

# **Factors influencing the use of road-crossing culverts by carnivores**

**João Paulo Martinho Craveiro**

Dissertation for the degree of Master of Science in  
**Management and Conservation of Natural Resources**

Advisors: Dr. Pedro Jorge Gonçalves Vaz  
Dr. António Paulo Pereira de Mira

**(Preliminary version)**

# **Factors influencing the use of road-crossing culverts by carnivores**

**João Paulo Martinho Craveiro**

Dissertação para a obtenção do Grau de Mestre em  
**Gestão e Conservação dos Recursos Naturais**

Orientadores: Doutor Pedro Jorge Gonçalves Vaz  
Doutor António Paulo Pereira de Mira

**(Versão provisória)**

*“Nothing has such power to broaden the mind as the ability to investigate systematically and truly all that comes under thy observation in life.”*

**Marcus Aurelios (121 AD – 180 AD)**

## Acknowledgments

“I cannot even imagine where I would be today were it not for that handful of friends who have given me a heart full of joy. Let's face it, friends make life a lot more fun.” I believe in this quote. It is my family and friends who give me strength to carry on in a society which I consider really unfair. However, I had the pleasure to know, during my life, people different from the others. These have accompanied and supported me in the most difficult moments. It is in them that I find my joy. And it is to them that I want to thank:

My advisor Pedro Vaz, for accepting me as his graduate student and helping me in this work. It was not just a doctor's office. He helped me in the field and we shared many funny moments that were happening throughout this work. He also shared his knowledge and experience, making me a better scientist. Without him, it would be impossible to do this thesis.

My co-advisor, António Mira, for accepting me on his team and for believing that I could do, at least, a good job. I am also grateful for the fact that he integrated me in a very important project as it is the *LIFELINES*. It was an opportunity that I will never forget.

To Project *LIFELINES* for financial support and to UBC for logistical support. And to the whole team from *UBC*. They are fantastic, with great fellowship and mutual assistance. It was with them that I spent most of the time I was in Évora. I loved all team dinners and barbecues at the end of work. With them I shared many joys. And thanks for taking me to the field when needed.

Bruno Silva, “my companion in arms”. We had many talks in the team dinners. Some of them unusual, encouraged by some wine and whiskey. You also saved me in some complicated situations. You know which. For you, I send you a big hug and good luck for your dream project. From you, I get your great friendship.

Silva Barreiro. Someone I know is lucky to have you. You're a great friend. You were always friendly to me, and I will never forget that. Remember, you like to walk at night like bats, but you won a friend to walk with you in the middle of its darkness whenever you need.

“People of the mud”, Tiago Pinto and Luís Sousa. Thank you for “adopting me”. We “sail” in different “kinds of waters”, but the “dirt” in the end of the day is the same (just a joke about

our work). Thank you for your friendship. Catch amphibians and reptiles with you was really funny. I appreciate all the time spent in the same office, laughing at the *facebook* jokes and videos made by idiots. But I have to ask you a favor. Take care of my desk. I hope it inspires others. A big hug for both.

Francesco Valério, the Italian guy. You are my fellow bus. I loved watching you beginning to tell jokes in a portuguese way. You are a great friend from another country. I hope you can solve the problem you shared with me in the retreat. You deserve it.

Rui Raimundo. You came later, but you revealed to be a spectacular person. You brought organization to the team. Everyone recognize your work. Also I consider you one of the persons with their “feet more firmly on the ground”. I wish you the best.

Pedro Costa, comrade. Thanks for the knowledge you shared with me. Without you I had never been able to identify the footprints. I really enjoyed having followed you in other works than my thesis. Sorry by missiles in the face thrown in the pool, but had to be. I leave you with a phrase: “long live the Jerónimo”.

Joana Bernardino, my “partner in crime”. I really enjoyed meet you and all the time spent with you. You came many times to the field with me. You did more than what they asked you when you went to Évora. I am eternally grateful to you. Anything I say will not be enough to show you that. So, I just tell you this: “Oi, Caramba”.

To my classmates, Pedro Nunes, Inês Marques, Marina Martins, Cláudia Penedos, Roberto Oliveira, Inês Ferreira, Beatriz Jorge and Tomás Araújo. We spent great moments and many laughs over these two years. I hope you can achieve all your dreams. We know it is difficult, but not impossible.

Pedro Nunes, Rui Martins and Pedro Bicho. We met only in Bachelor degree, but I feel that we are friends since forever. Thank you for what you have given me in my years as a university student. We shared many time protocols reductions and broke some test tubes and pipettes. We will be friends forever.

My childhood friends, some of which I do not see for a long time, that helped me to live my childhood.

To my mom. You made many sacrifices for me to be here. You lost a lot in your life because of me. You always gave me everything you could, but the most important was your love. Brother, we shared many plays. I remember playing *Pokémon*, *Digimon*, football and many other things with you. I will always be here to protect you. Uncle, you have always been there since I can remember. Thank you for all the support that you gave to our family. You three are the most precious thing I have. Everything I do and that I will make will be for you, including this thesis.

# Content

Abstract.....	ix
Resumo.....	x
Resumo alargado.....	xi
Scientific article.....	1
Abstract.....	1
1. Introduction.....	2
2. Materials and methods.....	4
2.1. Study area and study design.....	4
2.2. Crossings assessment.....	5
2.2.1. Data collection.....	7
2.2.2. Statistical analysis.....	11
3. Results.....	11
3.1. Predictors of probability of culvert usage by all carnivores.....	12
3.2. Species-specific predictors of probability of culvert usage.....	12
3.3. Factors influencing the number of crossings by carnivores.....	15
4. Discussion.....	16
4.1. How does water in culverts affects crossings by carnivores? .....	16
4.2. Other factors affecting culvert crossing by carnivores.....	18
4.3. Implications and conclusions.....	19
Acknowledgments .....	20
References.....	20

## Figures

Figure 1 – Drainage culverts sampled in southern Portugal and their nearest passages. N4, N18, N114 = Portuguese National roads.....	4
Figure 2 – Examples of the road-crossing drainage culverts sampled in this study. A – Circular; B – box; C – arch.....	5
Figure 3 – Examples of hardboard plates used in this study with rectangular wood cuboids (3x3x10 cm) nailed on the underside of each plate to raise it 3 cm above ground level and roof tiles to raise the plates up to a maximum of 10 cm whenever the culvert had water.....	6
Figure 4 – Scheme used to assign carnivore visits and complete crossings per sampling date in a culvert. The movement direction in one or two hardboard plates mounted in the culvert was assessed.....	7
Figure 5 – Examples of the double (A) and single (B) guard rail types, stream running inside one culvert (C) and a typical farm fence in Alentejo, southern Portugal.....	8
Figure 6 – Measurement scheme for the estimated percentages of grasses and shrubs within a 10 m radius from both culvert entrances and the averages of grass and of shrub heights (cm) measured at 12 points per entrance. Adapted from Chambers and Bencini (2015).....	9
Figure 7 – Topographic classes within 50, 100, and 150 m scales from the culvert entrance and within two 20 m strips parallel to both sides of the road: class UU = below grade; DD = raised; class UD = up and down sloped; class FF = road level; class DF = down sloped; class UF = up sloped.....	10
Figure 8 – Mean ( $\pm$ 95% CI) fitted values for the optimal logistic mixed-effects model predicting probability of drainage culvert usage by mid-sized carnivores in Évora district, southern Portugal.....	12
Figure 9 – Mean ( $\pm$ 95% CI) fitted values for the optimal logistic mixed-effects model predicting probability of drainage culvert usage by Egyptian mongoose in Évora district, southern Portugal.....	13
Figure 10 – Mean ( $\pm$ 95% CI) fitted values for the optimal logistic mixed-effects model predicting probability of drainage culvert usage by European badger in Évora district, southern Portugal.....	14
Figure 11 – Mean ( $\pm$ 95% CI) fitted values for the optimal logistic mixed-effects model predicting probability of drainage culvert usage by Common genet in Évora district, southern Portugal.....	14
Figure 12 – Mean ( $\pm$ 95% CI) fitted values for the optimal logistic mixed-effects model	15



predicting probability of drainage culvert usage by European otter in Évora district, southern Portugal.....

Figure 13 – Mean ( $\pm$  95% CI) fitted values for the optimal linear mixed-effects model predicting number of complete crossings per day of drainage culverts by mid-sized carnivores in Évora district, southern Portugal.....

16

## Tables

Table 1 – Summary of the 16 explanatory variables analyzed for the 30 culverts surveyed, including description and range.....	10
Table 2 – The fixed part of the optimal logistic mixed-effects model predicting probability of drainage culvert usage by mid-sized carnivores in Évora district, southern Portugal. SE = standard error; VIF= variance inflation factor.....	12
Table 3 – The fixed part of the optimal logistic mixed-effects model predicting probability of drainage culvert usage by the Egyptian mongoose in Évora district, southern Portugal. SE = standard error; VIF= variance inflation factor.....	13
Table 4 – The fixed part of the optimal logistic mixed-effects model predicting probability of drainage culvert usage by the European badger in Évora district, southern Portugal. SE = standard error; VIF= variance inflation factor.....	13
Table 5 – The fixed part of the optimal logistic mixed-effects model predicting probability of drainage culvert usage by the Common genet in Évora district, southern Portugal. SE = standard error; VIF= variance inflation factor.....	14
Table 6 – The fixed part of the optimal logistic mixed-effects model predicting probability of drainage culvert usage by the European otter in Évora district, southern Portugal. SE = standard error; VIF= variance inflation factor.....	15
Table 7 – The fixed part of the optimal linear mixed-effects model predicting number of complete crossings per day of drainage culverts by mid-sized carnivores in Évora district, southern Portugal. SE = standard error; VIF= variance inflation factor.....	15

# Factors influencing the use of road-crossing culverts by carnivores

## Abstract

A lot of effort has been put into minimizing the environmental impacts of roads, which promote habitat fragmentation, create barriers to movement, and directly kill many organisms. Many roads rely on drainage culverts as the only available infrastructures for road crossing by wildlife. However, culverts are poorly designed for the purpose as they may be flooded and unavailable to most terrestrial fauna in rainy periods. In this study, we conducted a field experiment partially covering the wet period in a Euro-Mediterranean area to assess the effects of flooding among factors influencing the usage of road-crossing drainage culverts by mid-sized carnivore mammals. Along three intermediate-level traffic roads in Évora district, Portugal, we used track stations inside 30 culverts to evaluate effective complete carnivore crossings and we developed mixed-effects models to quantify culvert usage as a function of factors driving wildlife passage way through these infrastructures. We determined the primary predictors of the probability of culvert usage and the main factors influencing the number of crossings. One main finding of our study was that the partial flooding of culverts did not represent per se an avoidance factor for carnivores, even when these infrastructures incorporate small streams inside. On the contrary, our findings suggest an important role of streams. However it is of paramount importance noticing that a dry band through the culvert tunnel was necessary to provide a natural crossing path for carnivores. Our findings provide a way in which to refine the paradigm of water effects in culverts with direct implications to management.

**Keywords:** flooding, drainage culvert, Euro-Mediterranean; mid-sized carnivores; dry ledge

# Factors influencing the use of road-crossing culverts by carnivores

## Resumo

Tem sido feito um grande esforço para minimizar os impactes ambientais das estradas pois estas promovem a fragmentação dos *habitats*, criam barreiras ao movimento, e causam mortalidade direta a muitos organismos. Em muitas estradas as passagens hidráulicas são as únicas infraestruturas disponíveis para o atravessamento por animais. No entanto, estas estruturas são inadequadas para esse propósito dado estarem muitas vezes inundadas nos períodos de maior pluviosidade. Neste estudo, foi realizada uma experiência de campo que cobriu parcialmente a época húmida numa região Euro-mediterrânica, para avaliar o efeito do alagamento entre outros fatores conhecidos que influenciam o uso das passagens hidráulicas para atravessamento das estradas por mamíferos carnívoros de médio porte. Em três Estradas Nacionais de tráfego intermédio do distrito de Évora, Portugal, usámos estações de pó de pedra dentro de 30 passagens hidráulicas para avaliar o seu atravessamento efetivo (completo) por carnívoros e desenvolvemos modelos de regressão mistos para quantificar a utilização em função dos fatores que influenciaram o atravessamento. Determinámos os principais fatores preditivos quer da probabilidade de atravessamento quer do número de passagens por dia. Um resultado fundamental foi que a inundação parcial das passagens hidráulicas não representou *per se* um fator impeditivo para os carnívoros mesmo quando estas estruturas eram atravessadas por ribeiras. Pelo contrário, os nossos resultados sugerem um papel relevante das ribeiras na promoção do atravessamento. No entanto, é importante salientar que dos nossos resultados resulta que a existência de uma faixa seca ao longo do túnel da passagem hidráulica é necessária para promover o atravessamento pelos carnívoros. Os nossos resultados permitem refinar o paradigma dos efeitos da inundação das passagens hidráulicas e têm implicações diretas em medidas de gestão.

**Palavras-chave:** alagamento, passagem hidráulica, Euro-Mediterrâneo, carnívoros de médio porte, passadiço seco

## Resumo alargado

Nos últimos anos tem havido um grande esforço para minimizar os impactes ambientais das estradas, dado que estas promovem a fragmentação de *habitats*, criam barreiras ao movimento, promovem o isolamento populacional e genético, e causam a mortalidade direta de muitos organismos. Os atropelamentos são apenas uma das faces mais visíveis destes impactes que são reconhecidos pelos agentes interessados em investir na mitigação dos mesmos. Numa tentativa para mitigar estas consequências, tornou-se cada vez mais comum incluir passagens para a fauna, especialmente ao longo de novas estradas auto-estradas, visando permitir o atravessamento da fauna selvagem. No entanto, devido ao elevado custo da implementação de passagens dedicadas para a fauna, muitas estradas dependem ainda, sobretudo, da existência de passagens hidráulicas pouco adequadas para esse propósito. A função das passagens hidráulicas é sobretudo permitir o escoamento das águas das chuvas e ribeiras que atravessam estradas e sobretudo prevenir inundações. Apesar disso, estas infraestruturas podem ser usadas por diferentes grupos de vertebrados, incluindo os mamíferos carnívoros, não obstante os períodos de alagamento comuns em períodos de maior pluviosidade poderem representar um obstáculo para os movimentos dos carnívoros.

Os carnívoros são vitais nos ecossistemas, nomeadamente devido ao seu papel enquanto reguladores da densidade das suas presas, super-predadores, e dispersores de sementes. No entanto, são animais particularmente vulneráveis aos impactes das estradas por apresentarem grandes áreas vitais, densidades populacionais e taxas de reprodução baixas, exibirem um comportamento territorial, e por terem necessidade de dispersar a grandes distâncias. Além disso, os carnívoros utilizam muitas vezes para dispersar as estruturas lineares, nomeadamente faixas ripícolas e estradas. Estas características aumentam a frequência com que encontram estradas, pelo que é fundamental anular os referidos impactes visando melhorar a conectividade entre áreas diferentes do seu território. Neste contexto, as passagens hidráulicas são uma solução económica que pode contribuir para o melhoramento da permeabilidade da paisagem ao movimento destes animais.

Vários estudos indicam que as características estruturais das passagens hidráulicas (e.g., índice de abertura) e outros fatores como a cobertura do solo nas imediações estão entre os fatores conhecidos mais relevantes e que influenciam a taxa de atravessamento pelos carnívoros. No entanto, poucos estudos avaliaram o efeito do alagamento na taxa de atravessamento. A literatura existente documenta que os carnívoros podem evitar passagens hidráulicas com uma profundidade de água superior a três centímetros e com elevadas percentagens de cobertura de água. Num outro estudo, a implementação de

passadiços elevados mostra ser uma solução para algumas espécies de carnívoros no sul de Portugal, mas não para outras.

Neste estudo, foi realizada uma experiência de campo que cobriu parcialmente a época húmida numa área Euro-mediterrânica para avaliar o efeito da inundação entre outros fatores conhecidos que influenciam o atravessamento de passagens hidráulicas por mamíferos carnívoros de médio porte. Em estradas nacionais com um nível de tráfego intermédio do distrito de Évora, Portugal, usámos estações de pó de pedra dentro de 30 passagens hidráulicas para avaliar o seu atravessamento completo por mamíferos carnívoros. De seguida, desenvolvemos modelos de regressão mistos para quantificar a utilização em função dos fatores que influenciaram o atravessamento.

Especificamente, tivemos como objectivo (i) determinar os principais fatores preditivos da probabilidade de atravessamento das passagens hidráulicas pelos carnívoros e (ii) determinar os principais factores que influenciam o número de atravessamentos sempre que uma passagem foi utilizada. Em ambos os objectivos, tivemos particular interesse nos efeitos do alagamento parcial ou completo das passagens na resposta dos carnívoros, considerando as espécies individualmente e no seu conjunto. Testámos a hipótese de que a inundação teria efeitos transitórios negativos sobre o atravessamento; as passagens seriam mais usadas quando secas, especialmente por espécies consideradas mais terrestres, como a geneta, o texugo, e o sacarrabos.

Contrariamente ao esperado, os nossos resultados mostraram que estas espécies de carnívoros não preferem necessariamente as passagens hidráulicas secas. Pelo contrário, as passagens continuaram a ser usadas para atravessamentos por todas as espécies de carnívoros quando não estavam inundadas, embora outros fatores tenham sido também essenciais. Os resultados sugerem que a presença de uma faixa seca ao longo da passagem hidráulica foi crucial para aumentar tanto a probabilidade como o número de atravessamentos bem-sucedidos pelas espécies de carnívoros estudadas. Uma excepção foi a Lontra europeia, uma espécie bem adaptada aos ambientes de água doce. Mais surpreendente foi o resultado de que a existência de uma ribeira dentro da passagem hidráulica influenciou positivamente tanto a probabilidade de atravessamentos como o número de atravessamentos por dia pelo conjunto das espécies. Sugerimos que passagens que incorporam ribeiras podem actuar como uma continuação destes corredores ripícolas, sendo possivelmente incorporados no habitat dos carnívoros. Valores intermédios de cobertura de água dentro das passagens hidráulicas foram também um importante fator explicativo da probabilidade de atravessamentos de sacarrabos.

Tal como esperado, a distância à passagem mais próxima seca influenciou o número de atravessamentos da passagem hidráulica alvo. No entanto, como a passagem mais próxima coincidiu em 95% dos casos com a mais próxima seca, tal resultado terá tido pouco a ver

com o alagamento da passagem próxima. A probabilidade de atravessamento diminuiu com a distância à passagem mais próxima. Uma possível explicação é a de que este resultado reflecte a probabilidade de descoberta da passagem hidráulica, isto é, a chegada de um animal à entrada da mesma. A descoberta de passagens hidráulicas mais isoladas terá sido menor. No entanto, são necessários mais estudos para esclarecer estas possibilidades.

A maior cobertura de vegetação herbácea na entrada das passagens também parece ter dificultado a descoberta da entrada da passagem hidráulica. Por outro lado, no nosso estudo a probabilidade de atravessamento parece também aumentar com a presença de uma vedação de gado a uma distância de 5 m da entrada, nomeadamente para o texugo. Uma explicação possível é a indicação de um papel de condução dos carnívoros ao longo destes cercados para a entrada da passagem hidráulica. No entanto, mais estudos poderão fornecer mais informações para entender este aparente paradoxo no futuro.

Não tão surpreendentemente, a topografia junto à estrada parece ter tido também nos nossos resultados um papel importante na condução dos carnívoros para a entrada da passagem hidráulica. No entanto, são manifestamente necessários mais estudos para percebermos exactamente através de que mecanismos a topografia na faixa de terreno junto à estrada influencia os movimentos dos carnívoros.

Curiosamente, o número de atravessamentos foi mais elevado quando a estrada apresentou um *rail* metálico protetor duplo. A partir dos nossos resultados sugerimos que *rails* duplos poderão funcionar como barreiras para carnívoros de médio porte que se aproximam da estrada orientando-os para a entrada da passagem hidráulica mais próxima.

Os nossos resultados permitem refinar o paradigma dos efeitos da inundação das passagens hidráulicas e têm implicações diretas para medidas de gestão. Por exemplo, será aconselhável a implementação de uma faixa seca de 0.5 a 2.5 m para fornecer um caminho de atravessamento. Uma segunda recomendação importante é a de que os passadiços secos nas passagens hidráulicas não devem ser ignorados, mesmo quando a passagem alberga um rio no seu interior. Do mesmo modo, o corte ocasional da vegetação herbácea junto à entrada da passagem hidráulica parece ser aconselhável para ajudar à descoberta da passagem pelos carnívoros. Além disso, a distância entre passagens ao longo da estrada deve seguir as recomendações técnicas já existentes, uma vez que parece ser de importância decisiva, tanto para aumentar o número de atravessamentos, como, possivelmente, para a sua descoberta. Por outro lado, intervir em passagens hidráulicas pré-existent é uma solução económica, especialmente em países com poucos recursos financeiros para implementar passagens para a fauna adequadas. Tais intervenções promovem a conectividade através das estradas, permitindo o movimento dos carnívoros entre diferentes populações. Quantificações como as apresentadas aqui são essenciais para documentar medidas de gestão e de mitigação eficazes.

# Scientific article

## Factors influencing the use of road-crossing culverts by carnivores

João Craveiro<sup>1</sup>, Joana Bernardino<sup>2</sup>, Pedro Costa<sup>2</sup>, António Mira<sup>2</sup>, Pedro G. Vaz<sup>3\*</sup>

1 ISA – Institute of Agriculture, University of Lisbon, Lisbon, Portugal

2 Conservation Biology Unit, Department of Biology / CIBIO-UE – Research Centre in Biodiversity and Genetic Resources. Pole of Évora / InBIO – Research Network in Biodiversity and Evolutionary Biology, University of Évora. Mitra, Évora, Portugal

3 CEABN – Centre for Applied Ecology “Prof. Baeta Neves” / CIBIO-InBIO – Research Centre in Biodiversity and Genetic Resources, ISA – Institute of Agriculture, University of Lisbon, Lisbon, Portugal

\*Corresponding authors e-mails: [pjgvaz@isa.ulisboa.pt](mailto:pjgvaz@isa.ulisboa.pt); [zasvaz@gmail.com](mailto:zasvaz@gmail.com)

### Abstract

A lot of effort has been put into minimizing the environmental impacts of roads, which promote habitat fragmentation, create barriers to movement, and directly kill many organisms. Many roads rely on drainage culverts as the only available infrastructures for road crossing by wildlife. However, culverts are poorly designed for the purpose as they may be flooded and unavailable to most terrestrial fauna in rainy periods. In this study, we conducted a field experiment partially covering the wet period in a Euro-Mediterranean area to assess the effects of flooding among factors influencing the usage of road-crossing drainage culverts by mid-sized carnivore mammals. Along three intermediate-level traffic roads in Évora district, Portugal, we used track stations inside 30 culverts to evaluate effective complete carnivore crossings and we developed mixed-effects models to quantify culvert usage as a function of factors driving wildlife passage way through these infrastructures. We determined the primary predictors of the probability of culvert usage and the main factors influencing the number of crossings. One main finding of our study was that the partial flooding of culverts did not represent per se an avoidance factor for carnivores, even when these infrastructures incorporate small streams inside. On the contrary, our findings suggest an important role of streams. However it is of paramount importance noticing that a dry band through the culvert tunnel was necessary to provide a natural crossing path for carnivores. Our findings provide a way in which to refine the paradigm of water effects in culverts with direct implications to management.

**Keywords:** flooding, drainage culvert, Euro-Mediterranean; mid-sized carnivores; dry ledge



# 1. Introduction

A lot of effort has been put into minimizing the environmental impacts of roads which promote habitat fragmentation, create barriers to movement, and directly kill many organisms (Seiler et al., 2001). In an attempt to mitigate these consequences, it is becoming increasingly common to include wildlife crossing passages, especially along new roads and highways, to allow wildlife crossing (Clevenger and Waltho, 2000, 2005; Forman et al., 2003). However, because of the high cost to implement these dedicated passages (Ascensão and Mira, 2007; Glista et al., 2009), some roads rely on drainage culverts poorly designed for the purpose. Moreover, being a structure that allows water to flow, a drainage culvert may be flooded and unavailable to many terrestrial fauna in rainy periods (Liu and Zhao, 2003). In this study, we evaluated which factors influence the crossing of carnivore mammals through drainage culverts, with particular attention to the effects of water flooding. Culverts are primarily engineered to allow the rainwater runoff and stream flow under the road in order to prevent flooding (Liu and Zhao, 2003). They can exhibit an extensive range in sizes and forms and be made of various materials (Ruediger and DiGiorgio, 2007; van der Ree et al., 2007). However, different groups of vertebrates worldwide also regularly use these infrastructures (Dodd et al., 2004; Ng et al., 2004; Crook et al., 2013), including Mediterranean habitats (Yanes et al., 1995; Rodríguez et al., 1996; Clevenger et al., 2001a; Mata et al., 2005), namely in Southern Portugal (Ascensão and Mira, 2007; Grilo et al., 2008; Mateus et al., 2011; Serronha et al., 2013; Villalva et al., 2013). Regrettably, the presence of water may represent an obstacle to animals' movements (Grilo et al., 2010).

Roads have several impacts on nature being recognized by governments and management agencies, which have invested lots of money to quantify and mitigate them (Seiler, 2001; Evink, 2002; Bekker and Luell, 2003; Forman et al., 2003; Donaldson, 2006; van der Ree et al., 2007; van der Grift et al., 2013). The wildlife-vehicle collisions can cause direct mortality (Glista et al., 2009; Grilo et al., 2009) and are the most visible impact of traffic on wildlife communities. Barrier effects are likely the major negative ecological impact (Bekker and Luell, 2003), because they lead to population isolation, by reducing animal movements (Grilo et al., 2009) and connections between populations (Ament, 2007) decreasing gene flow and causing behavioural changes such as avoidance (Jaeger and Fahrig, 2004). Culverts may represent an economical solution (Crook et al., 2013) for the enhancement of landscape permeability to movement of wildlife species across.

Numerous previous studies have shown the impact of roads in several vertebrate groups (Fahrig et al., 1995; Forman et al., 2002; Gibbs and Shriver, 2002; McGregor et al., 2008; Carvalho and Mira, 2011), including carnivores (Davis et al., 1987; Ascensão and Mira,

2007). Carnivores are vital to ecosystems and biodiversity, due to their role as regulators of prey density, evolution promoters, seed dispersers, super-predators, among many other attributes (Loureiro et al., 2012). They are particularly vulnerable to road impacts, because of their large home-ranges, low population densities, low reproductive rates (Ruediger and DiGiorgio, 2007), and dispersal needs (Grilo et al., 2015). Carnivores move for foraging and dispersal across and along roads throughout their territory (James and Stuart-Smith, 2000; Colón, 2002; Clevenger and Wierzchowski, 2006) and use roads for faecal marking (e.g., unpaved roads, roadside verges). These characteristics increase the frequency of road encounters and car collisions (Garrah, 2012). In southern Portugal, Grilo et al. (2009) estimate an annual roadkill rate of about 47 carnivores / 100 km in 314 km national roads.

Previous studies show that attributes such as culvert openness, its surrounding land-cover, and vegetation near entrance are among the most important factors influencing the culvert crossings by carnivores (Rodríguez et al., 1996; Ascensão and Mira, 2007; Grilo et al., 2008; Villalva et al., 2013; Grilo et al., 2015). However, very few studies evaluate the effect of water flooding on the usage of drainage culverts by carnivores. Serronha et al. (2013) founded that most species tend to avoid culverts with water depth higher than 3 cm and with an increase of water cover. Villalva et al. (2013) installed 50-cm dry ledges to allow the crossing for carnivores in Southern Portugal and found that red foxes and badgers avoid culverts with dry ledges.

In this study, we conducted a field experiment partially covering a wet period in a Euro-Mediterranean area to assess the effects of flooding among factors influencing the usage of road-crossing drainage culverts by mid-sized carnivore mammals. Along three intermediate-level traffic roads in Évora district, Portugal, we used track stations inside culverts to evaluate effective (complete) carnivore road crossings and we developed mixed-effects models to quantify culvert usage as a function of factors driving wildlife passageway through these infrastructures. Specifically, we aimed to (i) determine the primary predictors of the probability of culvert usage by carnivores and (ii) determine the main factors influencing the number of crossings whenever the culvert was used. In both objectives, we were particularly interested in the effects of partial/complete flooding on overall and species-specific carnivore responses. We hypothesized that water flooding would have detrimental transient effects on usage; culverts would be more used when drier, especially by species considered more terrestrial, such as the Common genet (*Genetta genetta*) and Egyptian mongoose (*Herpestes ichneumon*).

This knowledge is essential for assisting resource managers for future mitigation projects including adaptation of culverts towards restoring animal movement across roads.

## 2. Materials and Methods

### 2.1. Study area and study design

We conducted this study from 11 March 2016 to 6 May 2016 along three national roads (asphalt paved; two-lanes) in Alentejo region, southern Portugal (Fig. 1). The local climate is Mediterranean with cold, wet winters (mean annual precipitation is 620 mm) and hot, dry summers with temperatures exceeding 40° C (range: 7 to 43 °C). The area has undulating relief with altitudes ranging from 200 to 400 m above sea level. The landscape was dominated by cork oak (*Quercus suber*) and holm oak (*Quercus rotundifolia*) stands managed by an agro-forestry system called Montado (Dehesa in Spain). Pastures, meadows, and olive groves were common. The population density in the study area was 23 inhabitants / km<sup>2</sup> (INE, 2015).

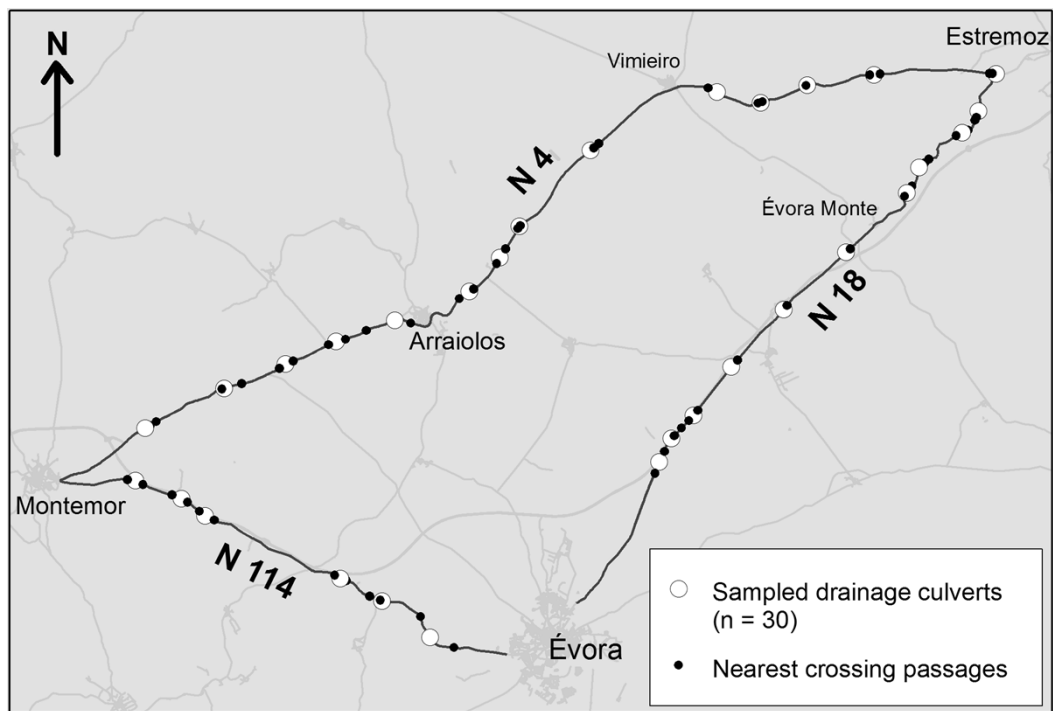


Figure 1. Drainage culverts sampled in southern Portugal and their nearest passages. N4, N18, N114 = Portuguese National roads.

The three road stretches, EN114, EN4, and EN18 (27.3, 56.9 and 43.2 km) were located in the Évora district and were traffic varies between 3000 and 10000 vehicles / day. These stretches have high levels of carnivore mortality (Santos et al, 2011a). Two nearby European Natura 2000 network Sites – Monfurado and Cabrela –, add contribution to a high carnivore richness (Santos-Reis and Petrucci-Fonseca, 1999) in the area. Carnivore species in the

study area include Egyptian mongoose (*Herpestes ichneumon*), Common genet (*Genetta genetta*), European otter (*Lutra lutra*), European badger (*Meles meles*), Stone marten (*Martes foina*), European polecat (*Mustela putorius*), Weasel (*Mustela nivalis*), Fox (*Vulpes vulpes*) and Wildcat (*Felis silvestris*).

In order to select the culverts for the survey, we first scanned all the road crossing passages – walking and driving at ~10-20 km / h. We geo-referenced 307 crossing passages, including culverts, bridges, viaducts, and underpasses for human and livestock use. We then selected 30 drainage culverts (Fig. 1) spaced 2 km apart on average (1.5 km minimum), the equivalent to the mean radius of the home range of the carnivore species in the area (Grilo et al., 2008), ensuring the independence of observations (Guisan and Zimmermann, 2000). The selected culverts included 22 boxes, four circular, and four arch culverts (Fig. 2). They were made of concrete and mostly had an irregular floor substrate with soil and sediments.



Figure 2. Examples of the road-crossing drainage culverts sampled in this study. A – Circular; B – box; C – arch.

## 2.2. Crossings assessment

We used track stations inside the 30 drainage culverts to assess their usage by carnivores. To record the animal footprints, we used marble dust covering two hardboard panels (width = 60 cm; height = 3 mm; length = culvert width), each placed transversely inside the culvert at 1.0 to 2.5 m from the entrance. In five cases, only one plate was used due to the large width of the culverts ( $\geq 3.97$  m); in those cases the hardboard was placed transversely at the midpoint of the tunnel. In order to minimize contact with the floor moisture, we nailed rectangular wood cuboids (3×3×10 cm) on the underside of each plate to raise it 3 cm above ground level. We further raised the plates up to a maximum of 10 cm using roof tiles whenever the culvert had water (Fig. 3). If no place with a water depth lower than 10 cm was available to mount the hardboard plates, we temporarily remove them and / or did not record tracks over that flooding period.



**Figure 3.** Examples of hardboard plates used in this study with rectangular wood cuboids (3x3x10 cm) nailed on the underside of each plate to raise it 3 cm above ground level and roof tiles to raise the plates up to a maximum of 10 cm whenever the culvert had water.

Each hardboard panel has been covered with 0.3 to 0.5 cm marble dust smoothed with a steel trowel. Marble dust is a scentless and persistent material that allows high-quality tracks (Yanes et. al., 1995). We identified carnivore species onsite or upon photographic record in a few cases. Every time we had doubts between two carnivore species the track was recorded as non-identified species. When we suspected that the uncertainty could have been from confusion with domestic animals the record was discarded.

Each culvert was sampled every two days for 29 operative days (i.e., days when tracks were recorded without water and / or wind damages). For logistic constraints, one sampling visit to all culverts took place with three days interval. Hence, each culvert was sampled 14 times ( $29 = 13 \times 2 + 1 \times 3$ ). At each sampling date, the marble dust was smoothed or replaced in order to prevent recount in the next visit. We then recorded the tracks / trails per species on each hardboard plate and the movement direction. When having tracks / trails from the same species on one hardboard panel, only clearly separated ones were considered as different records. These records were converted afterwards onto number of culvert crossings per species as follows.

In this study we defined culvert usage as complete carnivore crossings only, as opposed to simple visits to explore the interior of the culvert (see Martinig and Bélanger-Smith, 2016). We considered that the carnivore only visited the culvert when there were tracks in both directions in one of the two plates and we assigned a complete crossing to most of the other movement combinations (Fig 4). We have opted for considering two complete crossings in the particular situation of two-directions in one plate plus one direction in the other plate. Our

options took into consideration the possibility that carnivores may jump over track stations and ink-beds (van Vuurde and van der Grift, 2005; Costa, 2014).

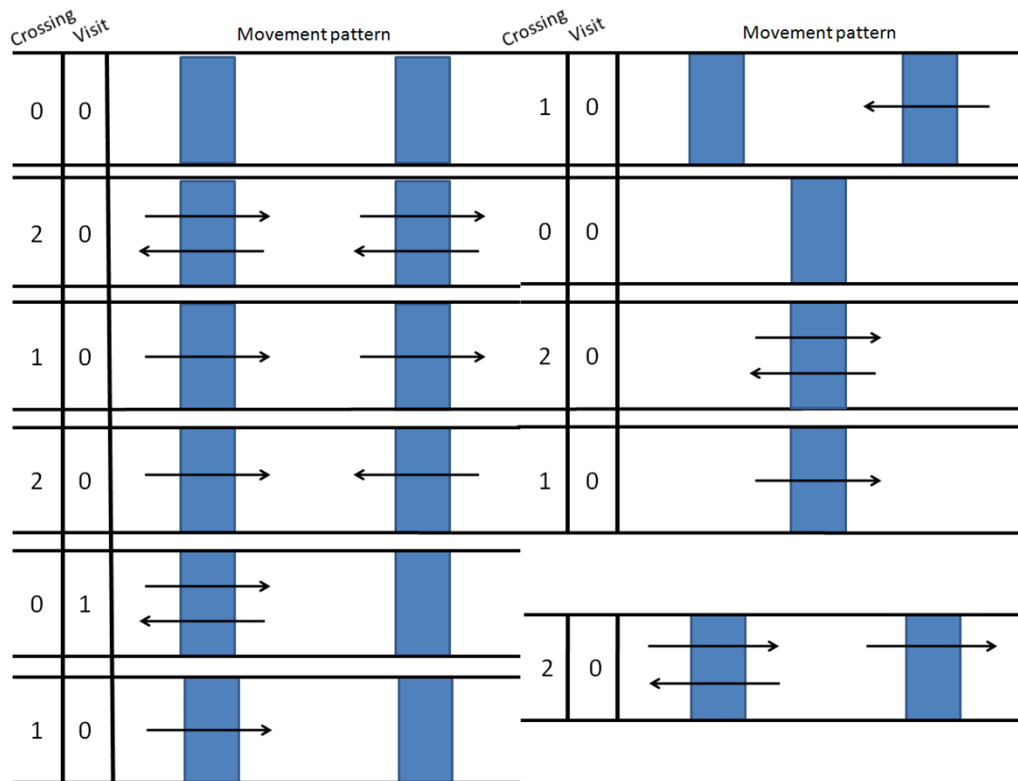


Figure 4. Scheme used to assign carnivore visits and complete crossings per sampling date in a culvert. The movement direction in one or two hardboard plates mounted in the culvert was assessed.

### 2.2.1. Data collection

In each sampling visit to a drainage culvert we recorded the number of complete crossings / day by carnivore species and we collected variables likely explaining that response. Our primary variables of interest reflected the water level inside the culvert, including:

- Dry width. – Average of three dry transverse widths inside the culvert as measured (m) at both entrances and at the midpoint of the culvert tunnel having the shortest dry transverse width. The latter was estimated when it was unreachable for direct measurement.
- Water depth. – Average of the water depths measured (cm) at the same points as dry width. For the analysis this variable was later simplified into four classes: 1 = 0 cm, 2 = ]0;3], 3 = ]3;6], and 4 ≥ 6 cm). Serronha et al. (2013) suggest that depths greater than 3 cm are unfavourable for culvert crossing by our carnivore species, so we used multiples of this value. Dry width and water depth were measured with a meter tape (precision = 1 mm).



- Water cover. – Visually estimated percentage of the culvert's ground covered with water. This was later simplified for the analysis using the Jenks Natural Breaks classification method (Jenks, 1967) to determine the best arrangement of the percentage values into four classes: 0 = 0, 2 = ]0,27], 3 = ]27,57], and 4 = ]57,100].

The following culvert features were also analysed:

- Culvert type. – The form of the culvert (box, circular, or arch);
- Openness. – Index calculated as the culvert cross-section area divided by the culvert tunnel length (Ascensão and Mira, 2007). Openness was derived from height and length measurements collected using a laser meter (precision: 1 mm).
- Fence distance. – Average of the two distances (m) from the culvert entrances to a perpendicular farm fence if present (barbed wire fences usually with mesh size >15 cm). This variable was later simplified into three classes: 0 = no fence, 1 = fence present at >5 m, 2 = fence within 5 m distance (Fig. 5D).
- Road distance. – Average of the two distances (m) from the road asphalt to the culvert entrances.
- Guard rail. – Presence of a rail guard on the road, considering three classes: 0 = no rail, 1 = single rail, 2 = double rail (Fig. 5A and B).
- Stream. – Persistence of a stream (yes / no) lasting running inside the culvert over the entire study sampling period (Fig. 5C).
- Rabbits. – Presence of rabbits as assessed by the presence of rabbit holes (yes / no) within a 20 m radius from each culvert entrance.



Figure 5. Examples of the double (A) and single (B) guard rail types, stream running inside one culvert (C) and a typical farm fence in Alentejo, southern Portugal.

- Slope. – Average of the two roadside slopes between asphalt and the culvert entrances. Slope was measured in percentage as the ratio of vertical drop per horizontal distance. Horizontality was assessed with a Carpenter's (spirit) level and distances were measured with a meter tape (precision = 1 mm).
- Grass cover and shrub cover. – Visually estimated percentages of grasses and shrubs within a 10 m radius from both culvert entrances.
- Grass height and shrub height. – Averages of grass and of shrub heights measured (cm) at 12 points per entrance within a 10 m radius (Fig. 6) using a meter tape.

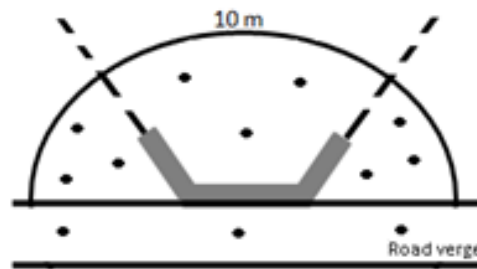
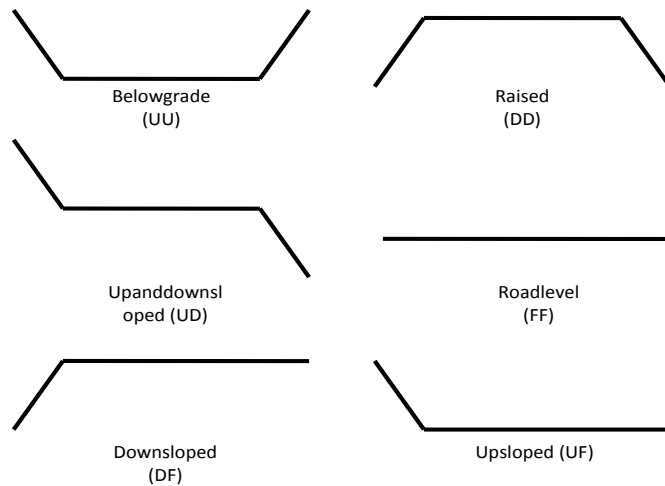


Figure 6. Measurement scheme for the estimated percentages of grasses and shrubs within a 10 m radius from both culvert entrances and the averages of grass and of shrub heights (cm) measured at 12 points per entrance. Adapted from Chambers and Bencini (2015)

The following variables characterized each culvert in a broader spatial context:

- Land cover. – Dominant land cover within a 1500 m buffer surrounding (and including) the culvert as assessed using GIS. Our base map was an eight-class legend simplification of the 2007 land-cover map of Portugal (reference scale 1 : 25 000; IGP, 2007) at level 5 detail. In our 1500 m buffers only Montado and Pasture classes were dominant.
- Distance to near driest passage. – Distance (km) along the road to the driest passage among the two nearest drainage passages. Subsequently to each of the 14 sampling visits to one culvert we also visited and assessed among the two nearest passages which one was driest and we then used GIS to compute this distance. We aimed to test the hypothesis that distance to a near dry passage would add influence the number of crossings in the target culvert.
- Roadside topography. – Six topographic classes visually assessed onsite within two 20 m strips parallel to both sides of the road (Fig. 7). We assign this variable at three different scales: within 50, 100, and 150 m from the culvert entrance along the road.





**Figure 7. Topographic classes within 50, 100, and 150 m scales from the culvert entrance and within two 20 m strips parallel to both sides of the road: class UU = below grade; DD = raised; class UD = up and down sloped; class FF = road level; class DF = down sloped; class UF = up sloped.**

In total, we analyzed 16 explanatory variables (Table 1).

**Table 1. Summary of the 16 explanatory variables analyzed for the 30 culverts surveyed, including description and range.**

Variable	Description	Range
<i>Culvert features</i>		
Dry width	Average of three dry transverse widths inside the culvert (m)	0–5.53
Water depth	Average of the water depth measured (cm) at the same points as dry width.	0–33.3
Water cover	Visually estimated percentage of the culvert's ground covered with water.	0–100
Culvert type	0 – circular; 1 – box; 2 – arches	0; 1; 2
Openness index	Culvert cross-section area/length	0.01–1.15
Fence distance	Average of the two distances (m) from the culvert entrances to a perpendicular farm fence if present (0 – absence; 1 – >5 m; 2 – 0–5m)	0; 1; 2
Road distance	Average of the two distances (m) from the road asphalt to the culvert entrances.	0.55–11.49
Guard-rails	Presence (yes / no) of a rail guard on the road (0 – absence; 1 – presence of single; 2 – double guard-rails)	0; 1; 2
Stream	Persistence of a stream (1 – yes / 0 – no) lasting running inside the culvert	0; 1
Rabbits	Presence of rabbits as assessed by the presence of rabbit holes (1 – yes / 0 – no) <sup>a</sup>	0; 1
Slope	Average of the two roadside slopes (%) between asphalt and the culvert entrances.	12.4–65.6
Grass and shrub cover	Visually estimated percentages of grasses and shrubs <sup>b</sup>	21–100 0–55
Grass and shrub height	Averages of grass and of shrub heights measured (cm) at 12 points per entrance <sup>b</sup>	35.6–127.5 0–200.6
<i>Spatial context</i>		
Land Cover (Montado or Pasture)	Dominant land cover within a 1500 m buffer surrounding (and including) the culvert	Montado = 15 Pasture = 15
Distance to near driest passage	Distance (km) along the road to the driest culvert among the two nearest drainage passages	27–1611
Roadside Topography	Six topographic classes visually assessed on site (DD – raised; UU – below grade; FF – road level; DF – down sloped; UF – up sloped; UD – up and down Topography in classes) <sup>c</sup>	DD = (18; 14; 13); DF = (6; 7; 2); FF = (6; 5; 3); UU = (0; 1; 2); UF = (0; 1; 3); UD = (0; 2; 7)

<sup>a</sup> within a 20 m radius

<sup>b</sup> within a 10 m radius from the entrance and between this and the road

<sup>c</sup> within 50, 100, and 150 m scales from the culvert entrance along the road and two 20 m strips parallel to both sides of the road

### 2.2.2. Statistical analysis

Each variable was expressed per species in each one of the 14 sampling visits to a culvert. Because groups of data records were nested within one culvert, we used mixed-effects models with culvert as the random factor to explore relationships between carnivore complete crossings and explanatory variables. A matrix of Spearman's correlations for explanatory variables revealed that openness was significantly correlated with dry width ( $r = 0.66$ ,  $p < 0.001$ ) and so we gave priority to using the latter when building the models. The other explanatory variables included in the final models below presented were not strongly collinear (Spearman correlation coefficient  $< 0.4$ ) and therefore could be included together in regression models.

To determine the primary predictors of the probability of culvert usage (objective one of this study), we used the presence of carnivore species as the binary response variable (0 = not present; 1 = present). For this objective one, we conducted logistic generalized mixed models (GLMM) with logit link using the lme4 package in R (Bates et al., 2011) to fit GLMM. To determine the main factors influencing the number of crossings whenever the culvert was used (objective two), the response variable was the number of complete crossings / day. For objective two, we log(x)-transformed the response variable and conducted linear mixed-effects models (LMM) with identity link using the nlme package in R to fit LMM (Pinheiro et al., 2011). We built logistic GLMM for the probability of culvert usage by all species considered together and then we built species-specific models. For objective two we only built one LMM for all carnivore species.

We assessed final models with a forward variable selection procedure. We entered predictors one at a time, recorded their significance values, and chose the most significant predictor ( $p < 0.05$  criteria) in addition to the model with the lowest AIC (Akaike Information Criterion value). The procedure stopped when no significant term could be added. Model fit was assessed using marginal  $R^2$  (proportion of variance explained by the fixed effects; Nakagawa and Schielzeth, 2013). We performed all statistical analysis using R version 3.3.1 (R Development Core Team, 2016).

## 3. Results

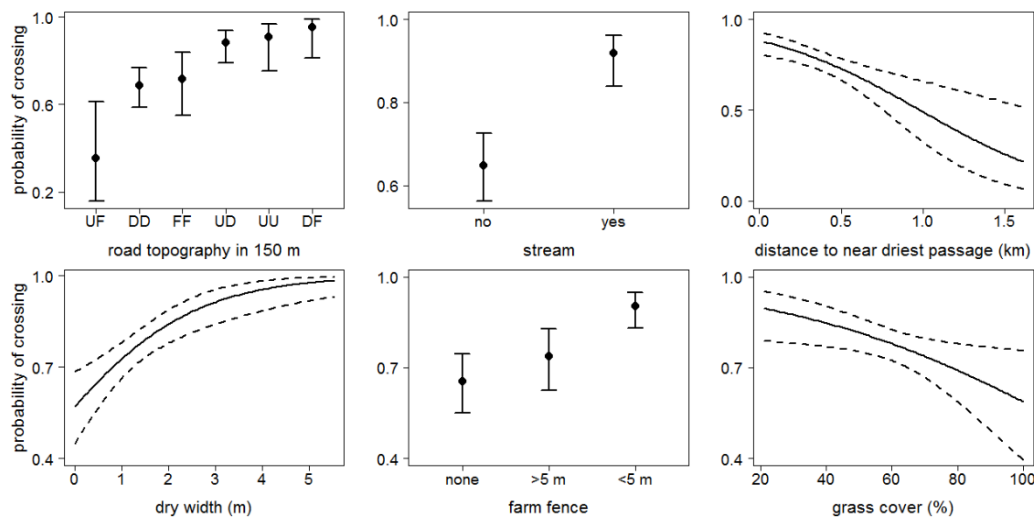
We recorded a total of 794 complete carnivore crossings for the 30 drainage culverts (mean  $0.96 \pm 0.17$  SE crossings /culvert / day). Crossings were from Egyptian mongoose (29 %), Eurasian badger (24 %), Common genet (19 %), Eurasian otter (10 %), Stone marten (3 %), and Red fox ( $<1$  %). In 15 % of cases the carnivore species was not identified.

### 3.1. Predictors of probability of culvert usage by all carnivores

The probability of crossing significantly increased with dry width and for drainage culverts further having a stream running inside it, while decreasing with the distance to the near driest passage (Table 2). The increase with dry width flattened around 2-3 m. Crossing probability was also significantly affected by the roadside topography within 150 m. Furthermore, the probability of usage increased with the presence of a farm fence within 5 m and decreased with higher grass covers outside the culvert (Fig. 8).

**Table 2.** The fixed part of the optimal logistic mixed-effects model predicting probability of drainage culvert usage by mid-sized carnivores in Évora district, southern Portugal. SE = standard error; VIF= variance inflation factor.

Variable	marginal $R^2 = 0.46$				
	Estimate	SE	z	Pr(> z )	VIF
intercept	1.534	0.639	2.40	0.016	
Roadside topography in 150 m (DF)	2.235	0.827	2.70	0.006	1.23
(FF)	0.142	0.451	0.32	0.752	1.71
(UD)	1.238	0.385	3.21	0.001	1.23
(UF)	-1.381	0.589	-2.34	0.019	1.36
(UU)	1.511	0.660	2.29	0.022	2.05
stream (yes)	1.820	0.487	3.73	<0.001	2.29
distance to near driest passage	-2.050	0.579	-3.54	<0.001	1.56
dry width	0.691	0.172	4.01	<0.001	1.45
farm fence (<5 m)	1.139	0.296	3.85	<0.001	1.57
(>5 m)	0.327	0.257	1.27	0.203	1.37
grass cover	-0.023	0.009	-2.35	0.018	2.30



**Figure 8.** Mean ( $\pm$  95% CI) fitted values for the optimal logistic mixed-effects model predicting probability of drainage culvert usage by mid-sized carnivores in Évora district, southern Portugal.

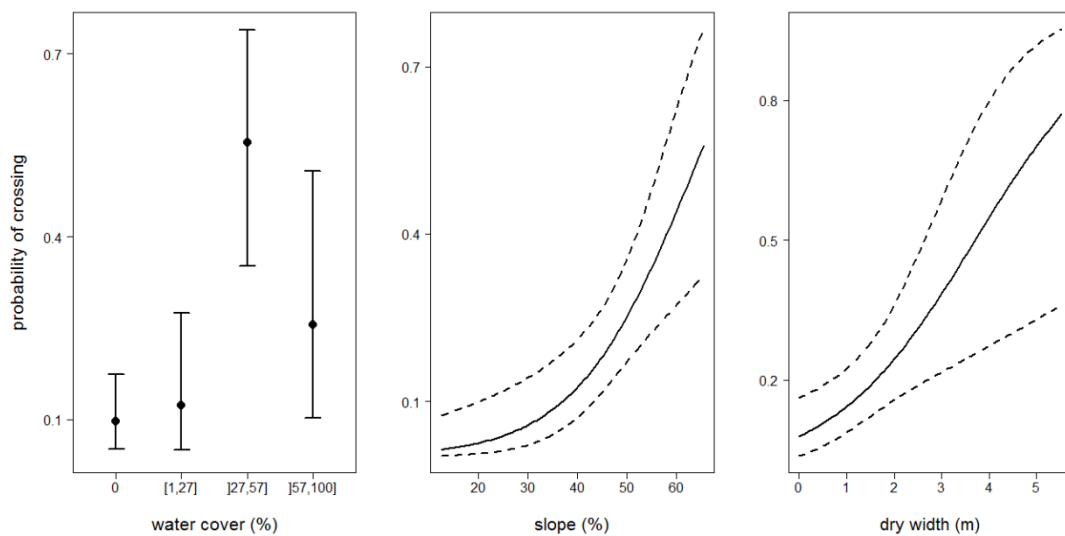
### 3.2. Species-specific predictors of probability of culvert usage

For Egyptian mongoose, the probability of crossing significantly increased with dry width and for drainage culverts further having intermediate values of water cover inside (27 to 57 %)

(Table 3). Moreover, the probability of culvert usage increased with greater roadside slopes, especially for slopes greater than 30-40 % (Fig. 9).

**Table 3.** The fixed part of the optimal logistic mixed-effects model predicting probability of drainage culvert usage by the Egyptian mongoose in Évora district, southern Portugal. SE = standard error; VIF= variance inflation factor.

Variable	marginal $R^2 = 0.39$				
	Estimate	SE	z	Pr(> z )	VIF
intercept	-5.935	1.256	-4.72	<0.001	
water cover ]0,27]	0.278	0.504	0.55	0.581	1.13
]27,57]	2.459	0.524	4.69	<0.001	1.28
]57,100]	1.169	0.634	1.84	0.065	1.43
slope	0.085	0.024	3.48	<0.001	1.13
dry width	0.662	0.215	3.08	0.002	1.31

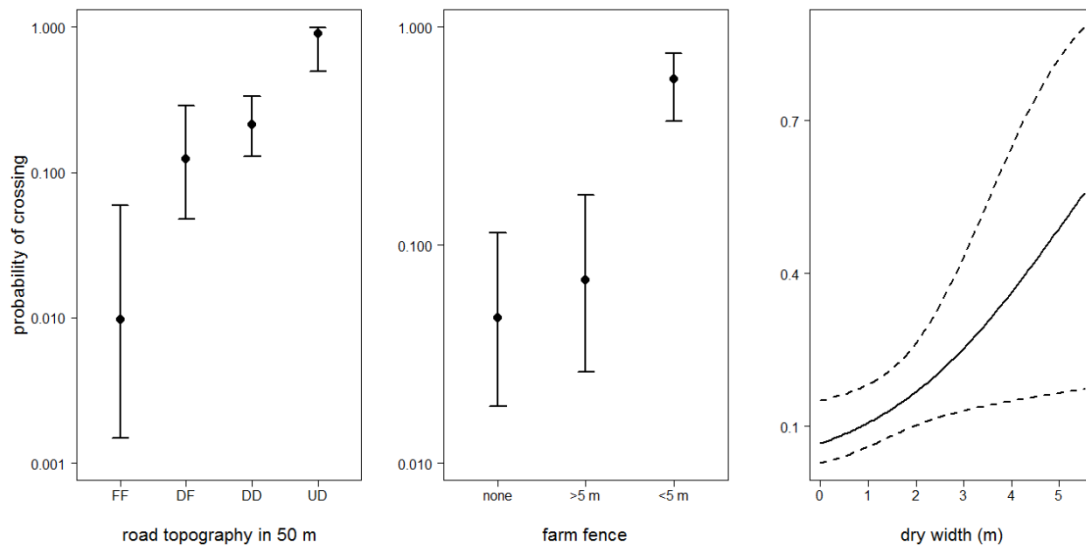


**Figure 9.** Mean ( $\pm$  95% CI) fitted values for the optimal logistic mixed-effects model predicting probability of drainage culvert usage by Egyptian mongoose in Évora district, southern Portugal

For European badger, the probability of crossing increased significantly with dry width (Table 4). Moreover, badger crossings were more likely to occur with the presence of a farm fence within 5 m. Probability of crossing was also significantly affected by the roadside topography within 50 m (Fig. 10).

**Table 4.** The fixed part of the optimal logistic mixed-effects model predicting probability of drainage culvert usage by the European badger in Évora district, southern Portugal. SE = standard error; VIF= variance inflation factor.

Variable	marginal $R^2 = 0.50$				
	Estimate	SE	z	Pr(> z )	VIF
intercept	-1.834	0.475	-3.86	<0.001	
roadside topography in 50 m (DF)	-0.650	0.603	-1.08	0.281	1.19
(FF)	-3.328	0.998	-3.33	<0.001	1.09
(UD)	3.585	1.210	2.96	0.003	1.19
farm fence (<5 m)	2.351	0.468	5.03	<0.001	1.41
(>5 m)	1.014	0.488	2.08	0.037	1.29
dry width	0.519	0.224	2.32	0.020	1.42

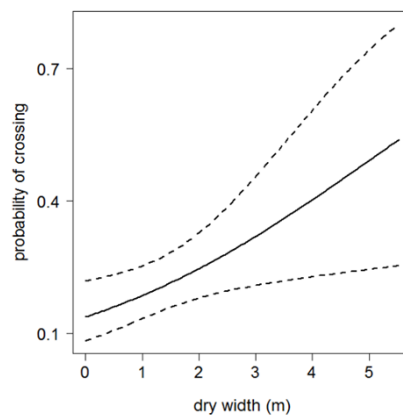


**Figure 10.** Mean ( $\pm$  95% CI) fitted values for the optimal logistic mixed-effects model predicting probability of drainage culvert usage by European badger in Évora district, southern Portugal.

For Common genet, the probability of complete crossings increased significantly with dry width only (Table 5, Fig. 11).

**Table 5.** The fixed part of the optimal logistic mixed-effects model predicting probability of drainage culvert usage by the Common genet in Évora district, southern Portugal. SE = standard error; VIF= variance inflation factor.

Variable	marginal $R^2 = 0.04$			
	Estimate	SE	z	Pr(> z )
intercept	-1.834	0.288	-6.35	<0.001
dry width	0.361	0.148	2.43	0.015

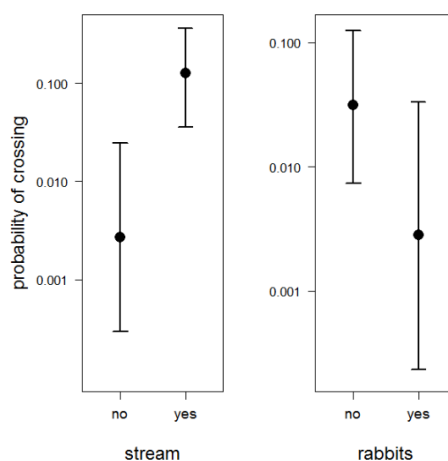


**Figure 11.** Mean ( $\pm$  95% CI) fitted values for the optimal logistic mixed-effects model predicting probability of drainage culvert usage by Common genet in Évora district, southern Portugal

For European otter, the probability of crossing significantly increased in drainage culverts having a stream running inside it, while being less likely to occur in culverts having rabbits within a 20 m radius from the entrance (Table 6, Fig. 12).

**Table 6. The fixed part of the optimal logistic mixed-effects model predicting probability of drainage culvert usage by the European otter in Évora district, southern Portugal.** SE = standard error; VIF= variance inflation factor.

Variable	marginal $R^2 = 0.49$				
	Estimate	SE	z	Pr(> z )	VIF
intercept	-4.738	1.028	-4.61	<0.001	
stream (yes)	3.962	1.177	3.37	<0.001	1.03
presence of rabbits (yes)	-2.444	1.194	-2.05	0.040	1.03



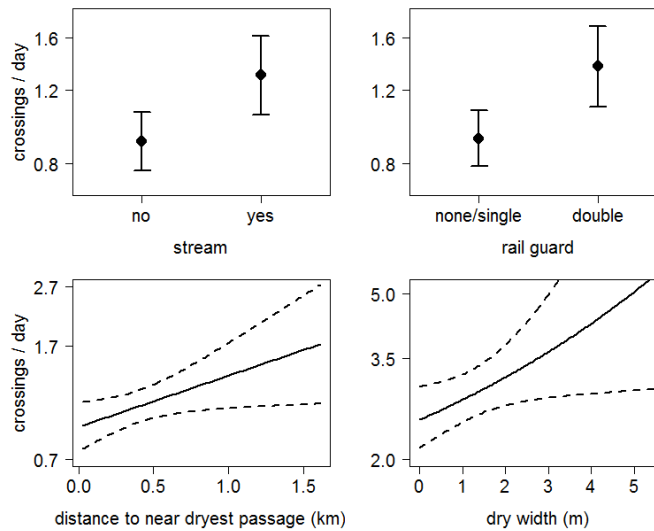
**Figure 12. Mean ( $\pm$  95% CI) fitted values for the optimal logistic mixed-effects model predicting probability of drainage culvert usage by European otter in Évora district, southern Portugal.**

### 3.3. Factors influencing the number of crossings by carnivores

Whenever the culvert was used by at least one carnivore species, the number of crossings per day significantly increased with dry width and in drainage culverts having also a stream inside it, while increasing with the distance to the near driest passage as well (Table 7). In addition, a significantly greater number of crossings per day were more likely to occur if the road had a double rail guard (Fig. 13).

**Table 7. The fixed part of the optimal linear mixed-effects model predicting number of complete crossings per day of drainage culverts by mid-sized carnivores in Évora district, southern Portugal.** SE = standard error; VIF= variance inflation factor.

Variable	marginal $R^2 = 0.25$				
	Estimate	SE	t	P	VIF
intercept	-0.535	0.121	-4.40	<0.001	
stream (yes)	0.363	0.142	2.56	0.016	1.17
rail guard (double)	0.396	0.137	2.88	0.007	1.05
distance to near driest passage	0.402	0.187	2.14	0.033	1.21
dry width	0.101	0.051	1.88	0.044	1.10



**Figure 13.** Mean ( $\pm$  95% CI) fitted values for the optimal linear mixed-effects model predicting number of complete crossings per day of drainage culverts by mid-sized carnivores in Évora district, southern Portugal.

## 4. Discussion

The success of drainage culvert design initiatives to offset the transient effects of water flooding will depend on understanding how water-related factors in addition to other driving features will determine usage for wildlife crossing. However, perhaps with the most notable exception being the work of Serronha et al. (2013) pointing out to lower crossing rates through passages with water depths greater than 3 cm, little progress has been made regarding this topic. Bridging this knowledge gap, our study determined that – as hypothesized – even partial water flooding had the potential to overwhelmingly affect the crossing success of mid-sized carnivore mammals. Moreover, water-related variables were important to explain carnivore successful crossings throughout our results. However, contrary to expectations, our outcome suggested that these carnivore species do not necessarily preferred dry culverts. On the contrary, culverts, when not completely flooded, were still used for successful crossings by all carnivores and other factors were crucial as well.

### 4.1. How does water in culverts affects crossings by carnivores?

Our results suggest that the presence of a dry band through the culvert tunnel was crucial both to increase the probability and the number of successful crossings for the carnivore species assessed. An exception was the European otter, a species well adapted to freshwater environments (Loureiro et al., 2012). This latter result was consistent with work from the same region where Serronha et al. (2013) predict that otters are more likely to use

culverts having more than 70 % of water cover and 50 cm of water depth. In general, our results for dry width confirm several technical recommendations advising the implementation of dry ledges or ledges-like structures in culverts (Cain et al., 2003; Glista et al., 2009; Clevenger and Huijser, 2011). Wood, concrete, steel, and natural substrate ledges that have been incorporated into culverts (Grilo et al., 2010). Villalva et al. (2013) highlight the importance of dry ledges for stone marten and genet. Accordingly, our results highlight the major importance of a dry band to augment the probability of crossings by genets as this was the only significant factor for this species. A common recommendation for a dry ledge width is a minimum of 50 cm (Ministerio de Agricultura, Alimentación y Medio Ambiente, 2015), which from our model prediction corresponds to ~65 % probability of successful carnivore crossing. Furthermore, according to our model, enlarging the dry width from 0.5 m up to 1 or 2 m increased the probability of crossing by ~7% and ~18%, respectively. Up to at least 2.5 m, our results clearly demonstrated that an increase in a dry band led to a marked augment in probability and in number of successful crossings per day for most carnivore species. This knowledge is important and follows Villalva et al. (2013) claim for further research on ledge design, in particular for carnivore species.

More surprisingly to our expectations was the finding that the persistence of a stream inside a culvert influenced positively both the probability of successful crossings and their number per day by all species assessed together, although a positive association was expected for otters. For example, Philcox et al. (1999) found that otters are most likely to occur where roads cross over watercourses. On the other hand, carnivores in general are known for having an association with riparian ecosystems, as these serve as movement and dispersal corridors or for food and water resources (Santos et al., 2011b; Grilo et al., 2016). Thus, we suggest that culverts embedding streams may act as the continuity of these corridors, possibly being incorporated into carnivores' habitat.

Water cover was an important driving factor only for Egyptian mongoose probability of crossing. Although we were expecting that dry culverts would be favored by this species, interestingly, intermediate values of water cover favored mongoose probability of crossing. However, it is noteworthy that this factor adds to the positive effect of a dry band through the culvert tunnel. Thus, both these results conveyed a certain preference for culverts with some water but not completely flooded. Grilo et al. (2008) also document that when water flows through a culvert or an underpass Egyptian mongoose crossing rates can be up to two times greater than when a stream parallels the crossing structure.



#### **4.2. Other factors affecting culvert crossing by carnivores**

As expected, distance to a near driest passage did influence the number of crossings in the target culvert. However, because it happened that the nearest crossing passage matched the near driest passage in 95 % of cases, we feel this result had little to do with the water content of the passage. Considering this, following Clevenger and Waltho (2005), we would anticipate a higher usage of target culverts having fewer passages nearby and lower usage otherwise. However, though our results for the number of crossings per day supported this trend, that was not the case for the probability of crossing which decreased with the distance to another passage. One possible explanation for this latter pattern may have been that it reflected the probability of culvert discovery, i.e., the arrival of an animal at the culvert entrance (Martinig and Bélanger-Smith, 2016), which could have been lower for more isolated culverts. More work is clearly still required to shed light on these possibilities.

A gradient in grass cover outside culverts also appeared to exaggerate differences in culvert discovery favouring more uncovered culverts. One possible explanation is that the presence of herbaceous vegetation difficult the discovery of the culvert entrance. However, once discovered, the vegetation could contribute to masking the passage structure, reducing reluctance to approach and cross through it (Rodriguez et al., 1997) or provide protection from predators (Grilo et al., 2008). Other studies founded that grass at entrance seems to promote carnivores crossing rate (Rodriguez et al., 1996, 1997; Ascensão and Mira, 2007; Grilo et al., 2008). However, these studies use different methods and do not make the same distinction we did between visits and complete crossings.

An apparently counterintuitive finding in our study was that the probability of crossing seems to increase with the presence of a farm fence within 5 m distance from the entrance, namely for the European badger. However, first, it is noteworthy that cattle farm fences present in this study area were highly permeable and not barriers for most carnivores assessed, having a quite large mesh size (>15 cm) with large holes in places, and soil excavations in many locations (e.g., from rabbits, wild boar and domestic animals). Second, our result may indicate a guidance role of the farm fence, i.e., it is possible that carnivores were conducted along the wire fence to the culvert entrance, thus helping in its discovery as well. Ascensão and Mira (2007) found numerous carnivore tracks along these fences and suggest a similar guidance role. However, more studies may provide further information to address this apparent paradox in the future.

Not as surprisingly, roadside topography seems to also have had an important role on carnivore's guidance to the culvert entrance in our results. Dickson et al. (2005) show that for cougars (*Puma concolor*) a riparian vegetation corridor should lie along routes with relatively gentle topographies. Likewise, Donaldson (2006) founded that hilly topographies seem to

serve as a natural guide for deer towards an underpass. However, more studies are clearly needed to examine through which mechanisms roadside topography influences carnivore's movements.

Interestingly, the number of culvert crossings was also higher once the road had a double rail guard. This brings novelty to the literature since to our knowledge only one study addresses the presence of guard rails. Malo et al. (2004), suggest that the presence of a guard rail acts to prevent collisions. From our own results, we suggest that double guard rails may have acted as barriers for mid-sized carnivores approaching the road, further guiding them towards the nearest culvert entrance.

Overall, most of the other significant factors assessed in our study besides water-related variables seem to be related to the ease of leading carnivores to the discovery of a certain culvert entrance. Still related to this, we were also expecting roadside slopes between asphalt and the culvert entrance to be a significant factor which did happen, but for the Egyptian mongoose only.

#### **4.3. Implications and conclusions**

Knowledge produced throughout this research provides useful information in developing guidelines for the design and management operations on road-crossing drainage culverts towards fostering their usage by mid-sized carnivores. Projects and practitioners incorporating technically sound measures are increasingly interested in road-crossing predictors such as those assessed here. As one key-message from our study we highlight that the partial flooding of culverts does not represent per se an avoidance factor for carnivores, even when these structures incorporate small streams inside. On the contrary, our findings suggest an important role of streams, possibly leading carnivores foraging and dispersing along the riparian corridor into the culvert entrance. However it is of paramount importance noticing that a dry band through the culvert tunnel is indeed necessary to provide a natural crossing path for most of these animals. A second highlight is that the influence of other non-water-related factors here assessed is likely attributable to their contribution to the discovery of the culvert by these animals. Nonetheless, this is still a new concept and a systematic study specifically investigating factors and mechanisms of wildlife passages discovery remains to be done.

Overall, our findings provide a way in which to refine the paradigm of water effects in culverts with direct implications to management. For example, it is overall advisable the implementation of a 0.5 up to ~2.5 m dry band to provide a crossing path. A second major recommendation is that practitioners should not ignore dry ledges in culverts incorporating small streams. Also, an important finding was that the cutting of herbaceous vegetation by

the culvert entrance is likely advisable for its discovery by these animals. In addition, distance between culverts along a road should follow available recommendations (Clevenger et al., 2001a; Smith, 2003; Ascensão and Mira, 2007) as this seem be of crucial relevance for its discovery and number of crossings. On the other hand, intervention in pre-existing culverts is an economical solution, especially in countries with low funds to implement dedicated wildlife passages. Such interventions promote connectivity across roads allowing the movement individuals and genes flow among carnivore populations. Quantifications as those presented here are essential to provide more effective mitigation programs to the management agencies and governments.

## **Acknowledgments**

This study was funded by the European project *LIFELINES* (LIFE14 NAT/PT/001081) under a protocol between *Infraestruturas de Portugal*, IP, Évora and Montemor-o-Novo municipal councils, *marca Montemor-o-Novo*, *University of Évora*, *Faculty of Sciences from University of Porto* and *University of Aveiro*. Special thanks to *Unidade de Biologia da Conservação (UBC)* for logistical support and to *UBC* team for the support given in fieldwork.

## **References**

- Ament, R.J., 2007. A Cohesive Multi-Partner Road Ecology Program. WTI-MSU Technical Report REP-07-02. Western Transportation Institute at Montana State University, pp. 22.
- Ascensão, F., Mira, A., 2007. Factors affecting culvert use by vertebrates along two stretches of road in southern Portugal. *Ecological Research* 22 (1), 57–66.
- Bates, D., Maechler, M., Bolker, B., 2011. lme4: linear mixed-effects models using Eigen and R syntax. R statistical package. Version 0.999375-42. R Project for Statistical Computing, Vienna, Austria.
- Bekker, H., Luell, B., 2003. Habitat fragmentation due to infrastructure. In: *Proceedings of the 2003 International Conference on Ecology and Transportation*, Eds. Irwin, C.L., Garrett, P., McDermott, K.P. Center for Transportation and the Environment, North Carolina State University, Raleigh, NC: pp. 1–14.
- Cain, A.T., Tuovila, V.R., Hewitt, D.G., Tewes, M.E., 2003. Effects of a highway and mitigation projects on bobcats in Southern Texas. *Biological Conservation*, 114 (2), 189–197.

- 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 (1), 157–174.
- Chambers, B., Bencini, R., 2015. Factors affecting the use of fauna underpasses by bandicoots and bobtail lizards. *Animal Conservation*, 18 (5), 424–432.
- Clevenger, A.P., Chruszcz, B., Gunson, K.E., 2001a. Drainage culverts as habitat linkages and factors affecting passage by mammals. *Journal of Applied Ecology* 38 (6), 1340–1349.
- Clevenger, A.P., Huijser, M.P., 2011. *Wildlife crossing structure handbook: design and evaluation in North America* (No. FHWA-CFL/TD-11-003).
- Clevenger, A.P., Waltho, N., 2000. Factors influencing the effectiveness of wildlife underpasses in Banff National Park, Alberta, Canada. *Conservation Biology*, 14 (1), 47–56.
- Clevenger, A.P., Waltho, N., 2005. Performance indices to identify attributes of highway crossing structures facilitating movement of large mammals. *Biological conservation*, 121 (3), 453–464.
- Clevenger, A.P., Wierzchowski, J., 2006. Maintaining and restoring connectivity in landscapes fragmented by roads. In *Conservation Biology*, Eds Crook, K., Sanjayan, M., 14, Cambridge University Press, pp. 502–535.
- Colón, C.P., 2002. Ranging behaviour and activity of the Malay civet (*Viverra zibetha*) in a logged and an unlogged forest in Danum Valley, East Malaysia. *Journal of Zoology*, 257 (4), 473–485.
- Costa, P., 2014. Plano de monitorização de fauna na linha do sul, variante ferroviária entre a estação do Pinheiro e o km 94, Relatório Anual (Ano2), Évora.
- Crook, N., Cairns, S.C., Vernes, K., 2013. Bare-nosed wombats (*Vombatus ursinus*) use drainage culverts to cross roads. *Australian Mammalogy* 35 (1), 23–29.
- Davies, J.M., Roper, T.J., Shepherdson, D.J., 1987. Seasonal distribution of road kills in the European badger (*Meles meles*). *Journal of Zoology*, 211 (3), 525–529.
- Dickson, B.G., Jenness, J.S., Beier, P., 2005. Influence of vegetation, topography, and roads on cougar movement in southern California. *Journal of wildlife Management*, 69 (1), 264–276.
- Dodd, C.K., Barichivich, W.J., and Smith, L.L., 2004. Effectiveness of a barrier wall and culverts in reducing wildlife mortality on a heavily traveled highway in Florida. *Biological Conservation* 118 (5), 619–631.
- Donaldson, B.M., 2006. Use of highway underpasses by large mammals and other wildlife in Virginia and factors influencing their effectiveness. In: *Proceedings of the 2005 International Conference on Ecology and Transportation*, Eds. Irwin, C.L., Garrett, P.,

- McDermott, K.P. Center for Transportation and the Environment, North Carolina State University, Raleigh, NC: pp. 433–441.
- Evink, G.L., 2002. Interaction between roadways and wildlife ecology, Vol. 305. Transportation Research Board.
- Fahrig, L., Pedlar, J.H., Pope, S.E., Taylor, P.D., Wegner, J.F., 1995. Effect of road traffic on amphibian density. *Biological conservation*, 73 (3), 177–182.
- Forman, R.T., Reineking, B., Hersperger, A.M., 2002. Road traffic and nearby grassland bird patterns in a suburbanizing landscape. *Environmental management*, 29 (6), 782–800.
- Forman, R.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.
- Garrah, E., 2012. Wildlife road mortality on the 1000 Islands Parkway in south eastern Ontario: Peak times, hot spots, and mitigation using drainage culverts. Master thesis of Environmental Studies at the Queen's University, Kingston, Ontario, Canada, May 2012.
- Gibbs, J.P., Shriver, W.G., 2002. Estimating the effects of road mortality on turtle populations. *Conservation Biology*, 16 (6), 1647–1652.
- Glista, D.J., DeVault, T.L., DeWoody, J.A., 2009. A review of mitigation measures for reducing wildlife mortality on roadways. *Landscape and urban planning*, 91 (1), 1–7.
- Grilo, C., Bissonette, J.A., Cramer, P.C., 2010. Mitigation measures to reduce impacts on biodiversity. In Jones, S.R. (ed.) *Highways: Construction, Management, and Maintenance*, Nova Science Publishers, Inc., Hauppauge, NY 11788. ISBN: 978-1-61728-862-3.
- Grilo, C., Bissonette, J.A., Santos-Reis, M., 2008. Response of carnivores to existing highway culverts and underpasses: implications for road planning and mitigation. *Biodiversity and Conservation* 17 (7), 1685–1699.
- Grilo, C., Bissonette, J.A., Santos-Reis, M., 2009. Spatial-temporal patterns in Mediterranean carnivore road casualties: consequences for mitigation. *Biological conservation*, 142 (2), 301–313.
- Grilo, C., Smith, D.J., Klar, N., 2015. Carnivores: struggling for survival in roaded landscapes. In *Handbook of Road Ecology*, First Edition. Eds van der Ree, R., Smith D.J., Grilo, C. John Wiley & Sons, Ltd.
- Grilo, F., Ferreira, E., Alcobia, S., Simões, L., Santos-Reis, M., 2016. Do fine-scale factors shape the use of riparian galleries by carnivores in a Mediterranean agro-forested environment?. *International Journal of Environmental & Agriculture Research*, 2 (4), 2454–1850.
- Guisan, A., Zimmermann, N.E., 2000. Predictive habitat distribution models in ecology. *Ecological modelling*, 135 (2), 147–186.

- Instituto Geográfico Português (IGP), 2007. Carta de uso e ocupação do solo de Portugal Continental 2007, <http://www.igeo.pt/>
- Instituto Nacional de Estatística (INE), I.P., 2015. Anuário Estatístico da Região Alentejo 2014, Lisboa.
- Jaeger, J.A., Fahrig, L., 2004. Effects of road fencing on population persistence. *Conservation Biology*, 18 (6), 1651–1657.
- James, A.R.C, Stuart-Smith, A.K., 2000. Distribution of caribou and wolves in relation to linear corridors. *Journal of Wildlife Management* 64:154-9.
- Jenks, G.F., 1967. "The Data Model Concept in Statistical Mapping", *International Yearbook of Cartography*, 7, 186–190.
- Liu, R., Zhao, D., 2003. Evaluation of Best Management Practices for Mitigating Impacts of Highways on Stream and Wildlife Ecology. First Progress Report.
- Loureiro, F., Pedroso, N.M., Santos, M.J., Rosalino, L.M., (eds.) 2012. Um olhar sobre os carnívoros portugueses. CARNIVORA. Lisboa. 252 pp.
- Malo, J.E., Suárez, F., Díez, A., 2004. Can we mitigate animal–vehicle accidents using predictive models?. *Journal of Applied Ecology*, 41 (4), 701–710.
- Martinig, A.R., Bélanger-Smith, K., 2016. Factors influencing the discovery and use of wildlife passages for small fauna. *Journal of Applied Ecology*, 53, 825–836.
- Mata, C., Hervás, I., Herranz, J., Suárez, F., Malo, J., 2005. Complementary use by vertebrates of crossing structures along a fenced Spanish motorway. *Biological Conservation*, 124 (3), 397–405.
- Mateus, A.R.A., Grilo, C., Santos-Reis, M., 2011. Surveying drainage culvert use by carnivores: sampling design and cost–benefit analyzes of track-pads vs. video-surveillance methods. *Environmental monitoring and assessment*, 181 (1-4), 101–109.
- McGregor, R.L., Bender, D.J., Fahrig, L., 2008. Do small mammals avoid roads because of the traffic?. *Journal of Applied Ecology*, 45 (1), 117–123.
- Ministerio de Agricultura, Alimentación y Medio Ambiente, 2015. Prescripciones técnicas para el diseño de pasos de fauna y vallados perimetrales (segunda edición, revisada y ampliada). Documentos para la reducción de la fragmentación de hábitats causada por infraestructuras de transportes, número 1. Ministerio de Agricultura, Alimentación y Medio Ambiente. 139 pp. Madrid.
- Nakagawa, S., Schielzeth, H., 2013. A general and simple method for obtaining R<sup>2</sup> from generalized linear mixed-effects models. *Methods in Ecology and Evolution*, 4, 133–142.
- Ng, S.J., Dole, J.W., Sauvajot, R.M., Riley, S.P., Valone, T.J., 2004. Use of highway undercrossings by wildlife in southern California. *Biological Conservation* 115 (3), 499–507.

- Philcox, C.K., Grogan, A.L., Macdonald, D.W., 1999. Patterns of otter *Lutra lutra* road mortality in Britain. *Journal of Applied Ecology*, 36 (5), 748–761.
- Pinheiro, J., Bates, D., Debroy, S., Sarkar, D. R., Development Core Team 2011. nlme: linear and non linear mixed effects models. R statistical package. Version 3.1-102. R Project for Scientific Computing, Vienna, Austria.
- R Development Core Team, 2009. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. R Development Core Team, Vienna, Austria, ISBN 3-900051-07-0 <http://www.r-project.org>
- Rodriguez, A., Crema, G., Delibes, M., 1996. Use of non-wildlife passages across a high speed railway by terrestrial vertebrates. *Journal of Applied Ecology* 33, 1527–1540.
- Rodriguez, A., Crema, G., Delibes, M., 1997. Factors affecting crossing of red foxes and wildcats through non-wildlife passages across a high-speed railway. *Ecography*, 20 (3), 287–294.
- Ruediger, W., DiGiorgio, M., 2007. Safe Passage: A user's guide to developing effective highway crossings for carnivores and other wildlife. Southern Rockies Ecosystem Project.
- Santos-Reis, M., Petrucci-Fonseca, F., 1999. Carnívoros. In: Mathias, M.L. (Eds.). *Mamíferos terrestres de Portugal Continental Madeira e Açores*. ICN/CBA, Lisboa, Portugal.
- Santos, S.M., Carvalho, F., Mira, A., 2011a. How Long Do the Dead Survive on the Road? Carcass Persistence Probability and Implications for Road-Kill Monitoring Surveys. *PLoS ONE* 6(9): e25383. doi:10.1371/journal.pone.0025383.
- Santos, M.J., Matos, H.M., Palomares, F., Santos-Reis, M., 2011b. Factors affecting mammalian carnivore use of riparian ecosystems in Mediterranean climates. *Journal of Mammalogy*, 92 (5), 1060–1069.
- Seiler, A., 2001. Ecological effects of roads: a review. Introductory research essay, 9, 1–40, at the Swedish University of Agricultural Sciences, Department of Conservation Biology, 2001, Uppsala.
- Serronha, A.M., Mateus, A.R.A., Eaton, F., Santos-Reis, M., Grilo, C., 2013. Towards effective culvert design: monitoring seasonal use and behavior by Mediterranean mesocarnivores. *Environmental monitoring and assessment* 185 (8), 6235–6246.
- Smith, D.J., 2003. Monitoring wildlife use and determining standards for culvert design. Final Report. Contract No. BC354-34, Florida Department of Transportation, Tallahassee, FL.
- van der Grift, E.A., van der Ree, R., Fahrig, L., Findlay, S., Houlahan, J., Jaeger, J.A.G., Klar, N., Madriñan, L.F., Olson, L., 2013. Evaluating the effectiveness of road mitigation measures. *Biodiversity and Conservation*, 22 (2), 425–448.
- van der Ree, R., van der Grift, E.A., Gulle, N., Holland, K., Mata, C., Suarez, F., 2007. Overcoming the barrier effect of roads—how effective are mitigation strategies? An

- international review of the use and effectiveness of underpasses and overpasses designed to increase the permeability of roads for wildlife. In: Proceedings of the International Conference on Ecology and Transportation. Center for Transportation and the Environment, North Carolina State University, Raleigh, pp. 423–431.
- van Vuurde, M.R., van der Grift, E.A., 2005. The effects of landscape attributes on the use of small wildlife underpasses by weasel (*Mustela nivalis*) and stoat (*Mustela erminea*). *Lutra*, 48 (2), 91–108.
- Villalva, P., Reto, D., Santos-Reis, M., Revilla, E., Grilo, C., 2013. Do dry ledges reduce the barrier effect of roads?. *Ecological engineering*, 57, 143–148.
- Yanes, M., Velasco, J.M., Suárez, F., 1995. Permeability of roads and railways to vertebrates: the importance of culverts. *Biological Conservation* 71 (3), 217–222.