto, A., Espinoza, N., Loja, G. & Tinoco, B. (2019) Assessing patterns of bird roadkills in a high Andean Ecuadorian national park. *Studies on Neotropical Fauna and Environment*, **54**, 149–156.

Amorim, D.M., Perez, R., Ávila, R.W. & De Moura, G.J.B. (2019) Diet and parasitism in Leposternon polystegum (Amphisbaenia, Amphisbaenidae) from coastal areas in the Brazilian Northeast. *https://doi.org/10.1080/00222933.2019.1667038*, **53**, 1799–1809.

Anderson, C. & Enge, K.M. (2012) Ctenosaura similis (Gray’s spiny-tailed iguana) and Iguana iguana (green iguana). Carrion feeding. *Herpetological Review*, **43**.

de Araújo, D.R., Ribeiro, P. & Teles, L.T. (2019) Can human demographic or biological factors influence mammal roadkill? A case study in the GO-060 highway. *Oecologia Australis*, **23**, 16–27.

Aresco, M. (2005) Mitigation Measures To Reduce Highway Mortality of Turtles and Other Herpetofauna At a North Florida Lake. *Journal of Wildlife Management*, **69**, 549–560.

Armendáriz, A. (2022) Mortalidad de vertebrados silvestres por atropellamiento en la ruta E40 (Guayaquil-Guayas).

Arteaga, A., Quezada, A., Vieira, J. & Guayasamin, J.M. (2022) Leaving no stone unturned: three additional new species of Atractus ground snakes (Serpentes, Colubridae) from Ecuador discovered using a biogeographical approach. *ZooKeys 1121: 175-210*, **1121**, 175–210.

Ascensão, F., Desbiez, A.L.J., Medici, E.P. & Bager, A. (2017) Spatial patterns of road mortality of medium-large mammals in Mato Grosso do Sul, Brazil. *Wildlife Research*, **44**, 135–146.

Ascensão, F., Yogui, D., Alves, M., Medici, E.P. & Desbiez, A. (2019) Predicting spatiotemporal patterns of road mortality for medium-large mammals. *Journal of*

Environmental Management, **248**, 109320.

Avgar, T., Baker, J.A., Brown, G.S., Hagens, J.S., Kittle, A.M., Mallon, E.E., Mcgreer, M.T., Mosser, A., Newmaster, S.G., Patterson, B.R., Reid, D.E.B., Rodgers, A.R., Shuter, J., Street, G.M., Thompson, I., Turetsky, M.J., Wiebe, P.A. & Fryxell, J.M. (2015) Space-use behaviour of woodland caribou based on a cognitive movement model. *Journal of Animal Ecology*, **84**, 1059–1070.

Avilova, L.I. & Gey, A.N. (2018) On the Construction Features of Wheeled Vehicles in Iran and Mesopotamia (Third To First Millennia Bc). *Archaeology, Ethnology & Anthropology of Eurasia*, **46**, 41–48.

Bager, A. & Fontoura, V. (2013) Evaluation of the effectiveness of a wildlife roadkill mitigation system in wetland habitat. *Ecological Engineering*, **53**, 31–38.

Barber, C.P., Cochrane, M.A., Souza, C.M. & Laurance, W.F. (2014) Roads, deforestation, and the mitigating effect of protected areas in the Amazon. *Biological Conservation*, **177**, 203–209.

Barrero, A., Llusia, D., Traba, J., Iglesias-Merchan, C. & Morales, M.B. (2021) Vocal response to traffic noise in a non-Passerine Bird: The Little Bustard Tetrax tetrax. *Ardeola*, **68**, 143–162.

De Barros Ferraz, K.M.P.M., De Siqueira, M.F., Martin, P.S., Esteves, C.F. & Do Couto, H.T.Z. (2010) Assessment of Cerdocyon thous distribution in an agricultural mosaic, southeastern Brazil. *Mammalia*, **74**, 275–280.

Bastos, D.F. de O., Souza, R.A.T., Zina, J. & da Rosa, C.A. (2019) Seasonal and spatial variation of road-killed vertebrates on br-330, Southwest Bahia, Brazil. *Oecologia Australis*, **23**, 388–402.

Bauni, V., Anfuso, J. & Schivo, F. (2017) Mortalidad de fauna silvestre por atropellamientos en el bosque atlántico del Alto Paraná, Argentina. *Ecosistemas*, **26**, 54–66.

Baxter-Gilbert, J.H., Riley, J.L., Neufeld, C.J.H., Litzgus, J.D. & Lesbarrères, D. (2015) Road mortality potentially responsible for billions of pollinating insect deaths annually. *Journal of Insect Conservation*, **19**, 1029–1035.

Beadnell, S. (1937) The Toll of Animal Life exacted by Modern Civilisation. *Proceedings of the Zoological Society*, **107A**, 173–82.

Belao, M., Bocon, R., Christo, S., de Souza, M. & Souza Junior, J. (2014) Incidentes De Mamíferos Na Rodovia Br-277, Paraná – Brasil. *Publicatio UEPG: Ciencias Biologicas e da Saude*, **20**, 37–41.

Berthinussen, A. & Altringham, J. (2012) The effect of a major road on bat activity and diversity. *Journal of Applied Ecology*, **49**, 82–89.

Bíl, M., Heigl, F., Janoška, Z., Vercayie, D. & Perkins, S.E. (2020) Benefits and challenges of collaborating with volunteers: Examples from National Wildlife Roadkill Reporting Systems in Europe. *Journal for Nature Conservation*, **54**, 125798.

BirdLife International (2016) Violet-throated Metaltail Metallura baroni. *The IUCN Red List of Threatened Species*.

Bischof, R., Steyaert, S.M.J.G. & Kindberg, J. (2017) Caught in the mesh: roads and their network-scale impediment to animal movement. *Ecography*, **40**, 1369–1380.

Boullosa, R.G., Costa-Neto, S.F., Morgado, L., Maldonado, A. & Gentile, R. (2021) A longitudinal survey of gastrointestinal parasites of the black-eared opossum Didelphis aurita at an urban-sylvatic interface area in Southeast Brazil: a morphological and ecological characterization based on helminth eggs. *Parasitology Research*, **120**, 3815–3825.

Bravo-Naranjo, V., Piñones-Cañete, C., Heraldo, Norambuena, V. & Zuleta, C. (2019) Puntos calientes y factores asociados al atropello de aves rapaces en una ruta costera de la zona semiárida de Chile Central. *Ornitología Neotropical*, **30**, 208–216.

Braz, V.D.S. & Rodrigues, F. (2016) Wild vertebrate roadkill in the Chapada dos Veadeiros National Park, Central Brazil. *Biota Neotropica*, **16**, 1–11.

Breiman, L. (2001) Random forests. *Machine Learning*, **45**, 5–32.

Brito, J., Camacho, M., Romero, V. & Vallejo, A. (2021) Mamíferos del Ecuador. Versión 2021.0. Museo de Zoología, Pontificia Universidad Católica del Ecuador.

Brito, J., Tinoco, N., Pinto, C.M., García, R., Koch, C., Fernandez, V., Burneo, S. & Pardiñas, U.F.J. (2022) Unlocking Andean sigmodontine diversity: five new species of Chilomys (Rodentia: Cricetidae) from the montane forests of Ecuador. *PeerJ*, **10**, e13211.

Brooks, T.M., Pimm, S.L., Resit Akç, H., Buchanan, G.M., Butchart, S.H.M., Foden, W., Hilton-Taylor, C., Hoffmann, M., Jenkins, C.N., Joppa, L., Li, B. V, Menon, V., Ocampo-Peñ, N. & Rondinini, C. (2019) Measuring Terrestrial Area of Habitat (AOH) and Its Utility for the IUCN Red List The International Union for Conservation of Nature (IUCN) Red List of Threatened Species includes assessment of extinction risk for. *Trends in Ecology & Evolution*, **34**.

Brown, C.R. & Bomberger Brown, M. (2013) Where has all the road kill gone? *Current Biology*, **23**.

Bueno, C., Faustino, M.T. & Freitas, S.R. (2013) Influence of landscape characteristics on capybara road-kill on highway BR-040, Southeastern Brazil. *Oecologia Australis*, **17**, 130–137.

Bueno, C., Sousa, C.O.M. & Freitas, S.R. (2015) Habitat or matrix: which is more relevant to predict road-kill of vertebrates? *Brazilian Journal of Biology*, **75**, 228–238.

Caceres, N.C. (2011) Biological characteristics influence mammal road kill in an Atlantic Forest-Cerrado interface in south-western Brazil. *Italian Journal of Zoology*, **78**, 379–389.

Caires, H.S., Souza, C.R., Lobato, D.N.C., Fernandes, M.N.S. & Damasceno, J.S. (2019) Roadkilled mammals in the northern amazon region and comparisons with roadways in other regions of Brazil. *Iheringia - Serie Zoologia*, **109**, 1–9.

Calles López, J. (2008) *Caracterización Ecológica de la Provincia del Napo. Proyecto Fortalecimiento a Gobiernos Locales*,.

Canova, L. & Balestrieri, A. (2019) Long-term monitoring by roadkill counts of mammal populations living in intensively cultivated landscapes. *Biodiversity and Conservation*, **28**, 97–113.

Cantor, M., Ferreira, L.A., Silva, W.R. & Setz, E.Z.F. (2010) Potential seed dispersal by Didelphis albiventris (Marsupialia, Didelphidae) in highly disturbed environment. *Biota Neotropica*, **10**, 45–51.

Carrete, M., Tella, J.L., Blanco, G. & Bertellotti, M. (2009) Effects of habitat degradation on the abundance, richness and diversity of raptors across Neotropical biomes. *Biological Conservation*, **142**, 2002–2011.

Carvalho, F. & Mira, A. (2011) Comparing annual vertebrate road kills over two time periods, 9 years apart: A case study in Mediterranean farmland. *European Journal of Wildlife Research*, **57**, 157–174.

Carvalho, N.C. de, Bordignon, M.O. & Shapiro, J.T. (2014) Fast and furious: a look at the death of animals on the highway MS-080, Southwestern Brazil. *Iheringia. Série Zoologia*, **104**, 43–49.

Castillo-R, J.C., Urmendez-M, D. & Zambrano-G, G. (2015) Mortalidad de fauna por atropello vehicular en un sector de la vía panamericana entre popáyan y patía. *Boletin Cientifico del Centro de Museos*, **19**, 207–219.

Cayuela, H., Bonnaire, É., Astruc, G. & Besnard, A. (2019) Transport infrastructure severely impacts amphibian dispersal regardless of life stage. *Scientific Reports*, **9**, 8214.

Cerqueira, R.C., Leonard, P.B., da Silva, L.G., Bager, A., Clevenger, A.P., Jaeger, J.A.G. & Grilo, C. (2021) Potential Movement Corridors and High Road-Kill Likelihood do not Spatially Coincide for Felids in Brazil: Implications for Road Mitigation. *Environmental Management*, **67**, 412–423.

Chen, H.L. & Koprowski, J.L. (2016) Barrier effects of roads on an endangered forest obligate: influences of traffic, road edges, and gaps. *Biological Conservation*, **199**, 33–40.

Cherem, J., Kammers, M., Ghizoni-Jr, I. & Martins, A. (2007) Mamíferos de Médio e Grande Porte Atropelados no Oeste de Santa Catarina, Brasil. *Biotemas*, **20**, 81–96.

Chyn, K., Lin, T.-E., Chen, Y.-K., Chen, C.-Y. & Fitzgerald, L.A. (2019) The magnitude of roadkill in Taiwan: Patterns and consequences revealed by citizen science. *Biological Conservation*, **237**, 317–326.

Chyn, K., Lin, T.E., Wilkinson, D.P., Tracy, J.L., Lawing, A.M. & Fitzgerald, L.A. (2021) Fine-scale roadkill risk models: understanding the intersection of wildlife and roads. *Biodiversity and Conservation*, **30**, 139–164.

Ciocheti, G., de Assis, J.C., Ribeiro, J.W. & Ribeiro, M.C. (2017) Highway widening and underpass effects on vertebrate road mortality. *Biotropica*, **49**, 765–769.

Cisneros-Heredia, D. (2021) Coniophanes dromiciformis. *The IUCN Red List of Threatened Species*.

Cisneros-Heredia, D. (2005) Rediscovery of the Ecuadorian snake Atractus dunni Savage, 1955 (Serpentes: Colubridae). *Časopis Národního muzea, Řada přírodovědná*, **174**, 87–114.

Clark, R.W., Brown, W.S., Stechert, R. & Zamudio, K.R. (2010a) Roads, interrupted dispersal, and genetic diversity in timber rattlesnakes. *Conservation Biology*, **24**, 1059–1069.

Clark, R.W., Brown, W.S., Stechert, R. & Zamudio, K.R. (2010b) Roads, interrupted dispersal, and genetic diversity in timber rattlesnakes. *Conservation Biology*, **24**, 1059–1069.

Clevenger, A.P., Chruszcz, B. & Gunson, K.E. (2003) Spatial patterns and factors influencing small vertebrate faunaroad-kill aggregations. **109**, 15–26.

Clevenger, A.P. & Kociolek, A. V (2013) Potential impacts of highway median barriers on wildlife: State of the practice and gap analysis. *Environmental Management*, **52**, 1299–1312.

Coelho, I.P., Kindel, A. & Coelho, A.V.P. (2008) Roadkills of vertebrate species on two highways through the Atlantic Forest Biosphere Reserve, southern Brazil. *European Journal of Wildlife Research*, **54**, 689–699.

Coelho, I.P., Teixeira, F.Z., Colombo, P., Coelho, A.V.P. & Kindel, A. (2012) Anuran road-kills neighboring a peri-urban reserve in the Atlantic Forest, Brazil. *Journal of Environmental Management*, **112**, 17–26.

Coffin, A., Oure, D., Bettez, N., Borda-de-Água, L., Daniels, A., Grilo, C., Jaeger, J., Navarro, L., Preisler, H. & Rauschert, E. (2021) The ecology of rural roads: effects, management, and research. *Issues in Ecology*, **23**, 2–35.

Coles, J.M. (1989) The World's Oldest Road. *Scientific American*, **261**, 100–107.

Colley, M., Lougheed, S.C., Otterbein, K. & Litzgus, J.D. (2017) Mitigation reduces road mortality of a threatened rattlesnake. *Wildlife Research*, **44**, 48–59.

Collins, C. & Kays, R. (2011) Causes of mortality in North American populations of large and medium-sized mammals. *Animal Conservation*, **14**, 474–483.

Collinson, W., Davies-Mostert, H., Roxburgh, L. & van der Ree, R. (2019) Status of Road

Ecology Research in Africa: Do We Understand the Impacts of Roads, and How to Successfully Mitigate Them? *Frontiers in Ecology and Evolution*, **7**, 479.

Cook, T.C. & Blumstein, D.T. (2013) The omnivore’s dilemma: Diet explains variation in vulnerability to vehicle collision mortality. *Biological Conservation*, **167**, 310–315.

Costa, I.M. da C., Ferreira, M.S., Mourão, C.L.B. & Bueno, C. (2022) Spatial patterns of carnivore roadkill in a high-traffic-volume highway in the endangered Brazilian Atlantic Forest. *Mammalian Biology*, **102**, 477–487.

Costa, R.R.G.F. & Dias, L.A. (2013) Mortalidade de vertebrados por atropelamento em um trecho da GO-164, no sudoeste goiano. *Revista de Biotecnologia & Ciência*, **2**, 58–74.

Covaciu-Marcov, S.D., Ferenţi, S. & Cicort-Lucaciu, A.S. (2012) Eryx jaculus (Reptilia, Boidae) north of Danube: a road-killed specimen from Romania. *Acta Herpetologica*, **7**, 41–47.

Cramer, P.C. & Portier, K.M. (2001) Modeling Florida panther movements in response to human attributes of the landscape and ecological settings. *Ecological Modelling*, **140**, 51–80.

Csapo, E. (2013) *The Dionysian Parade and the Poetics of Plenitude (UCL Housman Lecture)*,.

Cullen, L., Stanton, J.C., Lima, F., Uezu, A., Perilli, M.L.L. & Resit Akcakaya, H. (2016) Implications of Fine-Grained Habitat Fragmentation and Road Mortality for Jaguar Conservation in the Atlantic Forest, Brazil. *PLOS ONE*, **11**, e0167372.

Curatola Fernández, G.F., Obermeier, W.A., Gerique, A., López Sandoval, M.F., Lehnert, L.W., Thies, B. & Bendix, J. (2015) *Land cover change in the Andes of southern Ecuador-Patterns and drivers*,.

Cutler, D.R., Edwards, T.C., Beard, K.H., Cutler, A., Hess, K.T., Gibson, J. & Lawler, J.J. (2007) Random forests for classification in ecology. *Ecology*, **88**, 2783–2792.

Cuyckens, G.A.E., Mochi, L.S., Vallejos, M., Perovic, P.G. & Biganzoli, F. (2016) Patterns and Composition of Road-Killed Wildlife in Northwest Argentina. *Environmental Management*, **58**, 810–820.

D’Amico, M., Périquet, S., Román, J. & Revilla, E. (2016) Road avoidance responses determine the impact of heterogeneous road networks at a regional scale. *Journal of Applied Ecology*, **53**, 181–190.

D’Amico, M., Román, J., de los Reyes, L. & Revilla, E. (2015) Vertebrate road-kill patterns in Mediterranean habitats: Who, when and where. *Biological Conservation*, **191**, 234–242.

Dávila-Orozco, G., Sendoya, S.F., Kindel, A. & Dias, R.A. (2022) Multisite species-level roadkill across years reveals challenges and opportunities for monitoring and mitigation. *Austral Ecology*, **47**, 341–352.

Delgado-V, C. (2007) Muerte de mamíferos por vehículos en la vía del Escobero, Envigado (Antioquia), Colombia. *Actual Biol*, **29**, 235–239.

Diniz, M.F. & Brito, D. (2015) Protected areas effectiveness in maintaining viable giant anteater (Myrmecophaga tridactyla) populations in an agricultural frontier. *Natureza e Conservacao*, **13**, 145–151.

Duffett, D., Amico, M.D. & Mulero-Pázmány, M. (2020) Species ’ traits as predictors of avoidance towards roads and traffic. *Ecological Indicators*, **115**, 106402.

Dutra-Vieira, F.M., Silva, M.S., Vieira, G.S., Carvalho, A.S. & Schimming, B.C. (2021) Diet of crab-eating fox (Cerdocyon thous) in two conservation units of the Amazon rainforest, Brazil. *Brazilian journal of biology = Revista brasleira de biologia*, **84**.

Dvorak, M., Fessl, B., Nemeth, E., Kleindorfer, S. & Tebbich, S. (2012) Distribution and abundance of Darwin’s finches and other land birds on Santa Cruz Island, Galápagos: evidence for declining populations. *Oryx*, **46**, 78–86.

Ellenberg, H., Müller, K. & Stottele, T. (1981) Straßen-Ökologie: Auswirkungen von Autobahnen und Straßen auf Ökosysteme deutscher Landschaften. *Ökologie und Straße*, 19–122.

Englefield, B., Starling, M. & McGreevy, P. (2018) A review of roadkill rescue: Who cares for the mental, physical and financial welfare of Australian wildlife carers? *Wildlife Research*, **45**, 103–118.

Espinosa, S., Celis, G. & Branch, L.C. (2018) When roads appear jaguars decline: Increased access to an Amazonian wilderness area reduces potential for jaguar conservation. *PLoS ONE*, **13**, e0189740.

Fabrizio, M., Di Febbraro, M. & Loy, A. (2019) Where will it cross next? Optimal management of road collision risk for otters in Italy. *Journal of Environmental Management*, **251**.

Ferreguetti, A.C., Graciano, J.M., Luppi, A.P., Pereira-Ribeiro, J., Rocha, C.F.D. & Bergallo, H.G. (2020) Roadkill of medium to large mammals along a Brazilian road (BR-262) in Southeastern Brazil: spatial distribution and seasonal variation. *Studies on Neotropical Fauna and Environment*, **55**, 216–225.

Ferreras, P., Aldama, J.J., Beltrán, J.F. & Delibes, M. (1992) Rates and causes of mortality in a fragmented population of Iberian lynx Felis pardina Temminck, 1824. *Biological Conservation*, **61**, 197–202.

Ficetola, G.F. & De Bernardi, F. (2004) Amphibians in a human-dominated landscape: The community structure is related to habitat features and isolation. *Biological Conservation*, **119**, 219–230.

Filius, J., Hoek, Y., Jarrín-V, P. & Hooft, P. (2020) Wildlife roadkill patterns in a fragmented landscape of the Western Amazon. *Ecology and Evolution*, **10**, 6623–6635.

FONAG -EPMAPS (2021) *Anuario Hidrometeorológico 2020 Red Integrada De Monitoreo Hidrometeorológico*,.

Ford, S.G. (1980) *Evaluation of Highway Deer Kill Mitigation on SIE/LAS-395: Interim Report*, California-USA.

Forman, R.T.T., Sperling, D., Bissonette, J.A., Clevenger, A.P., Cutshall, C.D., Dale, V.H., Fahrig, L., France, R., Goldman, C.R., Heanue, K., Jones, J.A., Swanson, F.J., Turrentine, T. & Winter, T.C. (2003) *Road ecology: science and solutions*, Island Press, Washington, DC.

Fox, S., Potts, J.M., Pemberton, D. & Crosswell, D. (2019) Roadkill mitigation: Trialing virtual fence devices on the west coast of Tasmania. *Australian Mammalogy*, **41**, 205–211.

Frank, R., Melaun, C., Martins, M.M., Santos, A.L.Q., Heukelbach, J. & Klimpel, S. (2012) Tunga penetrans and further parasites in the giant anteater (Myrmecophaga tridactyla) from Minas Gerais, Brazil. *Parasitology Research*, **111**, 1907–1912.

Freile, J. & Poveda, C. (2019) Aves del Ecuador. Version 2019.0. Museo de Zoología, Pontificia Universidad Católica del Ecuador.

Freitas, D., de Oliveira, A., Ciocheti, G., Vieira, M. & Matos, D. (2015) How landscape features influence road-kill of three species of mammals in the Brazilian savanna? *Oecologia Australis*, **18**, 35–45.

Freitas, Justino, C.S. & Setz, E.Z.F. (2014) Road-kills of the giant anteater in south-eastern Brazil: 10 years monitoring spatial and temporal determinants. *Wildlife Research*, **41**, 673–680.

Funk, W., Caminer, M. & Ron, S.R. (2012) High levels of cryptic species diversity uncovered in Amazonian frogs. *Proceedings of the Royal Society B: Biological Sciences*, **279**, 1806–1814.

FWC (2016) *Annual Report on the Research and Management of Florida Panthers: 2015-2016*, Florida-USA.

GADPN (2018) *Proyecto de Actualización Plan de Desarrollo y Ordenamiento Territorial de la provincia de Napo.*, Ecuador.

Gaón, J. & Valdez, G. (2021) Mortalidad de la fauna silvestre por efecto vehicular en la carretera Calceta-Quiroga.

García-Carrasco, J.M., Tapia, W. & Muñoz, A.R. (2020) Roadkill of birds in Galapagos islands: A growing need for solutions. *Avian Conservation and Ecology*, **15**, 1–8.

Gaynor, K.M., Hojnowski, C.E., Carter, N.H. & Brashares, J.S. (2018) The influence of human disturbance on wildlife nocturnality. *Science,* **360**, 1232–1235.

Girardet, X., Conruyt-Rogeon, G. & Foltête, J.C. (2015) Does regional landscape connectivity influence the location of roe deer roadkill hotspots? *European Journal of Wildlife Research*, **61**, 731–742.

Gomes, D.J., Wierzbowska, I.A., Bevanger, K., O'Mahony, D.T. & Rola, K. (2020) Diet of the European badgers (Meles meles) in urban and rural areas of Norway. *European Journal of Wildlife Research*, **66**.

González-Gallina, A., Benítez-Badillo, G., Rojas-Soto, O.R. & Hidalgo-Mihart, M.G. (2013) The small, the forgotten and the dead: Highway impact on vertebrates and its implications for mitigation strategies. *Biodiversity and Conservation*, **22**, 325–342.

González-Suárez, M., Lucas, P.M. & Revilla, E. (2012) Biases in comparative analyses of extinction risk: Mind the gap. *Journal of Animal Ecology*, **81**, 1211–1222.

González-Suárez, M., Zanchetta Ferreira, F. & Grilo, C. (2018) Spatial and species-level predictions of road mortality risk using trait data. *Global Ecology and Biogeography*, **27**, 1093–1105.

González-Vélez, J.C., Torres-Madronero, M.C., Murillo-Escobar, J. & Jaramillo-Fayad, J.C. (2021) An artificial intelligent framework for prediction of wildlife vehicle collision hotspots based on geographic information systems and multispectral imagery. *Ecological Informatics*, **63**.

González, M. (2018) Mortalidad de fauna silvestre por efecto vehicular en el área de influencia de la Reserva Ecológica Manglares de Churute durante la época seca y húmeda.

Greenwell, B.M. (2017) pdp: An R Package for Constructing Partial Dependence Plots. *The R Journal*, **9**, 421–436.

Grilo, C., Borda-de-Água, L., Beja, P., Goolsby, E., Soanes, K., le Roux, A., Koroleva, E., Ferreira, F.Z., Gagné, S.A., Wang, Y. & González-Suárez, M. (2021) Conservation threats from roadkill in the global road network. *Global Ecology and Biogeography*, **30**, 2200–2210.

Grilo, C., Coimbra, M.R., Cerqueira, R.C., Barbosa, P., Dornas, R.A.P., Gonçalves, L.O., Teixeira, F.Z., Coelho, I.P., Schmidt, B.R., Pacheco, D.L.K., Schuck, G., Esperando, I.B., Anza, J.A., Beduschi, J., Oliveira, N.R., Pinheiro, P.F., Bager, A., Secco, H., Guerreiro, M., Carvalho, C.F., Veloso, A.C., Custódio, A.E.I., Marçal, O., Ciocheti, G., Assis, J., Ribeiro, M.C., Francisco, B.S.S., Cherem, J.J., Trigo, T.C., Jardim, M.M.A., Franceschi, I.C., Espinosa, C., Tirelli, F.P., Rocha, V.J., Sekiama, M.L., Barbosa, G.P., Rossi, H.R., Moreira, T.C., Cervini, M., Rosa, C.A., Silva, L.G., Ferreira, C.M.M., César, A., Casella, J., Mendes, S.L., Zina, J., Bastos, D.F.O., Souza, R.A.T., Hartmann, P.A., Deffaci, A.C.G., Mulinari, J., Luzzi, S.C., Rezzadori, T., Kolcenti, C., Reis, T.X., Fonseca, V.S.C., Giorgi, C.F., Migliorini, R.P., Kasper, C.B., Bueno, C., Sobanski, M., Pereira, A.P.F.G., Andrade, F.A.G., Fernandes, M.E.B., Corrêa, L.L.C., Nepomuceno, A., Banhos, A., Hannibal, W., Fonseca, R., Costa, L.A., Medici, E.P., Croce, A., Werther, K., Oliveira, J.P., Ribeiro, J.M., de Santi, M., Kawanami, A.E., Perles, L., do

Couto, C., Figueiró, D.S., Eizirik, E., Correia, A.A., Corrêa, F.M., Queirolo, D., Quagliatto, A.L., Saranholi, B.H., Galetti, P.M., Rodriguez-Castro, K.G., Braz, V.S., França, F.G.R., Buss, G., Rezini, J.A., Lion, M.B., Cheida, C.C., Lacerda, A.C.R., Freitas, C.H., Venâncio, F., Adania, C.H., Batisteli, A.F., Hegel, C.G.Z., Mantovani, J.A., Rodrigues, F.H.G., Bagatini, T., Curi, N.H.A., Emmert, L., Erdmann, R.H., Costa, R.R.G.F., Martinelli, A., Santos, C.V.F. & Kindel, A. (2018) BRAZIL ROAD-KILL: a data set of wildlife terrestrial vertebrate road-kills. *Ecology*, **99**, 2625.

Grilo, C., Koroleva, E., Andrášik, R., Bíl, M. & González-Suárez, M. (2020) Roadkill risk and population vulnerability in European birds and mammals. *Frontiers in Ecology and the Environment*, **18**, 323–328.

Guayasamin, J.M., Brunner, R.M., Valencia-Aguilar, A., Franco-Mena, D., Ringler, E., Armijos, A.M., Morochz, C., Bustamante, L., Maynard, R.J. & Culebras, J. (2022) Two new glassfrogs (Centrolenidae: Hyalinobatrachium) from Ecuador, with comments on the endangered biodiversity of the Andes. *PeerJ*, **10**, e13109.

Gunson, K.E., Mountrakis, G. & Quackenbush, L.J. (2011) Spatial wildlife-vehicle collision models: A review of current work and its application to transportation mitigation projects. *Journal of Environmental Management*, **92**, 1074–1082.

Heber, S., Wilson, K.J. & Molles, L. (2008) Breeding biology and breeding success of the blue penguin (Eudyptula minor) on the West Coast of New Zealand's South Island. *New Zealand Journal of Zoology*, **35**, 63–71.

Helgen, K.M., Kays, R., Helgen, L.E., Tsuchiya-Jerep, M.T.N., Pinto, C.M., Koepfli, K.-P., Eizirik, E. & Maldonado, J.E. (2009) Taxonomic boundaries and geographic distributions revealed by an integrative systematic overview of the mountain coatis, Nasuella (Carnivora: Procyonidae). *Small Carnivore Conservation*, **41**, 65–74.

Helldin, J.O. & Petrovan, S.O. (2019) Effectiveness of small road tunnels and fences in reducing amphibian roadkill and barrier effects at retrofitted roads in Sweden. *PeerJ*, **2019**, e7518.

Hibbitts, T., Fitzgerald, L., Walkup, D. & Ryberg, W. (2017) Why didn ' t the lizard cross the road ? Dunes sagebrush lizards exhibit road-avoidance behaviour. *Wildlife Research*, 194–199.

Hill, J.E., DeVault, T.L. & Belant, J.L. (2019) Cause-specific mortality of the world's terrestrial vertebrates. *Global Ecology and Biogeography*, **28**, 680–689.

Hill, J.E., De Vault, T.L. & Belant, J.L. (2021) A review of ecological factors promoting road use by mammals. *Mammal Review*, **51**, 214–227.

Hodson, N.L. & Snow, D.W. (1965) The road deaths enquiry, 1960–61. *Bird Study*, **12**, 90–99.

Holbrook, J.D., DeYoung, R.W., Caso, A., Tewes, M.E. & Young, J.H. (2012) Hog-nosed skunks (Conepatus leuconotus) along the Gulf of Mexico: Population status and genetic diversity. *Southwestern Naturalist*, **57**, 223–225.

Holderegger, R. & Di Giulio, M. (2010) The genetic effects of roads: A review of empirical evidence. *Basic and Applied Ecology*, **11**, 522–531.

Huijser, M., Mosler-Berger, C., Olsson, M. & Strein, M. (2015) *Wildlife warning signs and animal detections ystems aimed at reducing wildlife-vehicle collisions*. *Handbook of Road Ecology* (ed. by R. van der Ree), D.J. Smith), and C. Grilo), pp. 198–212. John Wiley & Sons, United Kingdom.

Instituto Geográfico Militar (2017) Infraestructura de datos espaciales para Instituo Geográfico Militar.

IUCN (2022) The IUCN Red List of Threatened Species. Version 2022-1.

IUCN SSC Amphibian Specialist Group (2018) Ceratophrys stolzmanni. The IUCN Red List of Threatened Species 2018: e.T56341A89204905.

Jackson, N.D. & Fahrig, L. (2011) Relative effects of road mortality and decreased connectivity on population genetic diversity. *Biological Conservation*, **144**, 3143–3148.

Jacobson, H.R. (1940) A history of roads from ancient times to the motor age.

Jacobson, S.L., Bliss-Ketchum, L.L., De Rivera, C.E. & Smith, W.P. (2016) A behavior-based framework for assessing barrier effects to wildlife from vehicle traffic volume. *Ecosphere*, **7**, 1–15.

Jared, C., Mailho-Fontana, P.L., Jared, S.G.S., Kupfer, A., Delabie, J.H.C., Wilkinson, M. & Antoniazzi, M.M. (2019) Life history and reproduction of the neotropical caecilian Siphonops annulatus (Amphibia, Gymnophiona, Siphonopidae), with special emphasis on parental care. *Acta Zoologica*, **100**, 292–302.

Ji, S., Jiang, Z., Li, L., Li, C., Zhang, Y., Ren, S., Ping, X., Cui, S. & Chu, H. (2017) Impact of different road types on small mammals in Mt. Kalamaili Nature Reserve. *Transportation Research Part D: Transport and Environment*, **50**, 223–233.

Johnson, T.F., Isaac, N.J.B., Paviolo, A. & González-Suárez, M. (2021) Handling missing values in trait data. *Global Ecology and Biogeography*, **30**, 51–62.

Jones, K.E., Bielby, J., Cardillo, M., Fritz, S.A., O'dell, J., David, C., Orme, L., Safi, K., Sechrest, W., Boakes, E.H., Carbone, C., Connolly, C., Cutts, M.J., Foster, J.K., Grenyer, R., Habib, M., Plaster, C.A., Price, S.A., Rigby, E.A., Rist, J., Teacher, A., Bininda-Emonds, O.R.P., Gittleman, J.L., Mace, G.M. & Purvis, A. (2009) PanTHERIA: a species-level database of life history, ecology, and geography of extant and recently extinct mammals. *Ecology*, **90**, 2648–2648.

Kang, X., Namgung, M., Fujiwara, A., Kim, W. & Wang, W. (2019) Analysis of Vehicle Maneuverability and Driving Characteristics on a Curved Road Condition. *KSCE Journal of Civil Engineering*, **23**, 420–432.

Khalaf, E.E.D.A.H. & El-Kheir, G.A. (2022) The Geological Heritage and Sustainable Development Proposed for the Project Geopark: an Example from Gabal Qatrani, Fayoum Depression, Western Desert, Egypt. *Geoheritage*, **14**, 1–30.

Kioko, J., Kiffner, C., Jenkins, N. & Collinson, W.J. (2015) Wildlife roadkill patterns on a major highway in Northern Tanzania. *African Zoology*, **50**, 17–22.

Kissling, W.D., Dalby, L., Fløjgaard, C., Lenoir, J., Sandel, B., Sandom, C., Trøjelsgaard, K. & Svenning, J.C. (2014) Establishing macroecological trait datasets: Digitalization, extrapolation, and validation of diet preferences in terrestrial mammals worldwide. *Ecology and Evolution*, **4**, 2913–2930.

Kleinschroth, F. & Healey, J.R. (2017) Impacts of logging roads on tropical forests. *Biotropica*, **49**, 620–635.

Kociolek, A., Grilo, C. & Jacobson, S. (2015) *Flight doesn't solve everything: Mitigation of road impacts on birds*. *Handbook of Road Ecology*, pp. 281–289. John Wiley & Sons.

Kouete, M.T. & Blackburn, D.C. (2020) Dietary Partitioning in Two Co-occurring Caecilian Species (Geotrypetes seraphini and Herpele squalostoma) in Central Africa. *Integrative*

Organismal Biology, **2**.

Kroll, G. (2015) An environmental history of roadkill: Road ecology and the making of the permeable highway. *Environmental History*, **20**, 4–28.

De La Ossa, J. & Galván-Guevara, S. (2015) Registro de mortalidad de fauna silvestre por colisión vehicular en. *Biota*, **Volumen 16**, 67–77.

Lala, F., Chiyo, P.I., Kanga, E., Omondi, P., Ngene, S., Severud, W.J., Morris, A.W. & Bump, J. (2021) Wildlife roadkill in the Tsavo Ecosystem, Kenya: identifying hotspots, potential drivers, and affected species. *Heliyon*, **7**, e06364.

Latham, J., Cumani, R., Rosati, I. & Bloise, M. (2014) *FAO Global Land Cover SHARE*,.

Laurance, W.F., Clements, G.R., Sloan, S., O'Connell, C.S., Mueller, N.D., Goosem, M., Venter, O., Edwards, D.P., Phalan, B., Balmford, A., Van Der Ree, R. & Arrea, I.B. (2014) A global strategy for road building. *Nature*, **513**, 229–232.

Laurance, W.F., Goosem, M. & Laurance, S.G.W. (2009) Impacts of roads and linear clearings on tropical forests. *Trends in Ecology and Evolution*, **24**, 659–669.

Lemke, A., Kowarik, I. & von der Lippe, M. (2019) How traffic facilitates population expansion of invasive species along roads: The case of common ragweed in Germany. *Journal of Applied Ecology*, **56**, 413–422.

Leonan, R., Novaes, M., Cláudio, V.C., Carrión-Bonilla, C., Abreu, E.F., Wilson, D.E., Maldonado, J.E. & Weksler, M. (2022) Variation in the Myotis keaysi complex (Chiroptera, Vespertilionidae), with description of a new species from Ecuador. *Journal of Mammalogy*, **103**, 540–559.

Leonan, R., Novaes, M., Laurindo, R.S., Dornas, R.A.P., Esbérard, C.E.L. & Bueno, C. (2018) On a collision course: The vulnerability of bats to roadkills in Brazil. *Mastozoología Neotropical*, **14**, 115–128.

Lesbarrères, D., Primmer, C.R., Lodé, T. & Merilä, J. (2006) The effects of 20 years of highway presence on the genetic structure of Rana dalmatina populations. *Ecoscience*, **13**, 531–538.

Liaw, A. & Wiener, M. (2002) Classification and Regression by randomForest. *R News*, **2**, 18–22.

Lodé, T., Cormier, J.-P. & Le Jacques, D. (2001) Decline in Endangered Species as an Indication of Anthropic Pressures: The Case of European Mink Mustela lutreola Western Population.

Loss, S.R., Will, T. & Marra, P.P. (2014) Estimation of bird-vehicle collision mortality on U.S. roads. *The Journal of Wildlife Management*, **78**, 763–771.

Lucherini, M. (2015) Crab-eating Fox Cerdocyon thous. *The IUCN Red List of Threatened Species*.

Madden, J.R. & Perkins, S.E. (2017) Why did the pheasant cross the road? Long-term road mortality patterns in relation to management changes. *Royal Society Open Science*, **4**.

MAE (2013) *Sistema de Clasificación de los Ecosistemas del Ecuador Continental. Subsecretaría de Patrimonio Natural*, Quito-Ecuador.

Maschio, G.F., Santos-Costa, M.C. & Prudente, A.L.C. (2016) Road-kills of snakes in a tropical rainforest in the Central Amazon Basin, Brazil. *South American Journal of Herpetology*, **11**, 46–53.

McAllister, J.M., Maida, J.R., Dyer, O. & Larsen, K.W. (2016) Diet of Roadkilled Western Rattlesnakes (Crotalus oreganus) and Gophersnakes (Pituophis catenifer) in Southern British Columbia . *Northwestern Naturalist*, **97**, 181–189.

Medrano-Vizcaíno, P. (2018) Efecto de atributos paisajísticos en los patrones de presencia de Ateles fusciceps en el noroccidente ecuatoriano. *Revista Peruana de Biología*, **25**, 241–248.

Medrano-Vizcaíno, P. (2015) Efecto de las carreteras en la mortalidad de vertebrados en un área megadiversa: Los Andes Tropicales del Ecuador.

Medrano-Vizcaíno, P. (2019) Predating behavior of the laughing falcon (Herpetotheres cachinnans) on the venomous amazonian pit viper Bothrops atrox (the use of roads as a prey source). *BioRisk*, **14**, 25–30.

Medrano-Vizcaíno, P., Bedoya, J. & Cadena-Ortiz, H. (2020) Dynamics of the distribution and hosts of Molothrus bonariensis (Passeriformes: Icteridae) in Ecuador. *Caldasia*, **42**, 38–49.

Medrano-Vizcaíno, P. & Brito-Zapata, D. (2021) Filling biogeographical gaps through wildlife roadkills: New distribution records for six snake species from Ecuador (Anilius scytale, Drymarchon corais, Erythrolamprus breviceps, Micrurus lemniscatus, Oxyrhopus vanidicus, Trilepida anthracina). *Neotropical Biodiversity*, **7**, 554–559.

Medrano-Vizcaíno, P., Brito-Zapata, D., Rueda, A., Jarrín-V, P., García-Carrasco, J.-M., Medina, D., Aguilar, J., Acosta, N. & Gonzalez-Suarez, M. (2023a) Road mortality data for Ecuadorian wildlife. *figshare. Dataset.*

Medrano-Vizcaíno, P. & Espinosa, S. (2021) Geography of roadkills within the Tropical Andes Biodiversity Hotspot: Poorly known vertebrates are part of the toll. *Biotropica*, **53**, 820–830.

Medrano-Vizcaíno, P., Grilo, C., Brito-Zapata, D. & González-Suárez, M. (2022a) The road of >1000 corpses: landscape and road-related features that promote mortality in the Amazon. *Research square Preprint*.

Medrano-Vizcaíno, P., Grilo, C. & González-Suárez, M. (2023b) Research and conservation priorities to protect wildlife from collisions with vehicles. *Biological Conservation*, **280**, 109952.

Medrano-Vizcaíno, P., Grilo, C. & Gonzalez Suarez, M. (2023c) Predicted vulnerability to roadkills of birds and mammals in Latin America. *figshare. Dataset.*

Medrano-Vizcaíno, P., Grilo, C., Pinto, F.A.S., Carvalho, W.D., Melinski, R., Schultz, E. & González-Suárez, M. (2022b) Roadkill rates and trait data for birds and mammals in Latin America. *figshare. Dataset.*

Medrano-Vizcaíno, P., Grilo, C., Silva Pinto, F.A., Carvalho, W.D., Melinski, R.D., Schultz, E.D. & González-Suárez, M. (2022c) Roadkill patterns in Latin American birds and mammals. *Global Ecology and Biogeography*, **31**, 1756–1783.

Medrano-Vizcaíno, P. & Gutiérrez-Salazar, P. (2020) Current and future suitable habitat areas for Nasuella olivacea (Gray, 1865) in Colombia and Ecuador and analysis of its distribution across different land uses. *Biodiversity Data Journal*, **8**, 1–13.

Meijer, J.R., Huijbregts, M.A.J., Schotten, K.C.G.J. & Schipper, A.M. (2018) Global patterns of current and future road infrastructure. *Environmental Research Letters*, **13**.

Melo-Sampaio, P.R., Passos, P., Prudente, A.L.C., Venegas, P.J. & Torres-Carvajal, O. (2021) Systematic review of the polychromatic ground snakes Atractus snethlageae complex reveals four new species from threatened environments. *Journal of Zoological Systematics and Evolutionary Research*, **59**, 718–747.

Meza-Joya, F.L., Ramos, E. & Cardona, D. (2019) Spatio-temporal patterns of mammal road mortality in middle magdalena valley, Colombia. *Oecologia Australis*, **23**, 575–588.

Ministerio del Ambiente del Ecuador (2018) Cobertura y Uso de la Tierra (CUT 2018).

Miotto, R.A., Cervini, M., Figueiredo, M.G., Begotti, R.A. & Galetti, P.M. (2011) Genetic diversity and population structure of pumas (Puma concolor) in southeastern Brazil: Implications for conservation in a human-dominated landscape. *Conservation Genetics*, **12**, 1447–1455.

Monroy, M.C., De La Ossa-Lacayo, A. & De La Ossa V, J. (2015) Tasa de atropellamiento de fauna silvestre en la vía San Onofre – María la baja, Caribe Colombiano. *Revista de la Asociación Colombiana de Ciencias Biológicas*, 88–95.

Morelli, F., Rodríguez, R.A., Benedetti, Y. & Delgado, J.D. (2020) Avian roadkills occur regardless of bird evolutionary uniqueness across Europe. *Transportation Research Part D: Transport and Environment*, **87**, 102531.

Mori, E., Sforzi, A., Menchetti, M., Mazza, G., Lovari, S. & Pisanu, B. (2015) Ectoparasite load in the crested porcupine Hystrix cristata Linnaeus, 1758 in Central Italy. *Parasitology Research*, **114**, 2223–2229.

MTOP (2016) *Plan estratégico de movilidad 2013 - 2037*, Ministerio de Transporte y Obras Públicas, Ecuador.

Mueller, M.A., Drake, D. & Allen, M.L. (2019) Using citizen science to inform urban canid management. *Landscape and Urban Planning*, **189**, 362–371.

Mulero-Pázmány, M., D’Amico, M. & González-Suárez, M. (2016) Ungulate behavioral responses to the heterogeneous road-network of a touristic protected area in Africa. *Journal of Zoology*, **298**, 233–240.

Mulero-Pázmány, M., Sullivan, T., Rollán C, L., D’amico, M. & González-Suárez, M. (2022) Road orientation affects the impact of roads on wildlife. *Wildlife Research*.

Myers, N., Mittermeler, R.A., Mittermeler, C.G., Da Fonseca, G.A.B. & Kent, J. (2000) Biodiversity hotspots for conservation priorities. *Nature*, **403**, 853–858.

Myhrvold, N.P., Baldridge, E., Chan, B., Sivam, D., Freeman, D.L. & Ernest, S.K.M. (2015) An amniote life-history database to perform comparative analyses with birds, mammals, and reptiles. *Ecology*, **96**, 3109–3109.

Newmark, W.D., Boshe, J.I., Sariko, H.I. & Makumbule, G.K. (1996) Effects of a highway on large mammals in Mikumi National Park, Tanzania. *African Journal of Ecology*, **34**, 15–31.

Nobre, C.A., Sampaio, G., Borma, L.S., Castilla-Rubio, J.C., Silva, J.S. & Cardoso, M. (2016) Land-use and climate change risks in the amazon and the need of a novel sustainable development paradigm. *Proceedings of the National Academy of Sciences of the United States of America*, **113**, 10759–10768.

Ogletree, K. & Mead, A. (2020) What Roadkills Did We Miss in a Driving Survey? A Comparison of Driving and Walking Surveys in Baldwin County, Georgia. *Georgia Journal of Science*, **78**, 8.

Paluszynska, A., Biecek, P. & Jiang, Y. (2020) randomForestExplainer: Explaining and Visualizing Random Forests in Terms of Variable Importance.

Paterson, J.E., Baxter-Gilbert, J., Beaudry, F., Carstairs, S., Chow-Fraser, P., Edge, C.B., Lentini, A.M., Litzgus, J.D., Markle, C.E., McKeown, K., Moore, J.A., Refsnider, J.M., Riley, J.L., Rouse, J.D., Seburn, D.C., Zimmerling, J.R. & Davy, C.M. (2019) Road avoidance and its energetic consequences for reptiles. *Ecology and Evolution*, **9**, 9794–9803.

Payán, E., Soto, C., Díaz-Pulido, A., Benítez, A. & Hernández, A. (2013) Wildlife road crossing and mortality: lessons for wildlife friendly road design in Colombia. *International Conference on Ecology and Transportation*, 1–18.

Peaden, J., Justin Nowakowski, A., Tuberville, T.D., Buhlmann, K.A. & Todd, B.D. (2017) Effects of roads and roadside fencing on movements, space use, and carapace temperatures of a threatened tortoise. *Biological Conservation*, **214**, 13–22.

Penone, C., Davidson, A.D., Shoemaker, K.T., Di Marco, M., Rondinini, C., Brooks, T.M., Young, B.E., Graham, C.H. & Costa, G.C. (2014) Imputation of missing data in life-history trait datasets: which approach performs the best? *Methods in Ecology and Evolution*, **5**, 961–970.

Périquet, S., Roxburgh, L., le Roux, A. & Collinson, W.J. (2018) Testing the value of citizen science for roadkill studies: A case study from South Africa. *Frontiers in Ecology and Evolution*, **6**, 15.

Pickles, W. (1942) Animal Mortality on Three Miles of Yorkshire Roads. *The Journal of Animal Ecology*, **11**, 37.

Pinto, F.A.S., Cirino, D.W., Cerqueira, R.C., Rosa, C. & Freitas, S.R. (2022) How Many Mammals Are Killed on Brazilian Roads? Assessing Impacts and Conservation Implications. *Diversity*, **14**, 835.

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**, 106337.

Pisso-Florez, G.A., Cardona-Giraldo, A., Pizo, H.M. & Ramírez-Chaves, H.E. (2022) A new threat for the rarest neotropical carnivore: the vulnerable Colombian weasel, Neogale felipei. *Mammalia*, **0**.

Plante, J., Jaeger, J.A.G. & Desrochers, A. (2019) How do landscape context and fences influence roadkill locations of small and medium-sized mammals? *Journal of Environmental Management*, **235**, 511–520.

de Quadros, R., Marques, S.M.T., de Moura, A.B. & Antonelli, M. (2014) Primeiro registro do nematoide Physaloptera praeputialis parasitando puma. *Neotropical Biology and Conservation*, **9**, 186–189.

Quintero-Ángel, A., Osorio-Dominguez, D., Vargas-Salinas, F. & Saavedra-Rodríguez, C.A. (2012) Roadkill rate of snakes in a disturbed landscape of Central Andes of Colombia. *Herpetology Notes*, **5**, 99–105.

Raymond, S., Schwartz, A.L.W., Thomas, R.J., Chadwick, E. & Perkins, S.E. (2021) Temporal patterns of wildlife roadkill in the UK. *PLoS ONE*, **16**.

Van Der Ree, R., Smith, D. & Grilo, C. (2015a) *Handbook of road ecology*, (ed. by John Wiley & Sons).

Van Der Ree, R., Smith, D.J. & Grilo, C. (2015b) The Ecological Effects of Linear Infrastructure and Traffic: Challenges and Opportunities of Rapid Global Growth. *Handbook of Road Ecology*, 1–9.

de Resende Assis, J., Carvalho-Roel, C.F., Iannini-Custódio, A.E., Pereira, W.G. & Veloso, A.C. (2020) Snakes roadkill on highways in the Cerrado biome: an intentional conduct? *Studies on Neotropical Fauna and Environment*.

Ribas, C.C., Fritz, S.C. & Baker, P.A. (2022) The challenges and potential of geogenomics for biogeography and conservation in Amazonia. *Journal of Biogeography*.

Ridgely, R.; Greenfield, P. (2006) *Aves del Ecuador, Guia de Campo*, Colibri Digital.

Rincón-Aranguri, M., Urbina-Cardona, N., Galeano, S.P., Bock, B.C. & Páez, V.P. (2019) Road Kill of Snakes on a Highway in an Orinoco Ecosystem: Landscape Factors and Species Traits Related to Their Mortality. *Tropical Conservation Science*, **12**, 194008291983083.

Rød-Eriksen, L., Skrutvold, J., Ivar Herfindal, |, Jensen, H. & Eide, N.E. (2020) Highways associated with expansion of boreal scavengers into the alpine tundra of Fennoscandia. *J Appl Ecol*, **57**, 1861–1870.

Ron, S., Merino-Viteri, A. & Ortiz, D. (2021) Anfibios del Ecuador. Version 2021.0. Museo de Zoología, Pontificia Universidad Católica del Ecuador.

Row, J.R., Blouin-Demers, G. & Weatherhead, P.J. (2007) Demographic effects of road mortality in black ratsnakes (Elaphe obsoleta). *Biological Conservation*, **137**, 117–124.

Ruedas, L. & Smith, A.T. 2019. (2019) Sylvilagus brasiliensis. The IUCN Red List of Threatened Species.

Rytwinski, T. & Fahrig, L. (2012) Do species life history traits explain population responses to roads? A meta-analysis. *Biological Conservation*, **147**, 87–98.

Safford, R.J., Ash, J.S., Duckworth, J.W., Telfer, M.G. & Zewdie, C. (1995) A new species of nightjar from Ethiopia. *Ibis*, **137**, 301–307.

Salazar-Sánchez, D. (2022) High density road kill event of Gymnopis multiplicata and first road kill report of Dermophis glandulosus, two caecilian species in Costa Rica. *Revista Latinoamericana de Herpetologia*, **5**, 65–67.

Sangermano, F., Toledano, J. & Eastman, R. (2012) Land cover change in the Bolivian Amazon and its implications for REDD+ and endemic biodiversity. *Landscape Ecology*, **27**, 571–584.

Santos, S.M., Carvalho, F. & Mira, A. (2011) How Long Do the Dead Survive on the Road? Carcass Persistence Probability and Implications for Road-Kill Monitoring Surveys. *PLoS ONE*, **6**, e25383.

Santos, S.M., Mira, A., Salgueiro, P.A., Costa, P., Medinas, D. & Beja, P. (2016) Avian trait-mediated vulnerability to road traffic collisions. *Biological Conservation*, **200**, 122–130.

Schwartz, A.L.W., Shilling, F.M. & Perkins, S.E. (2020) The value of monitoring wildlife roadkill. *European Journal of Wildlife Research*, **66**, 1–12.

Seo, C., Thorne, J.H., Choi, T., Kwon, H. & Park, C.H. (2013) Disentangling roadkill: the influence of landscape and season on cumulative vertebrate mortality in South Korea. *Landscape and Ecological Engineering*, **11**, 87–99.

Shyama Prasad Rao, R. & Saptha Girish, M.K. (2007) Road kills: Assessing insect casualties using flagship taxon. *Current Science*, **92**, 830–837.

Silva, I., Crane, M. & Savini, T. (2021) The road less traveled: Addressing reproducibility and conservation priorities of wildlife-vehicle collision studies in tropical and subtropical regions. *Global Ecology and Conservation*, **27**, 1–12.

Silva, Simões, M.P., Mira, A. & Santos, S.M. (2019) Factors influencing predator roadkills: The availability of prey in road verges. *Journal of Environmental Management*, **247**, 644–650.

Silveira Miranda, J.E., de Melo, F.R. & Keichi Umetsu, R. (2020) Are Roadkill Hotspots in the Cerrado Equal Among Groups of Vertebrates? *Environmental Management*.

Solís, F., Ibáñez, R., Hammerson, G., Hedges, B., Diesmos, A., Matsui, M., Hero, J., Richards, S., Coloma, L., Ron, S., La Marca, E., Hardy, J., Poweel, R., Bolaós, F., Chaves, G. & Ponce, P. (2009) Cane Toad Rhinella marina. *The IUCN Red List of Threatened Species*.

Stekhoven, D. (2013) *missForest: Nonparametric Missing Value Imputation using Random Forest. R package version 1.4.,*.

Stekhoven, D. & Bühlmann, P. (2012) Missforest-Non-parametric missing value imputation for mixed-type data. *Bioinformatics*, **28**, 112–118.

Stenseth, N.C., Leirs, H., Skonhoft, A., Davis, S.A., Pech, R.P., Andreassen, H.P., Singleton, G.R., Lima, M., Machang'u, R.S., Makundi, R.H., Zhang, Z., Brown, P.R., Shi, D. & Wan, X. (2003) Mice, rats, and people: The bio-economics of agricultural rodent pests. *Frontiers in Ecology and the Environment,* **1**, 367–375.

Stoner, D. (1925) The toll of the automobile. *Science*, **61**, 56–57.

Strassburg, B.B.N., Kelly, A., Balmford, A., Davies, R.G., Gibbs, H.K., Lovett, A., Miles, L., Orme, C.D.L., Price, J., Turner, R.K. & Rodrigues, A.S.L. (2010) Global congruence of carbon storage and biodiversity in terrestrial ecosystems. *Conservation Letters*, **3**, 98–105.

Street, S.E., Navarrete, A.F., Reader, S.M. & Laland, K.N. (2017) Coevolution of cultural intelligence, extended life history, sociality, and brain size in primates. *Proceedings of the National Academy of Sciences of the United States of America,* **114**, 7908–7914.

Swinnen, K.R.R., Jacobs, A., Claus, K., Ruyts, S., Vercayie, D., Lambrechts, J. & Herremans, M. (2022) "Animals under wheels": Wildlife roadkill data collection by citizen scientists as a part of their nature recording activities. *Nature Conservation*, **47**, 121–153.

Tanner, D. & Perry, J. (2007) Road effects on abundance and fitness of Galápagos lava lizards (Microlophus albemarlensis). *Journal of Environmental Management*, **85**, 270–278.

Taylor-Brown, A., Booth, R., Gillett, A., Mealy, E., Ogbourne, S.M., Polkinghorne, A. & Conroy, G.C. (2019) The impact of human activities on Australian wildlife. *PLoS ONE*, **14**, 1–28.

Teixeira, F.Z., Coelho, I.P., Esperandio, I.B., Oliveira, N.R., Peter, F.P., Dornelles, S.S., Delazeri, N.R., Tavares, M., Martins, M.B. & Kindel, A. (2013) Are Road-Kill Hotspots Coincident Among Different Vertebrate Groups? *Oecologia Australis*, **17**, 36–47.

Tejera, G., Rodríguez, B., Armas, C. & Rodríguez, A. (2018) Wildlife-vehicle collisions in lanzarote biosphere reserve, Canary Islands. *PLoS ONE*, **13**, e0192731.

Tinoco, B., Atudillo, P., S, L. & Graham, C. (2009) Distribution, ecology and conservation of an endangered Andean hummingbird: the Violet-throated Metaltail (Metallura baroni). *Bird Conservation International*, 63–76.

Tirira, D. (2017) *Mamíferos del Ecuador guía de campo*, Editorial Murciélago Blanco, Quito_Ecuador.

Torres-Carvajal, O., Pazmiño-Otamendi, G., Ayala-Varela, F. & Salazar-Valenzuela, D. (2022) Reptiles del Ecuador. Version 2022.1. Museo de Zoología, Pontificia Universidad Católica del Ecuador.

Tucker, M.A., Böhning-Gaese, K., Fagan, W.F., Fryxell, J.M., Van Moorter, B., Alberts, S.C., Ali, A.H., Allen, A.M., Attias, N., Avgar, T., Bartlam-Brooks, H., Bayarbaatar, B., Belant, J.L., Bertassoni, A., Beyer, D., Bidner, L., Van Beest, F.M., Blake, S., Blaum, N., Bracis, C., Brown, D., De Bruyn, P.J.N., Cagnacci, F., Calabrese, J.M., Camilo-Alves, C., Chamaillé-Jammes, S., Chiaradia, A., Davidson, S.C., Dennis, T., DeStefano, S., Diefenbach, D., Douglas-Hamilton, I., Fennessy, J., Fichtel, C., Fiedler, W., Fischer, C., Fischhoff, I., Fleming, C.H., Ford, A.T., Fritz, S.A., Gehr, B., Goheen, J.R., Gurarie, E., Hebblewhite, M., Heurich, M., Hewison, A.J.M., Hof, C., Hurme, E., Isbell, L.A., Janssen, R., Jeltsch, F., Kaczensky, P., Kane, A., Kappeler, P.M., Kauffman, M., Kays, R., Kimuyu, D., Koch, F., Kranstauber, B., LaPoint, S., Leimgruber, P., Linnell, J.D.C., López-López, P., Markham, A.C., Mattisson, J., Medici, E.P., Mellone, U., Merrill, E., De MirandaMourão, G., Morato, R.G., Morellet, N., Morrison, T.A., Díaz-Muñoz, S.L., Mysterud, A., Nandintsetseg, D., Nathan, R., Niamir, A., Odden, J., O’Hara, R.B., Oliveira-Santos, L.G.R., Olson, K.A., Patterson, B.D., De Paula, R.C., Pedrotti, L., Reineking, B., Rimmler, M., Rogers, T.L., Rolandsen, C.M., Rosenberry, C.S., Rubenstein, D.I., Safi, K., Saïd, S., Sapir, N., Sawyer, H., Schmidt, N.M., Selva, N., Sergiel, A., Shiilegdamba, E., Silva, J.P., Singh, N., Solberg, E.J., Spiegel, O., Strand, O., Sundaresan, S., Ullmann, W., Voigt, U., Wall, J., Wattles, D., Wikelski, M., Wilmers, C.C., Wilson, J.W., Wittemyer, G., Zięba, F., Zwijacz-Kozica, T. & Mueller, T. (2018) Moving in the Anthropocene: Global reductions in terrestrial mammalian movements. *Science*, **359**, 466–469.

Valencia, J., Garzón-Tello, K. & Barragán-Paladines, M. (2016) *Serpientes venenosas del Ecuador: sistemática, taxonomía, historia natural, conservación, envenenamiento y aspectos antropológicos*, Fundación Herpetológica Gustavo Orcés, Quito.

Valerio, F., Basile, M. & Balestrieri, R. (2021) The identification of wildlife-vehicle collision hotspots: Citizen science reveals spatial and temporal patterns. *Ecological Processes*, **10**, 1–13.

Vercayie, D. & Herremans, M. (2015) Citizen science and smartphones take roadkill monitoring to the next level. *Nature Conservation*, **11**, 29–40.

Vilela, T., Harb, A.M., Bruner, A., Da Silva Arruda, V.L., Ribeiro, V., Alencar, A.A.C., Grandez, A.J.E., Rojas, A., Laina, A. & Botero, R. (2020) A better Amazon road network for people and the environment. *Proceedings of the National Academy of Sciences of the United States of America*, **117**, 7095–7102.

Viteri-Salazar, O. & Toledo, L. (2020) The expansion of the agricultural frontier in the northern Amazon region of Ecuador, 2000–2011: Process, causes, and impact. *Land Use Policy*, **99**, 104986.

Wallach, V., Williams, K. & Boundy, J. (2014) *Snakes of the world. A catalogue of living and extinct species.*, Taylor & Francis Group, Boca Raton, FL.

Wang, Y., Yang, Y., Han, Y., Shi, G., Zhang, L., Wang, Z., Cao, G., Zhou, H., Kong, Y., Piao, Z. & Merrow, J. (2022) Temporal patterns and factors influencing vertebrate roadkill in China. *Transportation Research Interdisciplinary Perspectives*, **15**.

Weisbrod, G., Vary, D. & Treyz, G. (2003) Measuring economic costs of urban traffic congestion to business. *Transportation Research Record*, 98–106.

Wiedenfeld, D.A. (2006) Aves, The Galapagos Islands, Ecuador. *Check list*, **2**, 1–27.

Wilkinson, M. (2012) Caecilians. *Current Biology*, **22**, R668–R669.

Wilman, H., Belmaker, J., Simpson, J., De, C., Rosa, L.A., Rivadeneira, M.M. & Jetz, W. (2014) EltonTraits 1.0: Species-level foraging attributes of the world’s birds and mammals. *Ecology*, **95**, 2027–2027.

Wright, P.G.R., Coomber, F.G., Bellamy, C.C., Perkins, S.E. & Mathews, F. (2020) Predicting hedgehog mortality risks on British roads using habitat suitability modelling. *PeerJ*, **2020**, 1–22.

Wypustek, A. (2021) Laughing in the Face of Death: A Survey of Unconventional Hellenistic and Greek-Roman Funerary Verse-Inscriptions. *Klio*, **103**, 160–187.

Young, K.R. (1994) Roads and the Environmental Degradation of Tropical Montane Forests. *Conservation Biology*, **8**, 972–976.

Yue, S., Bonebrake, T.C. & Gibson, L. (2019) Informing snake roadkill mitigation strategies in Taiwan using citizen science. *Journal of Wildlife Management*, **83**, 80–88.

Yusefi, G.H., Brito, J.C., Soofi, M. & Safi, K. (2022) Hunting and persecution drive mammal declines in Iran. *Scientific Reports*, **12**, 1–10.

Zavala, J. (2020) Mortalidad de fauna silvestre en la carretera 483 tramo Jipijapa-Puerto Cayo.

Zhao, H., Yin, Z., Chen, R., Chen, H., Song, C., Yang, G. & Wang, Z. (2010) Investigation of 184 passenger car-pedestrian accidents. *International Journal of Crashworthiness*, **15**, 313–320.

Zizka, A., Barratt, C.D., Ritter, C.D., Joerger-Hickfang, T. & Zizka, V.M.A. (2021) Existing approaches and future directions to link macroecology, macroevolution and conservation prioritization. *Ecography*, **44**, 1–15.