# ECOLOGIA DE ESTRADAS

## ANÁLISE DE ESTUDOS SOBRE A MORTALIDADE DE VERTEBRADOS POR ATROPELAMENTO E O USO DE PASSAGENS HIDRÁULICAS POR VERTEBRADOS

ROAD ECOLOGY – OVERVIEW ON VERTEBRATE ROAD MORTALITY STUDIES AND THE USE OF CULVERTS BY VERTEBRATES

Fernando Ascensão

**Orientador – António Mira**

**Dissertação para a obtenção do Grau de Mestre em Biologia da Conservação**

**Évora, Outubro de 2005**

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UNIVERSIDADE DE ÉVORA

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"Roads are noisy, dirty and scary. The last place one would want to carry out ecological studies. But here in the pollutant-ridden corridor (...) lays the foundation of a field ripe to develop – road ecology...the science and application frontier of the next decade."

(A. Clevenger 1999)

# RESUMO

As estradas e tráfego automóvel são fontes de inúmeros impactes negativos nas populações animais, dos quais a mortalidade por atropelamento é um dos mais significativos.

Para a avaliação deste impacte foi criado um ranking de estradas que confronta o número de observações (*quantidade*), com a importância para a conservação das espécies detectadas (*qualidade*). Por outro lado pretendeu-se avaliar a eficácia das passagens hidráulicas como estruturas minimizadoras do risco de atropelamento, servindo de ligação entre as bermas das estradas.

Os resultados sugerem que a ordenação das estradas varia consoante o critério assumido: *quantidade* ou *qualidade* dos atropelamentos. Para garantir que locais onde foram detectadas espécies ameaçadas serão alvo de medidas mitigadoras, deve ser dada especial atenção ao seu estatuto de conservação. Também de acordo com os resultados obtidos, as passagens hidráulicas são usadas por várias espécies, embora com grande variabilidade, podendo ser um complemento importante em programas de mitigação dos impactes das estradas.



**Palavras-chave:** Mortalidade por atropelamento, índice de conservação, índice de mortalidade, uso de passagens hidráulicas, fragmentação de habitats, ordenação canónica.

# ROAD ECOLOGY
# OVERVIEW ON VERTEBRATE ROAD MORTALITY STUDIES AND THE USE OF CULVERTS BY VERTEBRATES


## SUMMARY

Roads and traffic are major sources of negative impacts on animal populations, of which road-kill is a most important one.

By road ranking proceedings we aimed to evaluate road-kill impact, comparing road prioritization when employing a mortality index (*quantity*) in opposition to conservation indexes (*quality*). Also, we aimed to evaluate culverts as linkage points between roadsides, and this way diminish road-kill hazard.

Results suggest that prioritization depended highly on the criteria used for doing it: number of specimens killed or conservation/ecological value of the species. Decisions on prioritization road sections should integrate less total number of casualties than conservation concern of species detected, in order to ensure that threaten species receive proper attention toward road kill mitigation. Also according to results, culverts are used by several species, although high variability was detected, providing an important complement on mitigation impact programs.



**Key words:** Road-kills, conservation index, mortality index, culvert use, habitat fragmentation, canonical ordination.

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# ÍNDICE

# INTRODUÇÃO

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## Fragmentação e efeito barreira

Uma das mais graves ameaças à sobrevivência da biodiversidade a nível mundial é a fragmentação da outrora contínua paisagem natural ou semi-natural. A fragmentação cria parcelas de menor tamanho e mais isoladas de habitat favorável, embebidas numa matriz mais adversa (Saunders et al. 1991), dificultando ou impossibilitando o estabelecimento de espécies ou comunidades sensíveis a esta fragmentação (e.g.: Crooks 2002).

Como resultado da fragmentação dos habitats, um dos impactes mais importantes nas populações animais é o efeito de barreira. Este processo não é facilmente visível e quantificável, mas tem consequências que poderão ser bastante graves para a manutenção das populações. Uma vez que as extinções locais ocorrem na natureza, por acção humana ou não, a sobrevivência das espécies está interligada com a sua capacidade de dispersão e recolonização das manchas de habitat que experimentaram essas extinções. Deste modo, as diferentes populações encontram-se em contacto, englobadas numa metapopulação, e não verdadeiramente isoladas, o que lhes seria prejudicial. De facto, o isolamento das populações é reconhecido como uma das causas mais comuns para as extinções locais (Fahrig & Merrian 1994). No entanto, o sucesso da (re)colonização depende da disponibilidade de indivíduos dispersantes e da facilidade de deslocação que a paisagem permite. Por sua vez, este processo depende da estrutura espacial da paisagem e em particular da presença de potenciais obstáculos naturais ou artificiais (St.Clair 2003). Se a estrutura espacial da paisagem restringe a movimentação entre manchas isoladas, então a área necessária para a sobrevivência de uma espécie, o número de manchas de habitat e o tamanho das populações têm de ser necessariamente maiores (Fahrig & Merrian 1994).

Para além da actividade agrícola em larga escala e das grandes áreas urbanas, um dos agentes que mais contribui para o incremento exponencial da fragmentação e destruição dos habitats é a expansão, a nível mundial, da rede rodoviária. Particularmente intenso nas regiões mais desenvolvidas do globo, a Europa e América do Norte, o ritmo de crescimento da rede rodoviária no séc. XX permitiu o seu alargamento até às mais remotas áreas naturais remanescentes e atingiu dimensões que poderão pôr em causa a integridade das populações de diversas espécies (Bekker 2002; Trocmé et al. 2003). Este crescente conflito, entre a necessidade de mais espaço para satisfazer a expansão das rodovias e a conservação da natureza, exige a tomada de medidas urgentes num futuro próximo, a nível das políticas, dos planos e dos projectos, que permitam conciliar estas dimensões aparentemente antagónicas.

Diversos estudos têm demonstrado que a presença das estradas é prejudicial tanto às movimentações como à sobrevivência dos vários grupos animais, sejam invertebrados (e.g.: Haskell 2001), anfíbios (e.g.: Carr & Fahrig 2001), répteis (e.g.: Gibbs & Shriver 2002), aves (e.g.: Kuitunen et al. 1998) ou mamíferos (e.g.: Philcox et al. 1999). As estradas e o tráfego automóvel podem actuar como uma autêntica barreira que impede ou dificulta fortemente a mobilidade dos animais. Este efeito de barreira pode manifestar-se na incapacidade dos animais ultrapassarem o obstáculo (Mader 1984), na mortalidade por atropelamento (Clevenger et al. 2003) ou em reacções de certas espécies com fraca tolerância à perturbação, que tendem a afastar-se das estradas (Reijnen et al. 1995). Deste modo, a limitação nos deslocamentos dos animais, diários ou sazonais, pode levar à diminuição da conectividade entre as populações de vertebrados. Daqui pode resultar uma perda na diversidade genética, aumento da consanguinidade e maior vulnerabilidade das populações a fenómenos estocásticos que podem levar à sua extinção local (van der Zande et al. 1980). [Para uma revisão mais alargada dos impactes das estradas consultar e.g.: Forman & Alexander (1998); Spellerberg (1998); Trombulak & Frissel (2000); Seiler (2001); Forman et al. (2002.)].

Na última década surgiu um novo ramo científico que estuda a interacção das estradas e tráfego rodoviário com a biodiversidade, que em português se pode traduzir por Ecologia de Estradas (Forman et al. 2002). A ecologia de estradas pretende fornecer uma matriz de indicadores que possibilitem o delineamento de planos, práticas e políticas de transporte que integrem os impactes das estradas nos meios bióticos e abióticos, permitindo assim a eliminação/diminuição desses impactes, através da criação de soluções ecologicamente viáveis.

## Mortalidade de vertebrados por atropelamento: por detrás dos dados

A mortalidade dos vertebrados por atropelamento constitui um dos impactes mais visíveis e quantificáveis das estradas nas comunidades animais. Este tema já foi objecto de estudo por vários autores em diversos países (e.g.: Hodson 1960; Oxley et al. 1974; Berthoud 1980; Vignes 1984; Garnica & Robles 1986; Davies et al. 1987; Rodda 1990; Prieto et al. 1993; Fahrig et al. 1995; Philcox et al. 1999; Huijser 2000; Mumme et al. 2000; Ascensão 2001; Carr & Fahrig 2001; Gibbs & Shriver 2002). Todos os autores obtiveram resultados que demonstram que um vasto número de espécies é atingido por este processo, podendo inclusivamente provocar a exinção local de populações (Fahrig et al. 1995). Espécies com maiores dimensões, com domínios vitais mais alargados, ou que entram regularmente em contacto com as estradas (e.g. espécies migradoras), são frequentemente as mais afectadas pelo atropelamento (Forman & Alexander 1998; Trombulak & Frissel 2000).

Para avaliar a importância ecológica da mortalidade por atropelamento numa determinada espécie, implica considerar não só as suas características biológicas, como sejam o tamanho da população, a fenologia, taxa de fecundação, maturidade sexual, especialização trófica, tamanho dos indivíduos, mas também as características biogeográficas, i.e., a sua distribuição ao longo do território. Um número elevado de atropelamentos numa certa espécie não implica necessariamente um risco para a sobrevivência dessa espécie, podendo o grande número de colisões ser consequência de uma distribuição e densidade elevadas (Seiler 2002). Não diminuindo a importância e o impacte negativo que essa elevada taxa de mortalidade possa ter nessa mesma espécie, nomeadamente a nível local, interessa no entanto relativizar a questão e saber afinal onde está o maior impacte a ocorrer: em espécies abundantes e dispersas, ou em espécies com populações reduzidas e isoladas? É então necessário criar um índice de caracterização das espécies de forma a avaliar o impacto dos atropelamentos do ponto de vista da *qualidade* das espécies em conjugação com a *quantidade* de atropelamentos. Deste modo, a elaboração de um ranking a nível regional ou nacional das espécies e consequentemente das vias rodoviárias, de acordo com a sua mortalidade, poderá ajudar na decisão de onde aplicar primeiramente medidas mitigadores, definindo quais os locais com um impacte superior. O trabalho de Palmeirim et al. (1994) foi o ponto de partida para desenvolver este objectivo. Para tal foi feita uma reunião dos trabalhos académicos sobre este tema que até à data haviam sido realizados em Portugal, da qual resultou igualmente a oportunidade de avaliar o nível de conhecimento nesta matéria, a nível nacional.

## Passagens hidráulicas: atenuantes do problema ou parte da solução?

Como foi referido, a quebra na conectividade entre populações constitui um dos factores mais significativos que contribuem para a fragmentação da paisagem e das populações animais. Estando já demonstrado em diversos trabalhos a acção negativa das estradas e tráfego rodoviário em populações animais, nomeadamente a fragmentação dos habitats e populações (Forman et al. 2002), é necessário investir na investigação de soluções que facilitem a conectividade entre as manchas de habitat e as diferentes populações e, simultaneamente, diminuam o risco de atropelamento. Uma das medidas mais importantes é o estabelecimento de estruturas para a passagem de fauna que sejam eficazes e garantam o cruzamento das rodovias em segurança. No entanto, como refere Luell et al. (2003), estas medidas não devem ser consideradas isoladamente, mas sim enquadradas numa perspectiva holística do problema, integradas num conceito geral de permeabilidade das estradas. Deste modo, devem ser considerados diversos factores para a localização destas estruturas, nomeadamente a distribuição espacial das manchas de habitat e das populações, a distância entre as manchas e as estradas, entre outras características da paisagem. Assim, as passagens para a fauna constituem apenas pequenos elementos na resolução do problema, mas com uma importância central para o seu sucesso.

Existem diversas soluções para a construção de pontos de passagens especialmente desenhadas para a fauna (Luell et al. 2003). Uma vez que a aplicação generalizada destas estruturas pode envolver custos acrescidos consideráveis, vários autores têm sugerido uma intervenção complementar nas passagens hidráulicas, de maneira a torná-las mais atractivas aos animais, como forma de se obter melhores resultados. Alguns trabalhos, realizados especificamente com passagens hidráulicas (e.g.: Yanes et al. 1995; Rodriguez et al. 1996, Clevenger et al 2001; Taylor & Goldingay 2003; Mata et al. 2005), demonstraram que estas estruturas podem ser usadas por um elevado número de espécies, embora não existam ainda dados conclusivos sobre os factores que determinam a sua utilização pelos diferentes vertebrados. O elevado número destas estruturas presentes nas vias rodoviárias e o reduzido custo necessário para realizar algumas alterações de carácter biofísico, tornam esta intervenção bastante atractiva, nomeadamente para os decisores de projectos rodoviários. Assim, as passagens hidráulicas, cujo propósito inicial é a condução de águas de escorrência, parecem ser estruturas que podem funcionar como passagens para fauna com um sucesso significativo, para um certo número de espécies. Deste modo, determinar as características das passagens que favorecem o seu uso pelos vertebrados reveste-se de uma grande importância. Dado que a actividade humana e o tráfego intenso podem exercer uma influência negativa significativa na tolerância e aproximação de alguns animais (Rodriguez et al. 1996), interessa avaliar a influência destas variáveis através do estudo de diferentes tipos de vias rodoviárias, com níveis distintos de tráfego rodoviário. Para o sucesso deste processo interessa também perceber quais as características da paisagem que encaminham os animais para estes pontos de passagem. Uma das soluções que tem vindo a ser apontada por alguns autores é o estabelecimento de corredores ecológicos em comunicação com as passagens hidráulicas existentes ao longo das vias rodoviárias. Estes corredores poderiam direccionar os animais para as manilhas das passagens hidráulicas e, deste modo, garantir o cruzamento da via rodoviária sem o risco de atropelamento, diminuindo assim o efeito de barreira. A existência dos corredores ecológicos, embora não seja consensual entre a comunidade científica, tem sido reconhecida como um factor muito importante na conservação da biodiversidade (Beier & Noss 1998). Os corredores permitem a ligação entre habitats e populações isoladas, facilitando assim a mobilidade dos animais, o que conduz a uma homogeneização das densidades populacionais, troca de variabilidade genética e maior resistência à extinção derivada de fenómenos estocásticos (Fahrig & Merrian 1994; Haddad et al. 2003; Lecomte et al. 2004). As galerias ripícolas são frequentemente utilizadas por diversas espécies, particularmente carnívoros, uma vez que fornecem abrigo e alimento principalmente em áreas agrícolas (Virgós 2001; Hilty & Merenlender 2004). Este facto facilita o estabelecimento de corredores em ligação com as passagens hidráulicas, uma vez que, naturalmente, muitas passagens se encontram no prolongamento das ribeiras e linhas de água.

## OBJECTIVOS

De acordo com o acima exposto, os objectivos gerais deste trabalho foram:

a. Reunir e analisar trabalhos realizados em Portugal sobre o impacte do atropelamento nos vertebrados

b. Avaliar a aplicação de índices de conservação para ordenar estradas de acordo com o nível de impacte nas espécies

c. Avaliar se as passagens hidráulicas poderão minimizar o efeito de barreira e o impacte do atropelamento nas populações

d. Perceber quais as características da paisagem (estrada e habitats envolventes) que influenciam o uso das passagens hidráulicas pelas diferentes espécies/grupos faunísticos

Os dois primeiros objectivos foram desenvolvidos no primeiro artigo "Evaluating wildlife road mortality: more than meets the eye", submetido à revista Biodiversity and Conservation a 11/10/2005; sendo os restantes dois objectivos a base de trabalho do segundo artigo "Factors affecting culvert use by vertebrates in two roads of southern Portugal" submetido à revista Ecological Research a 03/10/2005.

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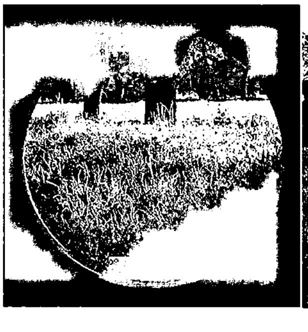
# ARTIGOS CIENTÍFICOS



# EVALUATING WILDLIFE ROAD MORTALITY: MORE THAN MEETS THE EYE

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**ABSTRACT:**

There are an increasing number of studies quantifying and evaluating the potential impact of road mortality in wildlife populations and ranking roads according to mortality indexes (*quantity* of causalities). However, high mortality indexes are expected for common species of usually non-threatened vertebrates, whereas rare species should experience lesser road mortality. Hence, road-kill of species of conservation concern might be overlooked. We suggest that *quality* of species that are affected by road mortality should also be taken in account in road ranking. Surveys from eight roads were used to evaluate road ranking differences. We intend to evaluate if the mortality indexes (MI, number of specimens/10km sampled/number sampling sessions) alone are appropriate descriptors of the mortality risk on wild species of higher conservation value. For that, two conservation-based indexes (CI's) were created, one derived from *Portuguese Red data book and European Status defined by Birds and Habitat Directives* and a second derived from *Biological and biogeographical status*.

A total of 5550 vertebrate road-killed specimens were collected and 129 species were identified. Casualties among vertebrate classes were significantly different, being higher on amphibians (n = 2590; 16 species) followed by birds (n = 1722; 68 species), mammals (n = 821; 28 species) and reptiles (n = 417; 17 species). The MI and CI's identified different species as priority in terms of vulnerability to road killing (*quantity*) and conservation concern (*quality*). Five species were high ranked on both groups suggesting high road killing rates of

species with conservation concern. Ranking of roads were significantly different using *quantity* and *quality* criteria.
We propose that road ranks probably should depend less on *quantity* classification than on *quality* levels of road-kills and conservation implications for road management are distinct according to the combination of both data types.

**Keywords**: Conservation Index, Mortality Index, Road-kills, Portugal, Vertebrates, Road Ecology

## INTRODUCTION

Road killing is one of the most recent and important sources of unnatural mortality in wildlife species, affecting from invertebrates (e.g.: Seibert and Conover 1991) to large mammals (e.g.: Groot et al. 1996), and ground, air-dwelling and aquatic species (Dodd et al. 1989; Mumme et al. 2000; Carr and Fahrig 2001). Road mortality modifies the demography and population structure of species, and in certain circumstances it can be the primary source of mortality in wild populations (Mumme et al. 2000; Clark et al. 1998), affecting the recovery of endangered species and/or contributing to the endangerment of others (Forman and Alexander 1998; Trombulack and Frissel 2000; Forman et al. 2002). The increasing complexity of road networks and number of circulating vehicles will most likely intensify the negative effect of road killing on wildlife populations in the following decades. Even for unpaved roads, road mortality is expected to increase, due to the current popularity of off-road vehicles (Bury et al. 1977). For these reasons, there are an increasing number of studies quantifying wildlife road mortality, evaluating the potential impact of road mortality in population structure of wildlife populations, and developing management strategies for mitigating this threat factor (e.g.: Philcox et al. 1999; Kerley et al. 2002; Clevenger et al. 2003).

Mitigation measures to diminish animal-vehicle collision are difficult to implement in all problematic road sections. Thus, road ranking according to mortality indexes might be a simple and expedite way to decide which roads should be considered priority for the application of mitigation measures. Species with higher conservation concern tend to have minor collision rates, has a result of their low population densities or as a consequence of restrict spatial distribution. Hence, roads with overall lower mortality indices but with casualties from species with high conservation concern might be overlooked when compared with roads with high mortality levels from common and broadly distributed species. Since most studies rely on total casualties counts (total number of specimens) or mortality indexes (number of specimens by a distance unit) to identify high risk roads or road sections (e.g.: Dodd et al. 1989; Fahrig et al. 1995; Clevenger et al. 2003), mortality indexes are taken as a measure of road mortality intensity and therefore high risk roads or road sections are selected to implement mitigation measures on the basis of *quantity* of road-killed specimens (Taylor and Goldingay 2004). Common victims of road traffic usually include several abundant and widespread amphibians representing, for instance, up to 90% of the total vertebrates found road-killed (e.g.: González-Prieto et al. 1993; Hels and Buchwald 2001). High mortality indexes are expected in common and/or widespread species of usually non-threatened vertebrates, whereas rare and/or geographically localized threatened species should experience lesser road mortality. Nevertheless, the *quality* of species that are most affected by road mortality should also be taken in account. As an example, one main cause of Iberian lynx (*Lynx pardinus*) non-natural death is road killing (Ferreras et al. 1992). Given the high conservation concern of this species, the most worldwide endangered felid, roads or road-sections in which casualties were detected must be taken has high priority to implement mitigation measures. Some authors (Malo et al. 2004; Ramp et al. 2005; Seiler 2005) proposed the use of predictive models of animal-vehicle collision in order to inform decisions on road alignment and on the exact location of crossing structures. Their works were based on total counts of vertebrate casualties and aimed to detect problematic sections along the studied roads. However, *quality* information should be integrated on this kind of work, in order to give weights to species (Millsap et al. 1990; Palmeirim et al. 1994) and consequently highlight important information not detectable at first sight. With this information it is possible to correct potential mislead priority ranking of roads or road sections and thus apply investment on mitigation measures where are most needed. Priority conservation indexes should be created based on legal status and information on biology and distribution of each species.

Thus, the aims of this work is to determine if mortality indexes used solely are suitable for identifying road or road sections with high risk for the conservation of wild species.

We also aim to provide the state of the art concerning vertebrate mortality studies on Portuguese roads, because the few data available is dispersed and remains unpublished (but see Brito and Álvares 2004). Mortality and conservation-based indexes will be used to evaluate differences in ranking of roads in terms of priority for the implementation of mitigation measures.

## METHODS

### *Road surveys*

Eight roads located in continental Portugal (Iberian Peninsula, SW Europe) were sampled by car at an average speed of 20 km/h (Fig. 1; Table 1). Data were collected by authors, except for Alcochete which came from an unpublished report (J. Marques, *personal communication*). All vertebrates found killed on the pavement were collected and identified to species level *in loco* whenever possible, or by analysis in the laboratory of skin, scales, feather or hairs, depending on the taxonomic group.

It should be emphasized that number of casualties found were most probably biased due several constrains, namely non-daily surveys, carrion foraging from other animals, climatic conditions, road physical characteristics, traffic levels, which can mislead correct counting and detection of corpses on roads (see Erritzoe et al. 2003; authors, unpublished data). Thus, records should be regarded merely as an underestimation of real carnage occurring on roads.

*Indexes for ranking species and roads*

Road mortality was evaluated by an index of mortality (MI) for *taxa* as number of specimens per 10 kilometers sampled per number of sampling sessions.

We created two indexes for the evaluation of priority conservation species (CI's):

*Threaten Status Index* (TSI), based on (1) Portuguese status, on the Revision of Portuguese Vertebrate Red Data Book (Cabral et al. *in press*); and (2) European Status, based on the annexes of Habitats Directive (92/43/CEE) and Birds Directive (79/409/CEE) (see table 2A).

*Biological and Biogeographical Status Index* (BGI). Species were ranked according to their intrinsic biological characteristics and the size of their distribution area. For the biological traits, six variables were considered: 1) Population size, being valorized the species with small number of mature individuals in Portugal; 2) Phenology, being valorized the species that are resident and/or breed in Portugal; 3) Annual fecundity, being valorized the low-fecundity species. This variable quantified the average number of eggs or embryos produced per year, but when two or more reproductive seasons occur per year, the average total number per year was used; 4) Female age at sexual maturation, being valorized species that mature at older ages; 5) Trophic specialization, being valorized the predator species; 6) Individual body mass, being valorized the heavier species. Data for these variables were obtained in several reference books on the biology of European and/or Portuguese vertebrates (McDonald and Barrett 1993; Salvador 1997; Snow and Perrins 1998; Barbadillo et al. 1999; Mathias 1999; Ferrand et al. 2001). Analysis of distribution was based on two descriptors: 1) Global distribution area, being valorized the Iberian-endemic species; 2) Portuguese distribution area, being valued the species with a restricted distribution area. Data for these variables were obtained in several European and Portuguese distribution atlas (Rufino 1989; McDonald and Barrett 1993; Gasc et al. 1997;

Snow and Perrins 1998; Mathias 1999; Mitchell-Jones et al 1999, Ferrand et al. 2001) (See table 2B).

In all variables, the minimum and maximum scores were 0 and 100 points, respectively. Each index was standardized by averaging its values by the number of variables considered on each case.

Comparisons between the three indexes were made using the threshold of the 29 most frequently road-killed species derived from the index of mortality (hereafter referred as 29M), and the 29 most highly ranked priority conservation species, derived from the TSI and BGI, respectively (hereafter referred as 29TS and 29BG). The threshold resulted from the fact that it was only possible to create eight score classes for TSI, and the uppermost six classes included about 22% of identified species, which are 29 *taxa*.

## RESULTS

*Overall mortality*

A total of 5414 vertebrate road-killed specimens were collected, 4797 of which were identified to species level (Table 3). A total of 129 species were recorded. Casualties among vertebrate classes were higher on amphibians (n = 2540) followed by birds (n = 1691), mammals (n = 782) and reptiles (n = 401). Vertebrate classes had also significant differences in species number; more bird species (n = 68) were identified than mammals (n = 28), reptiles (n = 17) and amphibians (n = 16).

Total number of species and specimens collected were not correlated with the sampling effort per road (Spearman rank correlation test, number of species: $r_s = 0.420$, $p = 0.333$; number of specimens: $r_s = 0.357$, $p = 0.385$).

The MI ranged from 0.007 on greater horseshoe bat (*Rhinolophus ferrumequinum*) to 9.031 on western spade foot toad (*Pelobates cultripes*) (MI mean = 0.447±1.26) (see annex for details on road mortality index results per road and per species). The CI's ranged from 0 on several species to highest value on Seoane's viper (*Vipera seoanei*) on both indexes (TSI=87.5, BGI=90).

*Mortality index*

A total of 3942 road-killed specimens representing the 29M were collected (Table 4a). This group summed an overall conservation index of 112.5 in TSI and 1157.5 in BGI. The total number of species and specimens within the 29M collected were not correlated with the sampling effort per road ($r_s$ = 0.12, $p$ = 0.978 and $r_s$ = 0.50, $p$ = 0.207, respectively). Similar result was found for number of species per vertebrate class within the 29M (amphibians: $r_s$ = -0.34, $p$ = 0.414; reptiles: $r_s$ = 0.15, $p$ = 0.721; birds: $r_s$ = 0.63, $p$ = 0.881; mammals: $r_s$ = 0.01, $p$ = 0.977). The number of specimens per vertebrate class within the 29M were also not correlated with the sampling effort, except for reptiles (amphibians: $r_s$ = 0.19, $p$ = 0.651; reptiles: $r_s$ = 0.922, $p$ = 0.001; birds: $r_s$ = 0.357, $p$ = 0.385; mammals: $r_s$ = 0.395, $p$ = 0.333).

Casualties among classes and class mortality rates within the 29M were higher on amphibians (n = 2406; MI = 30.7), followed by birds (n = 902; MI = 11.1), mammals (n = 359; MI = 5.1) and reptiles (n = 275; MI = 3.0) (see Table 4a and annex).

*Threaten status index*

A total of 242 road-killed specimens representing the 29TS were collected (Table 4b), summing 1275 on this conservation index. The total number of species within the 29TS was correlated with the sampling effort per road ($r_s$ = 0.88, $p$ = 0.007). The number of species per vertebrate class within the 29TS were not correlated with the sampling effort except for mammals (reptiles: $r_s$ = 0.17, $p$ = 0.678; birds: $r_s$ = 0.55, $p$ = 0.154; mammals: $r_s$ = 0.76, $p$ = 0.030). Only two species of amphibians were included in this index (Table 4b).

The total number of specimens collected was correlated with the sampling effort ($r_s$ = 0.88, $p$ = 0.004), although the number of specimens per vertebrate class within the 29TS were not correlated with the sampling effort (reptiles: $r_s$ = 0.41, $p$ = 0.307; birds: $r_s$ = 0.59, $p$ = 0.117; mammals: $r_s$ = 0.46, $p$ = 0.247).

Casualties among classes were again higher on birds (n=81), followed by reptiles (n=69), mammals (n=60) and amphibians (n=32) (Table 4b). Higher values of mortality rates were found for birds (MI=0.86), followed by mammals (MI=0.74), reptiles (MI=0.73) and amphibians (MI=0.41).

*Biological and biogeographical status index*

A total of 460 road-killed specimens representing the 29BG were collected (Table 4c), summing 1786 on this conservation index. The total number of species within the 29BG was not correlated with the sampling effort per road ($r_s = 0.52, p = 0.192$), nor were the number of species per vertebrate class within the 29BG correlated with the sampling effort (reptiles: $r_s = 0.26, p = 0.543$; birds: $r_s = 0.28, p = 0.498$; mammals: $r_s = 0.38, p = 0.346$). No amphibian species were included in this index (Table 4c).

The total number of specimens collected was not correlated with the sampling effort ($r_s = 0.66$, $p = 0.076$), and the number of specimens per vertebrate class within the 29BG were also not correlated with the sampling effort, except for reptiles (reptiles: $r_s = 0.92, p = 0.001$; birds: $r_s = 0.42, p = 0.307$; mammals: $r_s = 0.35, p = 0.399$).

Casualties among classes were higher on reptiles (n=244), followed by birds (n=166) and mammals (n=50). Higher values of mortality rates were found for reptiles (MI=2.48), followed by birds (MI=1.87) and mammals (MI=0.69).

None species presented in the 29M figure in either 29TS or 29BG. Two species, wild rabbit (*Oryctolagus cuniculus*, MI=0.640) and painted frog (*Discoglossus galganoi*, MI=0.404) are common to 29M and 29TS; and three others, Montpellier snake (*Malpolon monspessulanus*, MI=0.917), ladder snake (*Elaphe scalaris*, MI=0.697), and barn owl (*Tyto alba*, MI=1.308) are common to 29M and 29 BG (Table 4). Only 15 species were present in both 29TS and 29BG (Table 4).

*Mortality by road*

According to the MI, the roads Sendim and Portalegre were the most important in terms of *quantity* of road-killed vertebrates, representing over 65% of the 29M vertebrates found road-killed in all eight sampled roads (Fig. 2A). Road-killed amphibians were the vertebrate class that most contributed for this ranking, representing in these two roads, respectively, more than 90% and 69% of the total observations.

Regarding the conservation indexes, representing *quality* of road-killed vertebrates, higher MI values were found in the roads Portalegre and Vendas Novas, for both indexes, although in changed positions (Fig. 2B and 2C). Road-killed reptiles, birds and mammals were the vertebrate classes that most contributed for this ranking.

Interesting differences in the ranking of roads according to each index are noteworthy. The roads Sendim, Portalegre and Vendas Novas were, by this order, of highest-priority considering *quantity* data. However, according to *quality* data, Portalegre and Vendas Novas were the highest ranked roads, and Sendim was moved back to third place with TSI and to sixth with BGI. The latter index highlighted the Alcochete (third place) which was also moved ahead to fourth place with TSI. Moreover, Gerês, which had the lowest overall mortality rates, was repositioned to fifth place with both TSI and BGI.

## DISCUSSION

*Quantity versus quality data*

This study showed that roads with higher overall mortality index did not correspond to those with uppermost mortality index of highly ranked priority conservation species. Ranking of roads was different using *quantity* and *quality* criteria. Concerning conservation priorities, road ranks probably should depend less on *quantity* classification than on *quality* levels of animal road killed. For instance, Portalegre and Sendim were ranked as high priority when considering *quantity* data, mainly as a consequence of amphibian mass killing, however when *quality* data is highlighted, Alcochete and Vendas Novas become higher ranked due to high mortality of the other vertebrate groups.

Differences found between conservation scores of *quantity* and *quality* data ranks reflects what might be failed to notice at first sight. In fact, the specimens belonging to 29TS (n=242) and 29BG (n=460) had, respectively, overall conservation scores of TSI=1275 and BGI=1786, i.e., 11.3 and 1.5 times higher the one found for 29M group (TSI=112.5 and BGI=1157.5), which included 3942 specimens. Hence, *quantity* data might mask species of conservation value. Conversely, when integrating the *quality* data on road ranking we may deal with the species ecological importance. Nevertheless, results should be considered cautiously given that biases might have arisen as a result of differential sampling experimental design. In fact, amphibian mass killing occurs in few occasions throughout the year and always with specific climatic conditions. Since small vertebrate corpses remain detectable for few hours on pavement after collision, especially in high traffic roads (Erritzoe et al. 2003 and references therein; António Mira unpublished data), samples undertaken beyond this period are expected to underestimate significantly amphibian mortality rates, as well as other small bodied animals, such as passerines, small lizards and small mammals. Moreover, reptile observation seemed to be associated to sampling effort, which might confuse partially results interpretation. On the other hand, total number of specimens within the 29TS and number of reptiles' specimens within the 29BG collected were correlated with the sampling effort. This should be expected, given that rare species, either in distribution or in abundance, are likely to be easier to find with increasing sampling effort. These results emphasize that, equal experimental design should be implemented in future national surveys to prevent such biases.

*Conservation indexes*

Although highlighting different species assemblages, it is noteworthy that both conservation indexes emphasized, as most important, the same two roads. Moreover, a substantial number of species were selected by both indexes simultaneously, which suggests some concordance on choosing conservation priority species. Most probably, their conjunction on evaluation processes may help to identify more clearly vulnerable species to road killing and problematic road segments.

*Analysis within classes*

Most amphibian species have numerous and widespread populations in Iberian Peninsula (Ferrand et al. 2001; Pleguezuelos et al. 2002), thus significant road-kill levels occur leading to a taxonomically over-representation among 29M (Table 4). In fact, massive quantities of road-killed amphibians are usually reported during seasonal migrations (Fahrig et al. 1995; Hels and Buchwald 2001), as appeared to be the case of western spade foot toad and natterjack toad (*Bufo calamita*) in Sendim and Portalegre and common toad (*Bufo bufo*) in almost all sampled roads, contributing for their higher ranking in *quantity* terms (Fig. 2A). From the *quality* point of view, the single two 29TS amphibian species, golden-striped salamander (*Chioglossa lusitanica*) and painted frog, were detected in northern sampled roads. Despite the low number of road-killed specimens found, special attention should be addressed to the population status of these two Iberian endemisms in the vicinity of those roads, particularly to the latter one due its presence on both 29M and 29TS ranks.

For reptiles, four snake species and one lizard were found among 29M (Table 4). Several studies concerning traffic mortality on snakes (Bernardino and Dalrymple 1992; Rosen and Lowe 1994; Bonnet et al. 1999; Brito and Álvares 2004) showed that mortality may severely reduce snake populations to a level where reproductive output cannot replace road-killed individuals (Rosen and Lowe 1994). Although commonly poor acknowledged in overall road mortality studies due to their usually low *quantity* of mortality, in *quality* terms they can have a greater impact (Brito and Álvares 2004). In fact snakes were overrepresented among conservation indexes with seven species. Additionally, ladder snake and Montpellier snake were present in both 29M and 29TS, suggesting high mortality rates for these species with considerable high conservation value. Moreover, these two species had the highest score for 29BG among 29M. These species had higher mortality rates in Portalegre and Alcochete, respectively. Although Portalegre was higher ranked on both *quantity* and *quality* analysis, Alcochete was only upper ranked when *quality* data was considered. According to our results, Alcochete should receive prior attention, given its *quality* rank position, and this is largely due Montpellier snake casualties detected here. Additionally, Lataste's viper (*Vipera latastei*) and Seoane's viper were exclusively found road killed in Gerês and Montalegre, respectively. Brito and Álvares (2004) found for these roads male-biased road mortality, especially intense during the mating season. For females, road mortality was more frequent after parturition, when emaciated females searched for food. These suggests that mitigation measures especially addressed for these species might be developed and applied in specific time periods along the year in these roads, as reducing traffic speed or even stop traffic circulation at critical periods. Considering bird mortality, high rates were detected for several species, supporting previous studies reporting millions casualties per year across Europe (see Erritzoe et al. 2003). House Sparrow (*Passer domesticus*) was the commonest species, and its mortality rate contributed significantly to increase road ranks in *quantity* analysis, particularly Sendim. When considered *quality* data, three species casualties emerge from analysis, black-winged kite (*Elanus caeruleus*), red-backed shrike (*Lanius collurio*) and barn owl. The former one was observed in Portalegre and Vendas Novas, and it is a *Near Threatened* species with small population densities in Portugal (Cabral et al. *in press*). The second is a restricted-range species in Portugal and was only found road killed in Montalegre. The latter is present in both rankings of most road-killed and in one most high priority conservation species (29BG), suggesting significant casualties on a conservation concern species. This species had particularly high number of observations on Alcochete, where 57 individuals were detected in one year. In fact, road-kill has been stated as one major source of unnatural death, and a main cause of its population decreasing in England (Ramsden 2003).

As for mammals, carnivore and bat mortality should be underlined due their conservation concern. These mammalian groups are very exposed to traffic collision as consequence of their vast territory use. Several studies have demonstrated that road mortality can be a main cause of unnatural death for carnivores (Ferreras et al. 1992; Clark et al. 1998). In *quantity* terms, significant results were obtained for hedgehog (*Erinaceus europaeus*), one of the most affected species by road traffic (see Huijser 1999). Hedgehog had the highest score in 29BG among the mammals assembled on 29M, suggesting that conservation actions should be addressed to this species, particularly in Vendas Novas where a significant number of casualties occurred. Polecat (*Mustela putorius*) was the species most affected among carnivores, especially in Vendas Novas, which contributed for its rank increasing in *quality* analysis. One wild cat (*Felis silvestris*) specimen was found in Alcochete, which is related to this road higher *quality* rank position. The populations of this species are probably regressing to minima in Portugal as in Europe (Pierpaoli et al. 2003) and their future is probably following the world most endangered feline, the Iberian lynx, if conservation actions are not taken. Hence, mitigations measures should be developed to improve road permeability to carnivore daily movements, particularly for those with high conservation concern.

*Mortality by road*

Considering overall road ranks, Portalegre was placed in uppermost positions either when *quantity* data and *quality* data was taken account. Results suggest that this road should receive mitigation actions prior to remain roads here studied. This is more relevant in light of its location. In fact, PRT road section is located in the border of one of the most important Portuguese protected areas in Portugal, Serra de S.Mamede Natural Park. This area is located in a biogeographic crossroad assembling both Mediterranean and Atlantic climatic characteristics, which grants it with multiple habitat patches allowing high species diversity and richness. This is, reflected in the highest number of road-killed species here detected, particularly birds and mammals. As suggested by Spector (2002), biogeographic crossroads appear to be areas of high conservation priority and opportunity in both the short and long term and require increased attention in the process of setting conservation priorities. *Quality* data seems to support this prioritization, as it emphasizes Portalegre in overall studied roads context. In fact, great amount of 29TS and 29BG reptiles, birds and mammals were detected in this road.

*Experimental design issues*

Future road surveys should be experimentally designed in order to integrate national scale, assembling all different road categories concerning traffic levels, road width, habitat surrounding, and biogeographic regions. Also, intervals between samplings should be temporally narrow to allow detection of small bodied size vertebrates before vanishing due to traffic or carrion. Special attention should be attended to amphibian mass migration periods.

## CONCLUSION

Ranking roads or roads sections in order prioritize mitigations actions for road killings is fundamental process on road management. As shown above, results of this ranking depended highly on the criteria used for doing it: number of specimens killed or conservation/ecological value of the species. Threaten species are usually less abundant and/or have a narrower distribution range facing a higher risk of local or global extinction in a near future. More so, decisions on prioritization road sections should integrate less total number of casualties than conservation concern of species detected, in order to ensure that threaten species receive proper attention and conservation actions toward road kill mitigation occur where most needed.

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**Annex.** Mortality and conservation indexes (CI: TSI and BGI) by species and sampled road (see methods section for details on the calculations of indexes and Table 1 for the geographic location of roads). Species are displayed by alphabetic order.

| Species | Sampled roads | | | | | | | | TSI | BGI |
|---|---|---|---|---|---|---|---|---|---|---|
| | SND | PRT | VNV | SMN | ALC | BPS | MNT | GER | | |
| **Amphibians** | | | | | | | | | | |
| *Alytes cisternasii* | 0.162 | 0.014 | 0.021 | | | 0.021 | | 0.017 | 12.5 | 48 |
| *Alytes obstetricans* | 0.081 | | | | | 0.021 | | | 12.5 | 39 |
| *Bufo bufo* | 0.506 | 1.214 | 0.745 | 0.960 | | 0.150 | 1.117 | 0.447 | 0 | 29 |
| *Bufo calamita* | 4.575 | 3.128 | 0.559 | 0.053 | | 0.577 | | | 12.5 | 35 |
| *Chioglossa lusitanica* | | | | | | | | 0.009 | 75 | 51 |
| *Discoglossus galganoi* | 0.202 | 0.117 | | | | 0.085 | | | 37.5 | 50 |
| *Hyla arborea* | 0.344 | | | | | 0.021 | 0.013 | | 12.5 | 38 |
| *Hyla meridionales* | | 0.041 | | | | | | | 12.5 | 48 |
| *Pelobates cultripes* | 6.235 | 2.188 | 0.518 | 0.027 | | 0.064 | | | 12.5 | 44 |
| *Pelodytes sp.* | | 0.007 | | | | | | | 12.5 | 43 |
| *Pleurodeles waltl* | 1.174 | 0.727 | 0.352 | 0.053 | | 0.321 | | | 0 | 44 |
| *Rana iberica* | | | | | | | | 0.017 | 12.5 | 51 |
| *Rana perezi* | 0.364 | 0.034 | 0.021 | | | 0.064 | 0.051 | | 0 | 41 |
| *Salamandra salamandra* | 0.405 | 1.077 | 0.186 | 0.053 | 0.008 | 0.321 | 0.025 | 0.309 | 0 | 40 |
| *Triturus boscai* | 0.121 | | | | | 0.064 | | 0.009 | 0 | 39 |
| *Triturus marmoratus* | 0.729 | 0.075 | 0.021 | | | 0.427 | 0.013 | 0.009 | 12.5 | 33 |
| **Reptiles** | | | | | | | | | | |
| *Anguis fragilis* | | | | | | | 0.076 | 0.146 | 0 | 43 |
| *Chalcides striatus* | | | 0.021 | | | | | | 0 | 49 |
| *Coluber hippocrepis* | | 0.048 | 0.021 | 0.027 | | | | | 12.5 | 68 |
| *Coronella austriaca* | | | | | | | 0.025 | 0.017 | 50 | 63 |
| *Coronella girondica* | 0.040 | 0.007 | | | | 0.021 | 0.013 | 0.043 | 0 | 63 |
| *Elaphe scalaris* | 0.040 | 0.316 | 0.041 | 0.080 | 0.108 | 0.085 | | 0.026 | 0 | 64 |
| *Lacerta lepida* | 0.040 | 0.144 | | 0.027 | | 0.043 | 0.063 | 0.103 | 0 | 51 |
| *Lacerta schreiberi* | | | | | | | 0.038 | 0.043 | 37.5 | 58 |
| *Malpolon monspessulanus* | 0.142 | 0.192 | 0.145 | 0.053 | 0.317 | 0.043 | | 0.026 | 0 | 56 |
| *Mauremys leprosa* | | 0.082 | 0.083 | 0.027 | | | | | 37.5 | 61 |

| Species | Sampled roads | | | | | | | | TSI | BGI |
|---|---|---|---|---|---|---|---|---|---|---|
| | SND | PRT | VNV | SMN | ALC | BPS | MNT | GER | | |
| *Natrix maura* | 0.101 | 0.110 | 0.021 | | | 0.021 | 0.127 | 0.112 | 0 | 48 |
| *Natrix natrix* | 0.020 | 0.007 | 0.083 | 0.267 | 0.017 | | 0.025 | 0.052 | 0 | 51 |
| *Podarcis bocagei* | | | | | | | 0.013 | 0.112 | 0 | 54 |
| *Podarcis hispanica* | 0.020 | | | | | | | | 0 | 41 |
| *Psammodromus algirus* | 0.020 | 0.062 | 0.021 | 0.053 | 0.008 | 0.021 | | 0.026 | 25 | 43 |
| *Vipera latastei* | | | | | | | | 0.155 | 37.5 | 71 |
| *Vipera seoanei* | | | | | | | 0.051 | | 87.5 | 90 |
| **Birds** | | | | | | | | | | |
| *Acrocephalus arundinaceus* | | | | | 0.017 | | | | 0 | 46 |
| *Acrocephalus scirpaceus* | | | | | 0.017 | | | | 37.5 | 41 |
| *Aegithalos caudatus* | | | 0.021 | | | | | | 0 | 30 |
| *Alauda arvensis* | | | | | 0.017 | | | | 0 | 40 |
| *Alcedo atthis* | | 0.007 | | | 0.008 | | | | 25 | 45 |
| *Alectoris rufa* | | 0.014 | | | | | | | 0 | 39 |
| *Anthus pratensis* | | | | | 0.025 | | | | 0 | 38 |
| *Athene noctua* | | 0.055 | 0.021 | 0.133 | 0.067 | | | | 0 | 50 |
| *Bubulcus ibis* | | 0.007 | 0.021 | | 0.017 | | | | 0 | 53 |
| *Caprimulgus europaeus* | 0.020 | | | | 0.008 | | | | 62.5 | 49 |
| *Caprimulgus ruficollis* | | | | | 0.033 | | | | 37.5 | 51 |
| *Carduelis cannabina* | 0.020 | 0.014 | | | | | | | 0 | 28 |
| *Carduelis carduelis* | 0.040 | 0.103 | 0.518 | 0.187 | 0.175 | | | | 0 | 28 |
| *Carduelis chloris* | | 0.021 | | 0.053 | 0.025 | 0.021 | | | 0 | 28 |
| *Carduelis spinus* | | 0.007 | | | | | | | 0 | 24 |
| *Certhia brachydactyla* | | | | | | | | 0.009 | 0 | 33 |
| *Ciconia ciconia* | | 0.007 | | 0.027 | | | | | 25 | 61 |
| *Cisticola juncidis* | | 0.055 | 0.021 | 0.027 | 0.050 | | | | 0 | 36 |
| *Clamator glandarius* | | | 0.021 | | | | | | 37.5 | 53 |
| *Columba palumbus* | | | | | 0.008 | | | | 0 | 40 |
| *Corvus corone* | | | | | 0.008 | | | | 0 | 44 |
| *Delichon urbica* | | | 0.021 | 0.027 | 0.017 | | | | 0 | 34 |
| *Elanus caeruleus* | | 0.014 | 0.021 | | | | | | 50 | 74 |

| Species | Sampled roads | | | | | | | | TSI | BGI |
|---|---|---|---|---|---|---|---|---|---|---|
| | SND | PRT | VNV | SMN | ALC | BPS | MNT | GER | | |
| *Emberiza cia* | | | | | | | 0.038 | | 0 | 34 |
| *Emberiza cirlus* | 0.020 | | | | | | | | 0 | 40 |
| *Erithacus rubecula* | 0.182 | 0.213 | 0.166 | 0.240 | 0.158 | | 0.013 | | 0 | 38 |
| *Estrilda astrild* | | | | | 0.017 | | | | 0 | 32 |
| *Falco tinnunculus* | | 0.007 | | | | | | | 0 | 50 |
| *Ficedula hypoleuca* | 0.202 | 0.014 | | | | 0.021 | 0.013 | | 0 | 48 |
| *Fringilla coelebs* | 0.020 | 0.034 | 0.021 | | | | | | 0 | 33 |
| *Galerida cristata* | | 0.007 | | | | | | | 0 | 37 |
| *Gallinula chloropus* | | | | | 0.067 | | | | 0 | 42 |
| *Hirundo daurica* | | 0.075 | | 0.027 | | | | | 0 | 35 |
| *Hirundo rustica* | 0.061 | | | | 0.083 | | 0.025 | | 0 | 34 |
| *Lanius collurio* | | | | | | | 0.051 | | 62.5 | 67 |
| *Lanius meridionalis* | | 0.021 | | 0.027 | 0.017 | | | | 0 | 43 |
| *Lanius senator* | 0.020 | 0.096 | | 0.080 | 0.025 | | 0.013 | | 25 | 34 |
| *Locustella luscinioides* | | | | | 0.008 | | | | 37.5 | 59 |
| *Lullula arborea* | 0.020 | | | | | | | | 25 | 34 |
| *Luscinia megarhynchos* | | | | | | 0.021 | | | 0 | 34 |
| *Miliaria calandra* | | 0.309 | 0.104 | 0.080 | 0.042 | | | | 0 | 36 |
| *Motacilla alba* | 0.020 | 0.007 | | | 0.017 | | 0.013 | | 0 | 35 |
| *Motacilla flava* | | | | | | | 0.038 | | 0 | 44 |
| *Muscicapa striata* | | 0.007 | 0.021 | | | | | | 25 | 54 |
| *Parus caeruleus* | 0.101 | 0.165 | 0.373 | 0.187 | 0.017 | | 0.013 | | 0 | 33 |
| *Parus major* | | 0.027 | 0.021 | 0.080 | 0.050 | | | 0.009 | 0 | 35 |
| *Passer domesticus* | 0.688 | 0.549 | 0.559 | 0.293 | 0.658 | 0.064 | 0.038 | | 0 | 34 |
| *Passer hispaniolensis* | | 0.007 | | | | | | | 0 | 44 |
| *Passer montanus* | | | | 0.027 | | | | | 0 | 34 |
| *Petronia petronia* | | 0.007 | | | | | | | 0 | 40 |
| *Phoenicurus ochruros* | 0.040 | 0.007 | | | | | 0.013 | | 0 | 38 |
| *Phylloscopus collybita* | 0.020 | 0.226 | | | 0.033 | | | | 0 | 33 |
| *Phylloscopus trochilus* | 0.101 | 0.021 | | | | | | | 0 | 39 |
| *Pica pica* | | 0.007 | 0.021 | 0.080 | | | | | 0 | 42 |

| Species | Sampled roads | | | | | | | | TSI | BGI |
|---|---|---|---|---|---|---|---|---|---|---|
| | SND | PRT | VNV | SMN | ALC | BPS | MNT | GER | | |
| *Saxicola torquata* | 0.040 | 0.281 | 0.186 | 0.293 | 0.117 | | | | 0 | 35 |
| *Serinus serinus* | | 0.219 | 0.124 | 0.053 | 0.025 | | | | 0 | 31 |
| *Strix aluco* | | 0.137 | 0.124 | | 0.083 | | | | 0 | 56 |
| *Sturnus unicolor* | | | | | | | 0.013 | | 0 | 43 |
| *Sylvia atricapilla* | 0.061 | 0.254 | 0.228 | 0.160 | 0.050 | | | 0.026 | 0 | 38 |
| *Sylvia cantillans* | 0.101 | | | | | 0.021 | | | 0 | 38 |
| *Sylvia communis* | | | | | | | 0.013 | | 0 | 46 |
| *Sylvia melanocephala* | 0.020 | 0.226 | 0.455 | 0.293 | 0.125 | 0.021 | | | 0 | 38 |
| *Sylvia undata* | 0.142 | 0.178 | | | | | | | 25 | 36 |
| *Troglodytes troglodytes* | | 0.014 | | | 0.008 | | 0.025 | | 0 | 30 |
| *Turdus merula* | 0.040 | 0.041 | 0.041 | 0.160 | 0.017 | 0.021 | 0.025 | | 0 | 40 |
| *Turdus philomelos* | | 0.014 | | | | | | | 25 | 41 |
| *Tyto alba* | | 0.185 | 0.435 | 0.213 | 0.475 | | | | 0 | 53 |
| *Vanellus vanellus* | | | | | 0.017 | | | | 0 | 44 |
| **Mammals** | | | | | | | | | | |
| *Apodemus sylvaticus* | 0.061 | 0.254 | 0.290 | 0.320 | 0.033 | | 0.013 | 0.017 | 0 | 23 |
| *Barbastella barbastellus* | | | | | | | | 0.009 | 50 | 68 |
| *Crocidura russula* | 0.020 | 0.041 | 0.041 | | | | | | 0 | 33 |
| *Erinaceus europaeus* | 0.020 | 0.130 | 1.304 | 0.800 | 0.208 | | 0.038 | 0.026 | 0 | 44 |
| *Felis silvestris* | | | | | 0.008 | | | | 50 | 74 |
| *Genetta genetta* | | 0.034 | 0.062 | 0.027 | 0.025 | 0.021 | | | 0 | 53 |
| *Herpestes ichneumon* | | 0.014 | 0.021 | | 0.033 | | | | 0 | 61 |
| *Lepus granatensis* | | 0.075 | 0.021 | 0.027 | 0.083 | | | | 0 | 47 |
| *Lutra lutra* | | | | | 0.008 | | | | 37.5 | 60 |
| *Martes foina* | | 0.007 | 0.021 | 0.053 | | | | | 0 | 58 |
| *Meles meles* | | 0.034 | 0.021 | | | | | | 0 | 49 |
| *Microtus cabrerae* | | 0.021 | | | | | | | 75 | 44 |
| *Microtus duodecimcostatus* | | 0.021 | 0.021 | 0.053 | | | | | 0 | 36 |
| *Mus musculus* | 0.081 | | | | | | | | 0 | 20 |
| *Mus spretus* | | 0.062 | 0.021 | | | | | | 0 | 27 |
| *Mustela nivalis* | | | 0.021 | | 0.025 | | | | 0 | 40 |

| Species | Sampled roads | | | | | | | | TSI | BGI |
|---|---|---|---|---|---|---|---|---|---|---|
| | SND | PRT | VNV | SMN | ALC | BPS | MNT | GER | | |
| *Mustela putorius* | | 0.027 | 0.145 | 0.027 | 0.008 | | | | 12.5 | 74 |
| *Oryctolagus cuniculus* | | 0.082 | 0.352 | 0.027 | 0.167 | | 0.013 | | 25 | 30 |
| *Pipistrellus kuhli* | | 0.048 | | | | | | | 12.5 | 47 |
| *Pipistrellus pipistrellus/P.pygmeus* | | 0.034 | | | | | | | 12.5 | 46 |
| *Plecotus austriacus* | 0.040 | | | | | | | | 12.5 | 52 |
| *Rattus norvegicus* | | 0.014 | 0.062 | | | | | | 0 | 31 |
| *Rattus rattus* | | | 0.248 | 0.053 | 0.642 | | | | 0 | 34 |
| *Rhinolophus ferrumequinum* | | 0.007 | | | | | | | 75 | 54 |
| *Rhinolophus hipposideros* | 0.020 | | | | | 0.021 | | | 75 | 44 |
| *Sciurus vulgaris* | | | | | | | | 0.009 | 0 | 45 |
| *Talpa occidentalis* | 0.040 | 0.014 | 0.021 | | | | | 0.017 | 0 | 55 |
| *Vulpes vulpes* | 0.020 | 0.069 | 0.083 | 0.080 | 0.025 | 0.021 | | | 0 | 39 |

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Table 1. Sampled roads characteristics and sampling effort for quantifying wildlife road mortality in Portugal.

| *Road name* | *Code* | *Sampling period* | *Sampling extension (km)* | *Total distance sampled (km)* | *Median number of samples by month* |
|---|---|---|---|---|---|
| Alcochete | ALC | 10/1991 – 11/1992 | 20.0 | 1200 | 4 |
| Bemposta | BPS | 01/2001 – 12/2001 | 18.0 | 468 | 2 |
| Gerês | GER | 03/2000 – 11/2001* | 12.0 | 1344 | 6 |
| Montalegre | MNT | 05/2000 – 11/2001** | 12.5 | 788 | 3.5 |
| Portalegre | PRT | 03/1995 – 03/1997 | 27.0 | 1458 | 2 |
| S.Manços | SMN | 01/2000 – 07/2000 | 25.0 | 375 | 2 |
| Sendim | SND | 01/2001 – 12/2001 | 19.0 | 494 | 2 |
| Vendas Novas | VNV | 08/1999 – 07/2000 | 23.0 | 483 | 2 |

* - *No sampling between 12/2000 and 02/2001*

** - *No sampling between 10/2000 and 12/2000*

Table 2A. Variables and scores of the conservation index used for ranking species according to **Threaten status** (see methods section for details). *non detected species was categorized as *Critically Endangered*. This class is here included symbolically as a suggestion for further works.

| Variable | Category | Score |
|---|---|---|
| Portuguese Red Data Book status | Critically Endangered* | 200* |
| | Endangered (EN) | 100 |
| | Vulnerable (VU) | 75 |
| | Near Threatened (NT) | 50 |
| | Least Concern (LC) | 0 |
| | Data Deficient and Not Evaluated | 25 |
| European status (H -Habitats Directive; B – Birds Directive) | Priority species | 100 |
| | Species listed simultaneously in annexes HII and HIV | 75 |
| | Species listed in annexes HII or BI | 50 |
| | Species listed in annexes BIV or BII | 25 |
| | Species not listed in none of the annexes cited | 0 |

Table 2B. Variables and scores of the conservation index used for ranking species according to **Biological and Biogeographical status** (see methods section for details).

| Variable | Category | Score |
|---|---|---|
| ***Biological status*** | | |
| Population size | Less than 2500 mature individuals | 100 |
| | More than 2500 mature individuals | 0 |
| Phenology | Resident | 100 |
| | Breeding | 75 |
| | Wintering | 50 |
| | Migratory | 25 |
| | Occasional | 0 |
| Annual fecundity | Less than 3 offspring/year | 100 |
| | 3 to 5 offspring/year | 80 |
| | 5 to 10 offspring/year | 60 |
| | 10 to 15 offspring/year | 40 |
| | 15 to 100 offspring/year | 20 |
| | More than 100 offspring/year | 0 |
| Female age at sexual maturation | More than 5 years | 100 |
| | 3.5 to 5 years | 80 |
| | 1.5 to 3.5 years | 60 |
| | 1.0 to 1.5 years | 40 |
| | 0.2 to 1.0 years | 20 |
| | Less than 0.2 years | 0 |
| Trophic specialization | Secondary consumers (insectivorous and carnivores) | 100 |
| | Omnivorous | 50 |

| Variable | Category | Score |
| --- | --- | --- |
| | Primary consumers (herbivorous and frugivorous) | 0 |
| Individual body mass | More than 500 g | 100 |
| | 100 to 500 g | 80 |
| | 30 to 100 g | 60 |
| | 15 to 30 g | 40 |
| | 10 to 15 g | 20 |
| | Less than 10 g | 0 |
| ***Biogeographic status*** | | |
| Global distribution area | Iberian endemism | 100 |
| | 2/3 of the distribution area inside the Iberian peninsula | 75 |
| | Western Palearctic species (Europe, North Africa and Middle East) | 25 |
| | Cosmopolitan species | 0 |
| Portuguese distribution area | Less than 30% | 100 |
| | 31 to 60% | 50 |
| | More than 60% | 0 |

Table 3. Total number of observations and number of species by vertebrate class, by most frequently road-killed species *29M*, and by highly ranked priority conservation species *29TS*and *29BG* (see methods section for details).

| *Vertebrate class* | *Total number of run-over specimens (%)* | *Total number of run-over species (%)* | *Number of 29M species (%)* | *Number of 29TS species (%)* | *Number of 29BG species (%)* |
|---|---|---|---|---|---|
| Amphibians | 2540 (46.9) | 16 (12.4) | 9 | 2 | 0 |
| Reptiles | 401 (7.4) | 17 (13.2) | 5 | 6 | 10 |
| Birds | 1691 (31.2) | 68 (52.7) | 11 | 14 | 9 |
| Mammals | 782 (14.4) | 28 (21.7) | 4 | 7 | 10 |
| Total | 5414 | 129 | 29 | 29 | 29 |

Table 4. Most 29 highly ranked species according to its mortality index (MI) (table 4a); according to its Threaten status Index (TSI) (table 4b); and according to its Biological and Biogeographical Status Index (BGI) (table 4c). (See methods section for details). Species common to other rank: § - common to MI and TSI or BGI; £ - common to RBI and BGI

**TABLE 4A**

| Class | Species | MI | TSI | BGI |
|---|---|---|---|---|
| Amphibians | *Pelobates cultripes* | 9.031 | 12.5 | 44 |
| Amphibians | *Bufo calamita* | 8.892 | 12.5 | 35 |
| Amphibians | *Bufo bufo* | 5.138 | 0 | 29 |
| Birds | *Passer domesticus* | 2.850 | 0 | 34 |
| Amphibians | *Pleurodeles waltl* | 2.627 | 0 | 44 |
| Mammals | *Erinaceus europaeus* | 2.527 | 0 | 44 |
| Amphibians | *Salamandra salamandra* | 2.385 | 0 | 40 |
| Birds | *Tyto alba* § | 1.308 | 0 | 53 |
| Amphibians | *Triturus marmoratus* | 1.274 | 12.5 | 33 |
| Birds | *Sylvia melanocephala* | 1.142 | 0 | 38 |
| Birds | *Carduelis carduelis* | 1.023 | 0 | 28 |
| Mammals | *Apodemus sylvaticus* | 0.988 | 0 | 23 |

**TABLE 4A**

| | | | | |
|---|---|---|---|---|
| Birds | *Erithacus rubecula* | 0.971 | 0 | 38 |
| Mammals | *Rattus rattus* | 0.943 | 0 | 34 |
| Birds | *Saxicola torquata* | 0.918 | 0 | 35 |
| Reptiles | *Malpolon monspessulanus* [§] | 0.917 | 0 | 56 |
| Birds | *Parus caeruleus* | 0.855 | 0 | 33 |
| Birds | *Sylvia atricapilla* | 0.778 | 0 | 38 |
| Reptiles | *Elaphe scalaris*[§] | 0.697 | 0 | 64 |
| Mammals | *Oryctolagus cuniculus* [§] | 0.640 | 25 | 30 |
| Amphibians | *Rana perezi* | 0.534 | 0 | 41 |
| Birds | *Miliaria calandra* | 0.534 | 0 | 36 |
| Reptiles | *Natrix maura* | 0.492 | 0 | 48 |
| Reptiles | *Natrix natrix* | 0.470 | 0 | 51 |
| Birds | *Serinus serinus* | 0.422 | 0 | 31 |
| Reptiles | *Lacerta lepida* | 0.420 | 0 | 51 |
| Amphibians | *Discoglossus galganoi* [§] | 0.404 | 37.5 | 50 |
| Amphibians | *Hyla arborea* | 0.378 | 12.5 | 38 |
| Birds | *Turdus merula* | 0.346 | 0 | 40 |

**Table 4b**

| Class | Species | MI | TSI | BGI |
|---|---|---|---|---|
| Reptiles | *Vipera seoanei* [£] | 0.051 | 87.5 | 90 |
| Mammals | *Rhinolophus hipposideros* | 0.042 | 75 | 44 |
| Mammals | *Microtus cabrerae* | 0.021 | 75 | 44 |
| Amphibians | *Chioglossa lusitanica* | 0.009 | 75 | 51 |
| Mammals | *Rhinolophus ferrumequinum* [£] | 0.007 | 75 | 54 |
| Birds | *Lanius collurio* [£] | 0.051 | 62.5 | 67 |
| Birds | *Caprimulgus europaeus* | 0.029 | 62.5 | 49 |
| Reptiles | *Coronella austriaca* [£] | 0.043 | 50 | 63 |
| Birds | *Elanus caeruleus* [£] | 0.034 | 50 | 74 |
| Mammals | *Barbastella barbastellus* [£] | 0.009 | 50 | 68 |

**Table 4b**

| | | | | |
|---|---|---|---|---|
| Mammals | *Felis silvestris* [£] | 0.008 | 50 | 74 |
| Amphibians | *Discoglossus galganoi* [§] | 0.404 | 37.5 | 50 |
| Reptiles | *Mauremys leprosa* [£] | 0.192 | 37.5 | 61 |
| Reptiles | *Vipera latastei* [£] | 0.155 | 37.5 | 71 |
| Reptiles | *Lacerta schreiberi* [£] | 0.081 | 37.5 | 58 |
| Birds | *Caprimulgus ruficollis* | 0.033 | 37.5 | 51 |
| Birds | *Clamator glandarius* [£] | 0.021 | 37.5 | 53 |
| Birds | *Acrocephalus scirpaceus* | 0.017 | 37.5 | 41 |
| Mammals | *Lutra lutra* [£] | 0.008 | 37.5 | 60 |
| Birds | *Locustella luscinioides* [£] | 0.008 | 37.5 | 59 |
| Mammals | *Oryctolagus cuniculus* [§] | 0.640 | 25 | 30 |
| Birds | *Sylvia undata* | 0.320 | 25 | 36 |
| Birds | *Lanius senator* | 0.234 | 25 | 34 |
| Reptiles | *Psammodromus algirus* | 0.211 | 25 | 43 |
| Birds | *Ciconia ciconia* [£] | 0.034 | 25 | 61 |
| Birds | *Muscicapa striata* [£] | 0.028 | 25 | 54 |
| Birds | *Lullula arborea* | 0.020 | 25 | 34 |
| Birds | *Alcedo atthis* | 0.015 | 25 | 45 |
| Birds | *Turdus philomelos* | 0.014 | 25 | 41 |

**Table 4c**

| Class | Species | MI | TSI | BGI |
|---|---|---|---|---|
| Reptiles | *Vipera seoanei* [£] | 0.051 | 87.5 | 90 |
| Mammals | *Felis silvestris* [£] | 0.008 | 50 | 74 |
| Mammals | *Mustela putorius* | 0.207 | 12.5 | 74 |
| Birds | *Elanus caeruleus* [£] | 0.034 | 50 | 74 |
| Reptiles | *Vipera latastei* [£] | 0.155 | 37.5 | 71 |
| Mammals | *Barbastella barbastellus* [£] | 0.009 | 50 | 68 |
| Reptiles | *Coluber hippocrepis* | 0.095 | 12.5 | 68 |

**Table 4c**

| | | | | |
|---|---|---|---|---|
| Birds | *Lanius collurio* [£] | 0.051 | 62.5 | 67 |
| Reptiles | *Elaphe scalaris* [§] | 0.697 | 0 | 64 |
| Reptiles | *Coronella austriaca* [£] | 0.043 | 50 | 63 |
| Reptiles | *Coronella girondica* | 0.124 | 0 | 63 |
| Reptiles | *Mauremys leprosa* [£] | 0.192 | 37.5 | 61 |
| Mammals | *Herpestes ichneumon* | 0.068 | 0 | 61 |
| Birds | *Ciconia ciconia* [£] | 0.034 | 25 | 61 |
| Mammals | *Lutra lutra* [£] | 0.008 | 37.5 | 60 |
| Birds | *Locustella luscinioides* [£] | 0.008 | 37.5 | 59 |
| Reptiles | *Lacerta schreiberi* [£] | 0.081 | 37.5 | 58 |
| Mammals | *Martes foina* | 0.081 | 0 | 58 |
| Reptiles | *Malpolon monspessulanus* [§] | 0.917 | 0 | 56 |
| Birds | *Strix aluco* | 0.345 | 0 | 56 |
| Mammals | *Talpa occidentalis* | 0.092 | 0 | 55 |
| Mammals | *Rhinolophus ferrumequinum* [£] | 0.007 | 75 | 54 |
| Birds | *Muscicapa striata* [£] | 0.028 | 25 | 54 |
| Reptiles | *Podarcis bocagei* | 0.124 | 0 | 54 |
| Birds | *Clamator glandarius* [£] | 0.021 | 37.5 | 53 |
| Birds | *Tyto alba* [§] | 1.308 | 0 | 53 |
| Birds | *Bubulcus ibis* | 0.044 | 0 | 53 |
| Mammals | *Genetta genetta* | 0.169 | 0 | 53 |
| Mammals | *Plecotus austriacus* | 0.040 | 12.5 | 52 |

*Figure 1. Geographic location of sampled roads (see Table 1 for the names of the roads and sampling effort). Grey scale stand for altitude.*

*Figure 2. Mortality indexes by road of the (A) 29 most frequently road-killed species and of the 29 most highly ranked priority conservation species concerning (B)* Threaten status Index *and (C) the* Biological and Biogeographical Status Index *(see text for methodology, Table 1 for the names of the roads and figure 1 for the geographic location of roads).*

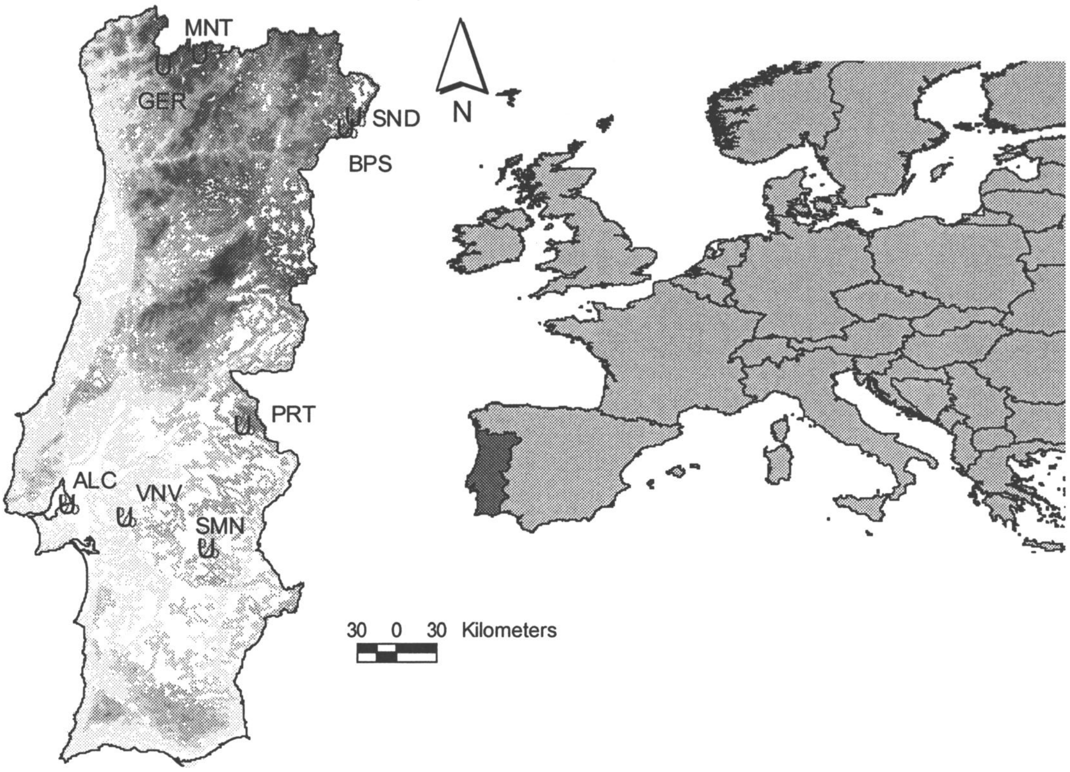


Figure 1

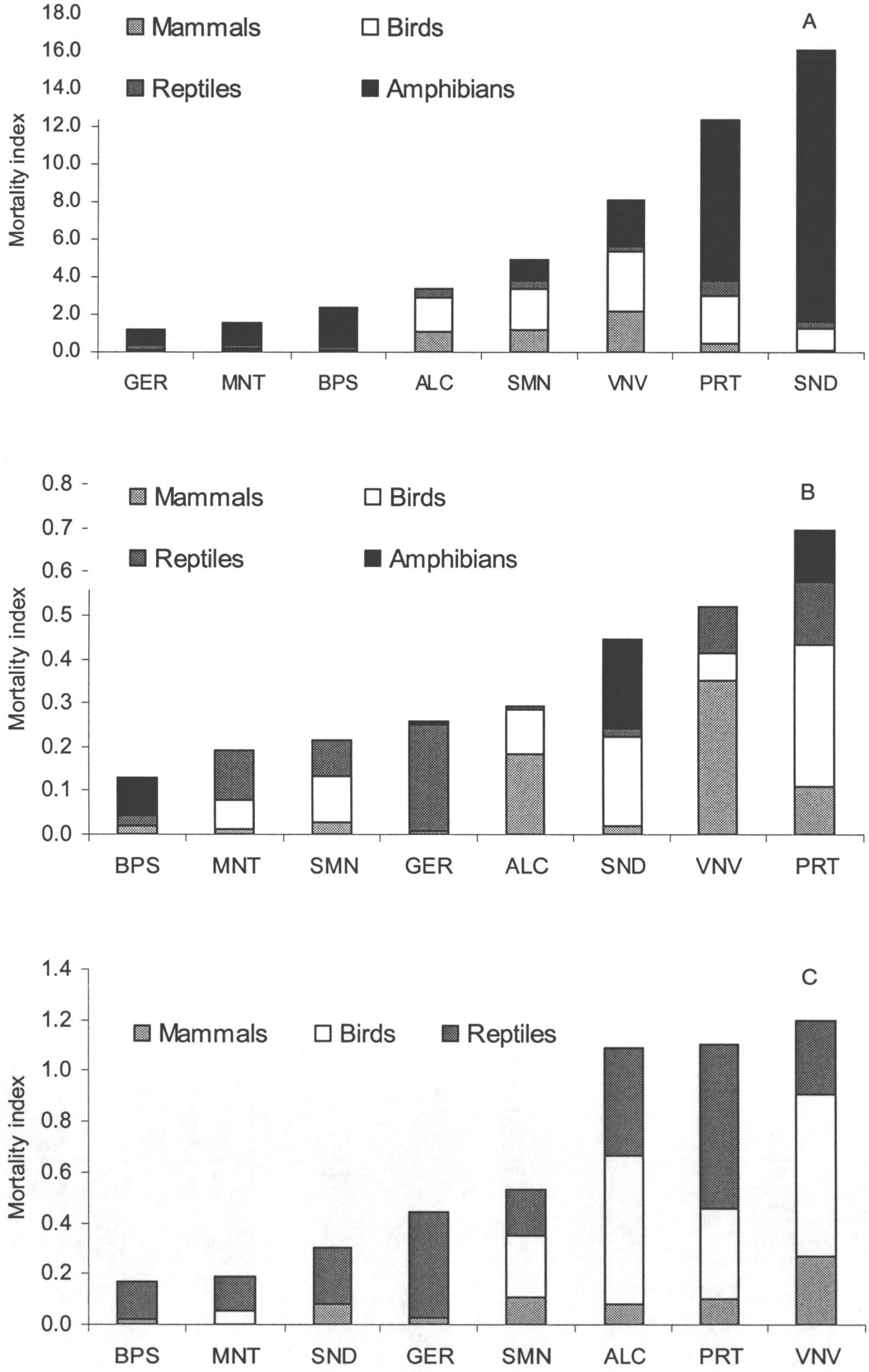
A
Mammals
Birds
Reptiles
Amphibians
Mortality index
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
GER MNT BPS ALC SMN VNV PRT SND
B
Mammals
Birds
Reptiles
Amphibians
Mortality index
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
BPS MNT SMN GER ALC SND VNV PRT
C
Mammals
Birds
Reptiles
Mortality index
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
BPS MNT SND GER SMN ALC PRT VNV

Figure 2

# FACTORS AFFECTING CULVERT USE BY VERTEBRATES IN TWO ROADS OF SOUTHERN PORTUGAL

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**ABSTRACT**

A major target for conservationists when trying to minimize the road-barrier effect on wildlife is to improve permeability to animal movements. Previous works have demonstrated that drainage culverts are used by vertebrates, although the knowledge of the main influencing factors is still scarce.

The use of 34 culverts from two roads of southern Portugal, differing in traffic volume, vehicle speeds and configuration, was evaluated by analysis of terrestrial vertebrate footprint data (408 passage-operative days). Culvert crossings were related to explanatory variables, concerning passage's *design* and *location* attributes, through canonical ordination techniques.

901 crossings were recorded, corresponding to an average of 2.2 crossings/culvert/operative day. Thirteen taxa were regularly detected which included reptiles, small mammals, lagomorphs, carnivores and domestic species. Our results suggest that fencing might be having a funneling effect, conducting larger animals toward culverts. Also, vegetation covering the culvert entrances seemed to have a positive effect, namely on genet; longer passages with entrances far from the pavement are apparently avoided by small sized animals; lower number of crossings was detected on passages with detritus pits; closest passages to urban areas are more used by domestic species; forestry species favoured passages with low open land cover nearby; and higher passage densities apparently increase culvert use by small sized species. Although not used by all species present in studied area, the implementation of numerous passages with different sizes without detritus pits and distributed along the roads might be an important step to mitigate road fragmentation effects on animal populations.

**Keywords** – Road ecology, culvert use, conservation, canonical ordination; vertebrates

## INTRODUCTION

Civilization has always interacted with the landscape, exploring natural resources and settling human communities in a vast range of different regions worldwide. As a consequence, humankind promoted the artificial landscape fragmentation on significant areas, particularly during the last century. This humanized landscape fragmentation is now recognized as one of the major threats to biodiversity sustainability (Saunders et al. 1991; Forman 1995). One major human agent of habitat fragmentation is the ever increasing and expanding road network world wide (Forman et al. 2002) which can be harmful to various faunal groups, such as invertebrates (e.g.: Haskell 2001), amphibians (e.g.: Carr and Fahrig 2001), reptiles (e.g.: Gibbs and Shriver 2002), birds (e.g.: Kuitunen et al. 1998) or mammals (e.g.: Philcox et al. 1999). Roads and traffic can act as barriers which difficult animal movements and reduces population connectivity, diminishing gene flow and disrupting sink and source population dynamics with consequent inbreeding and loss of genetic diversity (Ferreras 2001). Resultant isolation might increase population local extinction risks due to stochastic effects (van der Zande et al. 1980; Saunders et al. 1991; Fahrig and Merrian 1994; Cooper and Walters 2002). Roads also promote high animal-vehicle collision risks, particularly significant for larger species with wider home ranges as carnivores, being one of the most visible road impacts on wildlife (e.g.: Hodson 1960; Oxley et al. 1974; Fahrig et al. 1995; Philcox et al. 1999; Gibbs and Shriver 2002; Taylor and Goldingay 2004). All works provided clear evidence that a wide range of species may be affected by this threat, which can lead to local population extinction (Fahrig et al. 1995; Jones 2000). Additionally, roads can inhibit crossings by affecting animal behavior (Mader 1984, Goosem 2001), increasing this way the barrier effect. For a wider revision of road effects of on wildlife consult: Forman and Alexander (1998), Spellerberg (1998), Trombulak and Frissel (2000), Seiler (2001) or Luell et al. (2003).

Therefore, a major target for conservationists when trying to minimize negative effects of roads on wildlife is to improve road permeability to animal movements, in order to promote or establish contact between populations across both road sides. As referred by Forman (1995), a solution may lie in porous roadbeds with tunnels and underpasses. Specific wildlife passages were designed and incorporated on road planning and mitigation programs in numerous countries (Clevenger and Waltho 2000; Goosem et al. 2001; Keller 2002; Cain et al. 2003). However, specifically designed wildlife passages might be too expensive to build and implement in large scale. Although not designed for animal passage, previous works have demonstrated that drainage culverts are regularly used by vertebrates to cross roads (e.g.: Yanes et al. 1995; Rodríguez et al. 1996, 1997; Cain et al. 2003; Taylor and Goldingay 2003; Ng et al. 2004; Mata et al. 2005). These structures are widespread on every road and have a relative low cost when compared to specific wildlife passages. Nevertheless, the knowledge of the main factors driving the use of culverts by vertebrates is still scarce, particularly in Mediterranean environments (but see Yanes et al. 1995; Rodríguez et al. 1996; Mata et al. 2005).

The main goals of the present paper are: (i) to evaluate the extent of use of drainage culverts by vertebrates for road crossing, determining which taxa/species use them, on two different road categories; and (ii) determine the influence of culvert characteristics and landscape descriptors on vertebrate use of these structures.

## METHODS

### *Study area*

This study took place in two roads, 35km apart from each other, located in Alentejo, Southern Portugal (figure 1). The climate is Mediterranean with average rainfalls of 625 mm, minimum average temperatures of 9.9ºC and maximum average temperatures of 21.8ºC. Altitude lies between 100m and 400m a.s.l. Both roads vicinity is dominated by characteristic mediterranean agro-forestry areas, cork and holm oak tree stands (*Quercus suber* and *Q. rotundifolia*), hereafter referred as "montado"; open land as pastures, meadows or extensive agriculture (cereal, fodder); and olive groves.

The two roads have dissimilar traffic volumes, vehicle speeds and configuration. The first road section, hereafter named EN370, is a 16km national road section with very low traffic density (no official data available; personal observations); six meters width, without paved verges, and with two lanes along all its length. Except for cattle closure, there are no fences along the road. There are two small villages along this section: *Escoural,* in the extreme south and *Giesteira* in the middle of the studied section.

The second road section, hereafter named IP2, is a 30km main road section, having an annual average daily traffic volume of 5121 vehicles per day, from which close to 11% (562 vehicles) circulate on the night period (EP, Portuguese national road service, unpublished report from 2001). Paved section, including verges, varies between 12 and 20 meters width, with one lane for each direction or, where a high slope is present, two lanes on upper direction. Verges are bordered by progressive fences with 1.5m high on its entire length, which did not enclose any of the studied culvert entrances. There are three urban areas along this section: two villages, *S.Manços* and *M.Trigo*, and one small city, *Portel.* Human presence is higher along the road comparatively to EN370, but is restricted mainly to the vicinity of urban areas. There are eight upper passages and eight under passages for traffic crossing along this section, which connect to unpaved trails. Traffic crossing passages and border trails allowed us to reach both sides of the studied culverts without having to stop on the main road.

Albeit the large number of passages present along the studied road sections (52 culverts on EN370 and 74 on IP2), most culverts had no access to both entrances, particularly on IP2. Hence, culvert selection was basically dependent on degree of accessibility for surveys.

*Culvert use*

Culvert use was evaluated by analysis of animal footprints found inside the passages. Thin layers of marble dust, with less than 1cm thick and 60 to 100cm wide were placed inside each culvert, on both sides, near the entrance. 34 culverts (17 on each road) were sampled regularly. Sampling was carried out on three seasons (spring, summer and autumn of 2004). Each period consisted of four operative days. Each operative day represented two sampling days given that tracks were checked every second day. We considered an operative day when tracks could be read clearly, i.e., with no damage from water or wind flow. Presence-absence data for each species on each sampling day were used for estimates of crossing rates (CR). For each culvert, species CR were computed as the ratio between the number of days with species tracks and the number of operative days. CR varies between zero when no tracks are detected and one when taxa are detected in all operative days. This avoided problems of pseudo-replication associated with the repeating counting of tracks on the same passage. Species identification was restricted to mammals. Other vertebrate classes were also considered: reptiles: lizards and ophidians; small mammals not including hedgehog (*Erinaceus europaeus*): rats, mice, voles and shrews; lagomorphs: rabbit (*Orictolagus cuniculus*) and hare (*Lepus granatensis*); and wild carnivores not including domestic cat (*Felis catus*) and dog (*Canis familiaris*). Amphibians were excluded from analysis due to the low number of tracks recorded.

*Explanatory variables*

We evaluated the relation between culvert use and nine explanatory variables related to passage's *design* and *location* attributes (table 1). Culvert openness (CO) was derived from the formula culvert cross section area (CCS) / culvert length (CL) (adapted from Yanes et al. 1995). Detritus pits (DP) are designed to collect debris that may otherwise block the culverts, and are located at the entrance and below the bottom, across the full width of the culverts. None of the passages studied had detritus pits on both entrances.

Land cover surrounding each culvert was assessed for a radius of 300 meters through orthofotomap analysis (A.M.D.E. 2002/3), with corrections from field work observations. Although several classes of land cover could be identified along both road sections, we grouped them in four main types (open, olive groves, montado with shrubs and montado without shrubs), which covered about 95% of total circle areas (appendix 1).

The density of crossing structures along the road corridor may be one confounding variable when the main aim is to find the major characteristics of culvert and landscape that influence culvert use. As stated by Clevenger and Waltho (2005), high crossing rates might be a consequence of the non-existence of nearby passages, as well as lower use might be a result of higher densities of passages. Although these authors applied this correction on specific designed macro fauna passages, we also incorporated it in our study. We defined for each culvert the "Probability of use" ($P_n$), taking into account its distance to the nearby culverts, underpasses or flyovers: $P_n = (d_{n-1} + d_{n+1})*2 / L$, where $P_n$ is the absolute probability of an animal to use culvert $n$, $d_{n-1}$ is the distance from passage $n$-1 to passage $n$, $d_{n+1}$ is the distance from culvert $n$ to passage $n$+1 and $L$ is the road section length (adapted from Clevenger and Waltho 2005). According to the formula, passages have higher probability of use when they are more isolated from other crossing structures.

Road type (RT), that is EN370 or IP2, and Season (spring, summer and autumn) were considered nominal variables. All GIS data treatment was made using ArcView GIS 3.2 (ESRI, 1999).

*Data analysis*

We assumed that each studied culvert was independent from the others. However, this assumption may not verify always because some passages were less than 600 meters apart (n=9 on EN370 and n=5 on IP2), and therefore share adjoining landscape attributes. Also, different passages might have been visited by the same animals. Mean distance between studied culverts was 802 meters on EN370 and 1717 meters on IP2.

Differences on culvert use related to categorical variables were evaluated with Mann-Whitney U test (MW) or Kruskal-Wallis test (KW) (Zar 1999). For numerical variables we performed Spearman rank correlations ($r_s$). Non-parametric tests were preferred because of small sample size.

For multivariate analysis we used canonical ordination techniques. Detrended correspondence analysis (DCA) was performed to evaluate grouping of culverts from the two roads, with downweight of rare species and detrending by segments options (Jongman et al. 1995). A Direct gradient analysis (Canonical Correspondence Analysis - CCA) was then executed with crossing rates data and all spatial explanatory variables considered, except for land cover from which we select only open land (OP). This option was made due the strong correlation observed among land cover descriptors, and the achievement of a compromise of obtaining the maximum percentage of variance explained and the significance of both eigenvalues and correlations of species-explanatory variables with the axis. Significance of species-environment correlation was tested by the Monte Carlo test (499 permutations). Ordination axes were interpreted using the intraset correlations that allow inference on the relative importance of each variable for predicting community composition (ter Braak 1986). CANOCO for Windows version 4.5 (ter Braak and Šmilauer 2002) was used for DCA and CCA.

## RESULTS

### *Culvert usage*

During the study period, 901 vertebrate tracks denoting culvert crossings were recorded, corresponding to an average of 2.2 crossings per culvert per operative day. Of those, only 7 (0.8%) tracks were of amphibians, 32 (3.6%) were of reptiles, 289 (32.1%) were of small mammals, 55 (6.1%) were of hedgehog, 71 (7.9%) were of lagomorphs and 378 (42.0%) were of carnivores not including domestic cat and dog which accounted for 55 (6.1%) and 14 (1.6%) crossings, respectively. Stone marten (*Martes foina*) was the most common carnivore species accounting for 93 (10.3%) of total crossing records, followed by Egyptian mongoose (*Herpestes ichneumon*) (n=82, 9.1%), genet (*Genetta genetta*) (n=65, 7.2%), badger (*Meles meles*) (n=55, 6.1%), fox (*Vulpes vulpes*) (27, 3.0%), weasel (*Mustela nivalis*) (n=16, 1.8%) and otter (*Lutra lutra*) (n=2, 0.2%). Table 2 presents the results of culvert use by species/taxa known to have passed trough one or more passages, number of culverts used (N) and their average crossing rate (MCR), their standard deviation (SD) and maximum and minimum values.

Small mammals were detected in all passages and had the highest average CR for both roads. Several species used numerous passages, namely stone marten (n=28), Egyptian mongoose (n=21), genet (n=20) and domestic cat (n=18). Otter and weasel were the carnivorous detected with the lowest number of crossings (n=2 and n=9, respectively) being the former detected only on IP2.

Although mean CR of badger did not differ greatly among both roads, noteworthy results were detected on culvert use. In fact, this species was detected in only one passage of EN370, a small number when compared with the use of nine culverts on IP2. Moreover, the use of EN370 culvert was alike the average one observed for the nine culverts of IP2, that is, it was detected in more than one third of total operative days, and reached the highest average CR among carnivores for both roads, denoting a frequent and continuous use of this passage. Also fox was detected in only two EN370 passages, while in IP2 tracks were found on 10 passages. In contrast, hedgehog showed a generalized use of passages on EN370 (n=12), while on IP2 it crossed only three culverts.

*Seasonal variation*

Vertebrate crossings were mostly observed during spring (table 3). Culvert use did not differ significantly among seasons for all considered groups, except for small mammals (KW: $K$=14.784; $p<0.01$) which had the highest recordings on spring and summer. Carnivores had higher values in autumn and summer. Considering lagomorphs, highest CR were recorded on summer and lowest on autumn. Reptiles had the greatest seasonality in use of culverts, in opposition of carnivore group which was the one with lower variability.

*Influence of explanatory variables*

Significant differences on culvert use between the two roads were found for several *taxa*, namely small mammals (MW: $U$=73.0, $n_{1,2}$=17; $p<0.05$), hedgehog (MW: $U$=5.0, $n_{1,2}$=17; $p<0.10$), badger (MW: $U$=5.0, $n_{1,2}$=17; $p<0.01$), Egyptian mongoose (MW: $U$=81.0, $n_{1,2}$=17; $p<0.05$), fox (MW: $U$=74.5, $n_{1,2}$=17; $p<0.01$) and dog (MW: $U$=89.5, $n_{1,2}$=17; $p<0.05$), which represent 57.9% of overall records. For small mammals and hedgehog crossing rates were higher on EN370, while for remaining species higher values were observed on IP2. This differential species composition of the two roads is reflected in the DCA ordination results, presented in figure 2. The eigenvalues of DCA analysis were 0.292 in the first axis (horizontal) and 0.239 in the second axis (vertical). The plot shows a clear segregation of culverts, being IP2 culverts generally positioned on the upper right quadrant, opposed to EN370 passages. Also, species assemblages associated with each road seemed to be related with body size. In fact, all small taxa are gathered around EN370 culverts, while larger carnivores are positioned close to IP2 passages. Still, some IP2 culverts - passages number 19, 20, 31 and 34 - had similar composition to EN370 culverts, due its overall low use of carnivores. Conversely, passage number 10, from EN370, is embedded among the IP2 culverts. This passage had one of the highest overall values of carnivore crossings and was the only one used by both badger and fox, on this road.

The direct gradient analysis (CCA) results are shown in figure 3. The eigenvalues were 0.230 in the first axis and 0.186 in the second. Mont Carlo test was significant for both, first canonical axis ($F=5.415$, $P<001$) and all canonical axis ($F=2.753$, $P<001$). First two axes explained 63.2% of data variability. First axis reflects mainly the effects of vegetation covering the entrance, distance to urban areas, and the proportion of open land cover adjoining each culvert; while the second one reflects principally the culvert length, probability of use and road type effects (table 4). A noteworthy result is that, except for cat, all larger animals are once again apart from small taxa, placed on the upper quadrants of the plot, being genet, weasel, Egyptian mongoose and stone marten assembled on first quadrant, while badger, fox and dog are gathered on the fourth one.

Crossings were significantly different on culverts with vegetation covering the entrances (VC) for lagomorphs (MW: $U=41.5$, $n_1=13$, $n_2=21$; $p<0.01$), badger (MW: $U=49.0$, $n_1=13$, $n_2=21$; $p<0.01$), genet (MW: $U=70.0$, $n_1=13$, $n_2=21$; $p<0.05$), fox (MW: $U=70.5$, $n_1=13$, $n_2=21$; $p<0.01$) and dog (MW: $U=83.0$, $n_1=13$, $n_2=21$; $p<0.01$). Except for genet, all other taxa had higher crossing rates on culverts with no vegetation covering the entrances. Significant differences of CR were found on passages with detritus pits (DP) for hedgehog and lagomorphs (MW: $U=35.0$, $n_1=5$, $n_2=29$; $p<0.05$, for both). Hedgehog and lagomorphs, along with badger, weasel, otter, fox, cat and dog, were not detected on any of the five studied passages containing detritus pits. The passage with the deepest pit (passage number 34, 1.80m) was only visited by

Egyptian mongoose, once, and tracks orientation revealed that it did not climb the box walls. Albeit tracks from small mammals were regularly present on both entrances of this passage, it is unlikely that individuals could climb the pit walls, given its depth, resulting in unsuccessfully crossings.

Several significant correlations were obtained between culvert use and explanatory variables (table 5), although with noteworthy differences between groups. Concerning *culvert design* attributes, in a general way, opposite results were once more observed regarding animal body size. In fact, for small sized animals like small mammals and reptiles, shorter (CL) and less isolated (DFP) culverts seemed to be preferred for crossings, while for carnivores as genet and Egyptian mongoose, more tracks were detected on culverts with distant entrances from the pavement (DFP), a variable associated with culvert length (CL). Hedgehog seemed to have a preference on passages with higher cross section area (CCS) and openness (CO), which reflects its significant higher use of EN370 passages. As for lagomorphs, narrow passages (CCS) and located near the pavement (DFP) appeared to be favored for road crossing. Regarding culvert location attributes, typical forestry species, as genet and stone marten, seemed to avoid passages dominated by open land (OP) on road vicinity, in opposition to lagomorphs, fox and dog results. Closest passages to urban areas (DU) were favored only by domestic cat and hedgehog. Concerning the probability of use in relation to passage density ($P_n$), only hedgehog obtained a significant positive result, denoting that its use of culverts might be conditioned to number of passages present along the road. However, observing the CCA plot, again there seems to be a clear differentiation between species size, concerning this variable. In fact, small bodied taxa are all displayed on the positive segment of Pn, in opposition to all carnivores, suggesting that culvert use by less mobile species may be maximized when a high number of passages are present.

*Less used passages by carnivores*

Among the 34 studied passages, six had some considerable low use by carnivores (CR lower than 0.1), which might be a reflex of some inhibitor factor. Both first and last passages of EN370 are inside this group (passages number 1 and 17). The former is the closest passage to a main road and a highway, with heavier traffic volumes and disturbance, while the latter is the closest passage to urban areas (appendix 1). Also in this road, passage number 4 was used only by weasel. This is one of the narrowest passages, a factor that might restrain other corpulent animals to use it. On IP2, two passages had carnivore CR lower than 0.1, passages 19 and 34. The latter has the deepest detritus pits, already referred, while the former had one of its entrances partially obstructed by debris, which reduced considerably its openness, and probably its attractiveness to animal use.

## DISCUSSION

Our discussion is based on the assumption that crossing rates reflects the response of species/taxa to culvert, road and landscape features. However we keep in mind that different population abundances on road vicinity and variability among individual response to culverts may certainly had some influence on the results.

The results clearly support previous works (e.g.: Yanes et al. 1995; Rodriguez et al. 1996; Taylor and Goldingay 2003; Dodd et al 2004; Ng et al. 2004; Mata 2005) confirming that many species, including conservation concern species, regularly use culverts to cross roads. Moreover, and particularly for carnivores, the results suggest that animals incorporate culverts into their paths and movements regardless traffic levels. In fact, despite the low traffic intensity of EN370, culvert crossing rates were common in spite of the apparently low collision threat for larger species. During field work on EN370 only two stone marten road-killed were detected. On the other hand, higher carnivore crossings observed in IP2 might partially have resulted from a mixed effect of road fencing and higher traffic disturbance and collision risk. Road fencing may reinforce the barrier effect on the surrounding animal populations, and medium and large vertebrates are induced to use the transverse passages, as culverts, in order to cross roads (Mata et al. 2005). Several studies have demonstrated that fences placed along the roads, highly enough and correctly berried maximize the funnel effect on larger animals toward culverts (Foster 1995; Putman 1997; Clevenger et al. 2001; Cain et al. 2003). Although fences on IP2 verges were not properly berried in some points, and they do not constitute always an insurmountable obstacle, even for bigger species as fox or badger, it is likely that they induce in some extent the animal movement toward culverts. In fact, numerous carnivore tracks going along and parallel to fences were detected in several places during field work and, generally crossing rates for bigger species are higher on the fenced road (IP2). Also, higher traffic volumes might discourage attempts to cross the road and persuade animals to search alternative passageways. If this result is validated in future works, it assumes a major importance for road connectivity improvement actions, since it might maximize culvert use, and thus reduces animal-vehicle collisions. However, further investigation is needed in view of the fact that 33 carnivore specimens, all belonging to the identified species using the culverts, were found road-killed along IP2 study section during field work (authors, data unpublished). Moreover, only 11% of vehicles (n=562) circulate during the night period (8 hours), which represents roughly an average of one vehicle per minute. Apparently such low traffic density may represent a considerable risk of collision for carnivores. On IP2 there is a crossing structure on every 400m, a passage density that should be enough to allow a good permeability of this road to larger animals. This way, results suggest that culverts might not be entirely efficient in reducing road-kills and improving connectivity between road sides for all medium size species. On the other hand, road-kills may reflect the absence of more effective structures (vegetation or artificial) that promote animal movement towards culverts.

As stated by Clevenger and Waltho (2005) and citations therein, there have been mixed results concerning the relative importance of road and landscape attributes affecting culvert crossings. Most papers argue whether design or location is the most important element influencing animal response. Nevertheless, discrepancies in how animals react may certainly be explained by specific species dynamics (Clevenger and Waltho 2005). On our study, several significant effects were detected from the former two explanatory variables categories.

Regarding design attributes, vegetation covering the culvert entrances seemed to have a major effect on crossing rates, namely on genet. From the CCA plot we observe that many *taxa* are located on the positive segment of first axis, which had the highest correlation with this variable (table 4). Presence of cover near entrances may reduce distrust for such structures in some species, and thus maximize their use. Other authors found similar results concerning carnivores (Rodriguez et al. 1996) and small mammals (McDonald and St Clair 2004), but see Mata et al. (2005). However, as represented in CCA plot, species like badger, fox and lagomorphs seemed to prefer more clear passages.

Culvert physical attributes seemed to have considerable influence on animal use, being the longer passages apparently avoided by small sized animals, in opposition to most carnivores (figure 3, table 4). Smallest bodied species - reptiles, small mammals and lagomorphs - are all placed on the underside of the diagram suggesting that they preferred short passages with higher openness values. These three taxa had higher crossing rates on EN370, which has a narrow width of pavement. Therefore, the average length of its passages is smaller, increasing its culverts openness. Hence, this result suggests that longer passages might discourage these animals to use them. According to some authors (Mader 1984; Goosem 2001), some small mammals' species are inhibited to cross wider roads, an effect that might occur also when trying to cross longer culverts. Also, previous works described a high preference of small mammals for narrow passages (Yanes et al. 1995; Rodriguez et al. 1996; McDonald and St Clair 2004; Mata et al. 2005). Longer culverts usually have entrances further apart from the roadway, and so less conspicuous species as genet might prefer to use them. In fact, results from the variable distance from pavement (DFP) support this relation. Passages with greater underneath distance from the pavement, that is passages more isolated, had a significant less number of crossings from reptiles, small mammals and lagomorphs. Conversely, observing the CCA plot, these passages seemed to be preferred by carnivores as stone marten, genet and Egyptian mongoose, the latter two with significant correlations (table 5).

Lower number of crossings was detected on passages with detritus pits (DP). As most species can negotiate pits up to 1.5m, others will hardly transpose successfully such obstacles. Furthermore, the deepest studied pit was used only once by one Egyptian mongoose. These findings highlight the importance of design studies to improve the effectiveness of passages on animal movements, namely to prevent formation of obstacles that limit or inhibit such movements.

Regarding the second variable category, location of passage, the most important factor driving differences in species crossing rates seemed to be the road type. Culverts from the two roads had clear distinct species composition and use as expressed by the DCA plot (figure 2). As previously discussed, traffic levels and fencing effects might have had a major importance on species composition segregation. However, one should be aware that features as species population density, human disturbance, topography and many other external factors not considered in the present study may also have had significant influence on the results.

Culverts located far from urban areas were preferred by several taxa, most probably due lower human activity and disturbance nearby. Closest passages to urban areas seemed to be more used by anthropogenic species as domestic cat (table 5), as expressed in the CCA plot. Hence, implementation of mitigation measures should give priority to passages located far from these areas, given that it is more likely to benefit a great number of wild species.

Considering land cover, most species favored passages with low open land cover nearby. In fact, according to CCA plot, only lagomorphs, badger fox and dog were clearly on the positive segment of this variable. Probably, the former ones use the culverts as protection against predators, as raptors, while foraging on road verges. Also hares inhabit mostly open areas which may have influenced the results for the lagomorphs.

Regarding the probability of use ($P_n$) effect, we found that culvert density apparently influence in some extent its use, mainly by small sized species. Observing the CCA plot, culvert use by these taxa seemed to be positively influenced by culvert proximity, particularly for hedgehog, for which crossings were positively correlated with this variable (table 5). This result suggests that culvert use by small species might be relatively random, that is, individuals probably use passages when they found them on their foraging or dispersal movements, instead of actively seek for them.

All vertebrate groups showed seasonal variability on passage use, although significant differences were found only for small mammals, which had its lowest crossing rates in autumn. Same pattern was described by Yanes et al. (1995) which suggests a lesser use during high breeding activity. Lagomorphs also showed a seasonal pattern of use similar to the one observed by Yanes et al. (1995), with higher rates on summer. As for reptiles, their high use of culverts in this season may be a consequence of thermoregulatory activity rather than crossing movements (Rodriguez et al. 1996). During summer season we recorded mid-day temperature levels on ten different culverts, simultaneously inside the culvert and outside at shadow. Differences in temperature varied from 4.0ºC to 14.0ºC (mean=9.3±3.3). This supports evidence that culverts might function has refreshing spots for temperature regulation which might be favorable to reptile activities, and also increase its attractiveness to other animals on hottest periods. In fact, not only reptiles take advantage of cooler effect. Larger animals, as bobcats *Lynx rufus*, have been observed using culverts as refreshing and resting spots (Cain et al. 2003). As referred by Yanes et al. (1995), the important fact is that culverts allow the passage of reptiles and possibly increase the permeability of roads for these animals. Nevertheless, given the observed seasonality response among the groups, future culvert survey should be extended year around in order to avoid bias from variable seasonal activity of most species. On our work it was not possible to extend field work through winter which might have caused miss evaluation for some species' crossings, particularly amphibians. In fact, on two rainy night surveys (authors, unpublished data) we detected several amphibians crossing these structures.

Finally, one should have in mind that culverts by it self do not solve connectivity problems. Several species present on both roads vicinity, as wild boar (*Sus scrofa*) (personal observations) and wild cat (*Felis silvestris*) (Santos-Reis and Mathias 1996), were not detected using these passages. This way, implementation of specific fauna passages should also be considered on conservation actions.

Applied conservation biology must provide solutions for the protection of species in modern landscapes, where prime habitats are being continuously fragmented and altered, and animals are being restricted to small, nonviable populations. As referred by Luell et al. (2003), we should consider a holistic vision of fragmentation scenario, and measures to improve road permeability should integrate information from diverse domains, as predictive models of road design and road alignment (Malo et al. 2004; Ramp et al. 2005; Seiler 2005) in order to guarantee success on conservation programs. Culvert improvement might be a small but crucial step to mitigate fragmentation effects on animal populations.

## CONCLUSIONS

Overall, our results corroborate previous works, indicating that culverts are commonly used by vertebrates when attempting to cross roads. As found by other authors, road fencing seems to promote the use of passages by medium and large size vertebrates Although not used by all species present in studied area, culverts, at least during dry weather conditions, can act as fauna passages. If properly designed (without detritus pits and with different sizes) and located they may be an important tool to minimize road fragmentation effects on animal populations.

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Figure legends

Figure 1. Map of the studied area. Dark solid lines represent the two road sections, EN370 and IP2. White circles represent studied culverts. Dark thin lines represent road network and white squares represent most important localities. Grey scale areas stand for altitude (varying from 100m to 400m a.s.l.)

Figure 2. DCA plot ordination of 17 EN370 culverts and 17 IP2 culverts, and 13 vertebrate taxa. Road types are superimposed (full square: IP2; grey square: EN370). Numbers represent culvert identification.

Figure 3. CCA ordination of 13 vertebrate taxa that crossed culverts on roads EN370 and IP2 (triangles), with explanatory variables (numerical and ordinal variables – arrows; nominal variable: square). Longer vector lines represent stronger "intraset correlations" (ter Braak 1986). CL - Culvert length, CCS - Culvert cross section area, CO - culvert openness, DP - detritus pits, DFP - distance of the culvert from the pavement, VC - vegetation covering the passage entrances, DU - distance to urban areas, OP - open land, Pn - probability of use, RT - road type.

Table 1 – Explanatory variables concerning the 34 culverts (17 on each road) surveyed in this study, code, variable type and its median and range values, in EN370 and IP2 roads. (See text for details).

| | | Variable | Code | Type | EN370 | IP2 |
|---|---|---|---|---|---|---|
| Design attributes | | Culvert length (m) | CL | Numerical | 9.0 (8.0 – 25.0) | 19.5 (8.0 – 37.0) |
| Design attributes | | Culvert cross section area ($m^2$) | CCS | Numerical | 0.7 (0.24 – 1.20) | 0.5 (0.38 – 0.79) |
| Design attributes | | Culvert openness (x 100) (m) | CO | Numerical | 7.11 (2.67-13.33) | 2.72 (1.37-13.09) |
| Design attributes | | Detritus pits (number of passages with attribute) | DP | Ordinal | n=2 | n=3 |
| Design attributes | | Distance of the culvert from the pavement (m) | DFP | Numerical | 2.5 (1.0-7.0) | 2.5 (1.7-15.0) |
| Design attributes | | Vegetation covering the passage entrances (number of passages) | VC | Ordinal | n=14 | n=7 |
| Location attributes | | Distance to urban areas (m) | DU | Numerical | 901 (90-4116) | 2310 (657-4265) |
| Location attributes | Land cover (ha) | Open land | OP | Numerical | 10.06 (0.00-20.89) | 13.44 (0.00-28.12) |
| Location attributes | Land cover (ha) | "Montado" with shrubs | MSh | Numerical | 14.10 (0.00-28.12) | 0.00 (0.00-14.09) |
| Location attributes | Land cover (ha) | "Montado" without shrubs | MNT | Numerical | 0.00 (0.00-12.52) | 1.17 (0.00-28.12) |
| Location attributes | Land cover (ha) | Olive groves | OL | Numerical | 0.00 (0.00-15.18) | 0.00 (0.00-25.50) |
| Location attributes | | Probability of use (x 100) | Pn | Numerical | 2.02 (0.97-5.22) | 1.07 (0.53-2.29) |

Table 2 – Culvert use by road expressed in number of passages where *taxon* was detected (N) its mean crossing rate (MCR), standard deviation (SD), maximum (Max) and minimum (Min) values.

| **Taxa** | **EN370** | | | | | **IP2** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **N** | **MCR** | **SD** | **Max** | **Min** | **N** | **MCR** | **SD** | **Max** | **Min** |
| Reptiles | 8 | 0.24 | ±0.11 | 0.40 | 0.09 | 5 | 0.13 | ±0.09 | 0.29 | 0.00 |
| Small mammals | 17 | 0.70 | ±0.24 | 1.00 | 0.09 | 17 | 0.54 | ±0.20 | 0.86 | 0.08 |
| Hedgehog | 12 | 0.34 | ±0.23 | 0.91 | 0.10 | 3 | 0.12 | ±0.04 | 0.14 | 0.07 |
| Lagomorphs | 5 | 0.50 | ±0.32 | 0.82 | 0.07 | 10 | 0.30 | ±0.26 | 0.69 | 0.06 |
| Weasel | 5 | 0.15 | ±0.09 | 0.29 | 0.07 | 4 | 0.11 | ±0.08 | 0.23 | 0.06 |
| Stone marten | 14 | 0.20 | ±0.11 | 0.43 | 0.07 | 14 | 0.32 | ±0.20 | 0.79 | 0.07 |
| Badger | 1 | 0.36 | ±0.00 | 0.36 | 0.36 | 9 | 0.40 | ±0.27 | 0.92 | 0.13 |
| Otter | - | - | - | - | - | 2 | 0.07 | ±0.00 | 0.07 | 0.07 |
| Genet | 11 | 0.22 | ±0.16 | 0.57 | 0.07 | 9 | 0.27 | ±0.18 | 0.50 | 0.06 |
| Egyptian mongoose | 8 | 0.20 | ±0.13 | 0.43 | 0.07 | 13 | 0.35 | ±0.21 | 0.69 | 0.07 |
| Fox | 2 | 0.11 | ±0.05 | 0.14 | 0.07 | 10 | 0.17 | ±0.13 | 0.43 | 0.07 |
| Domestic dog | 1 | 0.18 | ±0.00 | 0.18 | 0.18 | 8 | 0.11 | ±0.04 | 0.17 | 0.07 |
| Domestic cat | 10 | 0.26 | ±0.15 | 0.55 | 0.07 | 8 | 0.14 | ±0.06 | 0.21 | 0.06 |
| All | 17 | 0.32 | ±0.26 | 1.00 | 0.07 | 17 | 0.28 | ±0.22 | 0.92 | 0.06 |

Table 3 – Mean CR and number of culverts (N) used by fauna groups by season (small mammals including hedgehog). Seasonal variability of mean values is expressed using the coefficient of variation (CV=100 x standard deviation/mean) (Yanes et al. 1995).

| | **Spring** | **N** | **Summer** | **N** | **Autumn** | **N** | **CV** |
|---|---|---|---|---|---|---|---|
| Reptiles | 0.57 | 7 | 0.46 | 7 | 0.67 | 1 | 18.5 |
| Small mammals | 0.87 | 33 | 0.91 | 28 | 0.68 | 27 | 15.0 |
| Lagomorphs | 0.60 | 10 | 0.75 | 8 | 0.54 | 8 | 17.2 |
| Carnivores | 1.03 | 28 | 0.84 | 27 | 1.05 | 27 | 11.9 |
| All | 2.10 | 33 | 1.82 | 34 | 1.85 | 33 | 8.0 |

Table 4: Correlations between explanatory variables and axes of species, from the canonical correspondence analysis (CCA).

| Variable | Code | Axis 1 | Axis 2 |
|---|---|---|---|
| Culvert length | CL | 0.271 | 0.446 |
| Culvert cross section | CCS | 0.334 | -0.241 |
| Culvert openness | CO | -0.016 | -0.352 |
| Detritus pits | DP | 0.150 | -0.049 |
| Vegetation cover | VC | 0.802 | -0.283 |
| Distance from pavement | DFP | 0.500 | 0.247 |
| Open land | OP | -0.541 | 0.026 |
| Distance to urban areas | DU | -0.463 | 0.378 |
| Probability of use | $P_n$ | -0.190 | -0.507 |
| Road type | RT | -0.285 | 0.723 |

Table 5 – Spearman rank correlation results between culvert crossing rates and explanatory variables. N=34 for all analysis. See tests for variables names. Significant results are underlined; symbols * and ** represents P<0.05 and P<0.01, respectively.

| | CL | CCS | CO | DFP | OP | MSh | MNT | OL | DU | Pn |
|---|---|---|---|---|---|---|---|---|---|---|
| Reptiles | -0.340* | -0.133 | 0.220 | -0.377* | 0.090 | -0.097 | -0.117 | 0.248 | -0.052 | 0.025 |
| Small mammals | -0.460** | -0.137 | 0.269 | -0.477** | 0.261 | 0.088 | -0.172 | 0.277 | 0.009 | 0.180 |
| Hedgehog | -0.321 | 0.374* | 0.459** | -0.102 | -0.118 | 0.340* | 0.025 | 0.159 | -0.349* | 0.348* |
| Lagomorphs | -0.153 | -0.354* | -0.082 | -0.491** | 0.322 | -0.507** | 0.295 | 0.024 | 0.500** | 0.088 |
| Weasel | -0.050 | -0.064 | -0.036 | 0.116 | -0.157 | 0.012 | 0.314 | 0.110 | -0.076 | 0.044 |
| Stone marten | 0.108 | 0.025 | -0.057 | 0.270 | -0.393* | 0.074 | 0.323 | -0.179 | 0.038 | -0.185 |
| Badger | 0.029 | -0.173 | -0.076 | -0.143 | 0.261 | -0.315 | 0.013 | -0.336 | 0.320 | -0.161 |
| Otter | 0.284 | 0.008 | -0.227 | 0.076 | 0.005 | -0.231 | 0.091 | -0.189 | 0.328 | -0.041 |
| Genet | 0.166 | 0.202 | -0.041 | 0.503** | -0.585** | 0.304 | 0.172 | 0.058 | -0.155 | -0.097 |
| E. mongoose | 0.493** | 0.095 | -0.313 | 0.347* | -0.143 | 0.036 | 0.029 | -0.039 | 0.302 | -0.500** |
| Fox | 0.140 | 0.035 | -0.070 | 0.002 | 0.269 | -0.386* | 0.091 | -0.394* | 0.266 | -0.189 |
| Dog | 0.044 | 0.207 | 0.058 | -0.179 | 0.215 | -0.413* | 0.216 | -0.188 | 0.117 | -0.231 |
| Cat | -0.176 | 0.480** | 0.452** | 0.148 | -0.254 | 0.327 | -0.068 | -0.082 | -0.607** | 0.076 |

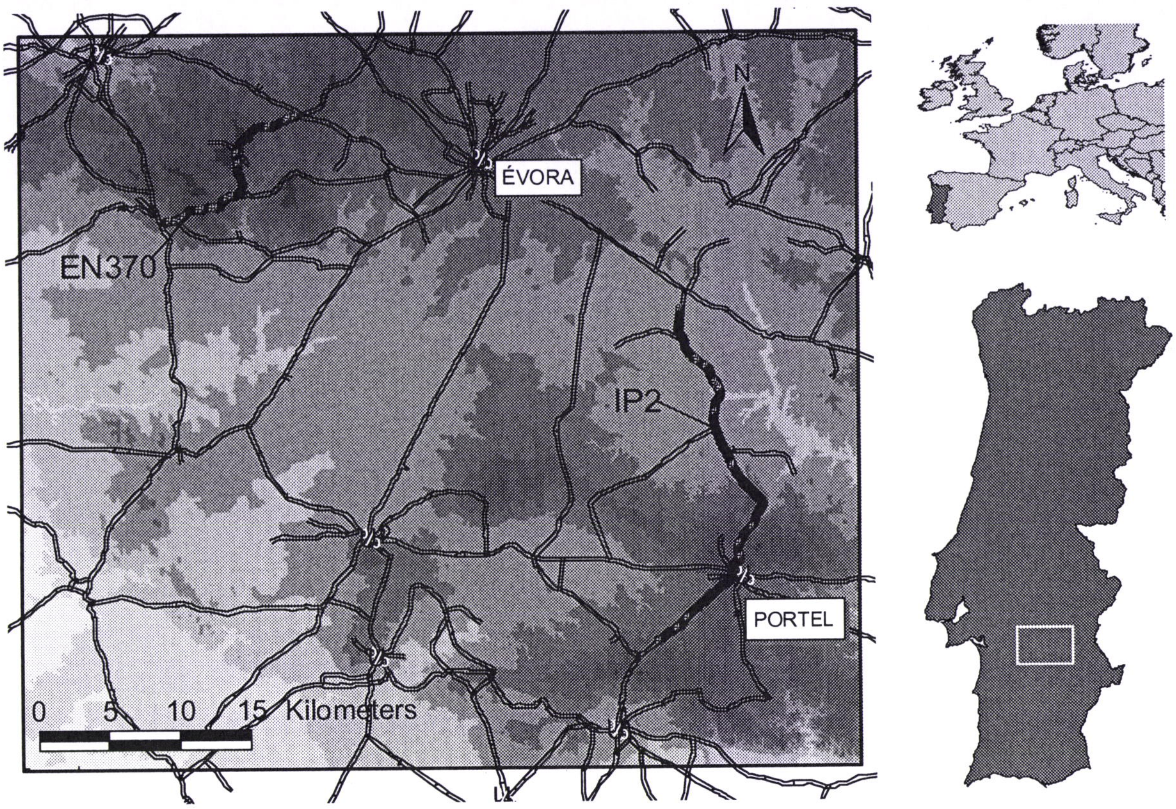
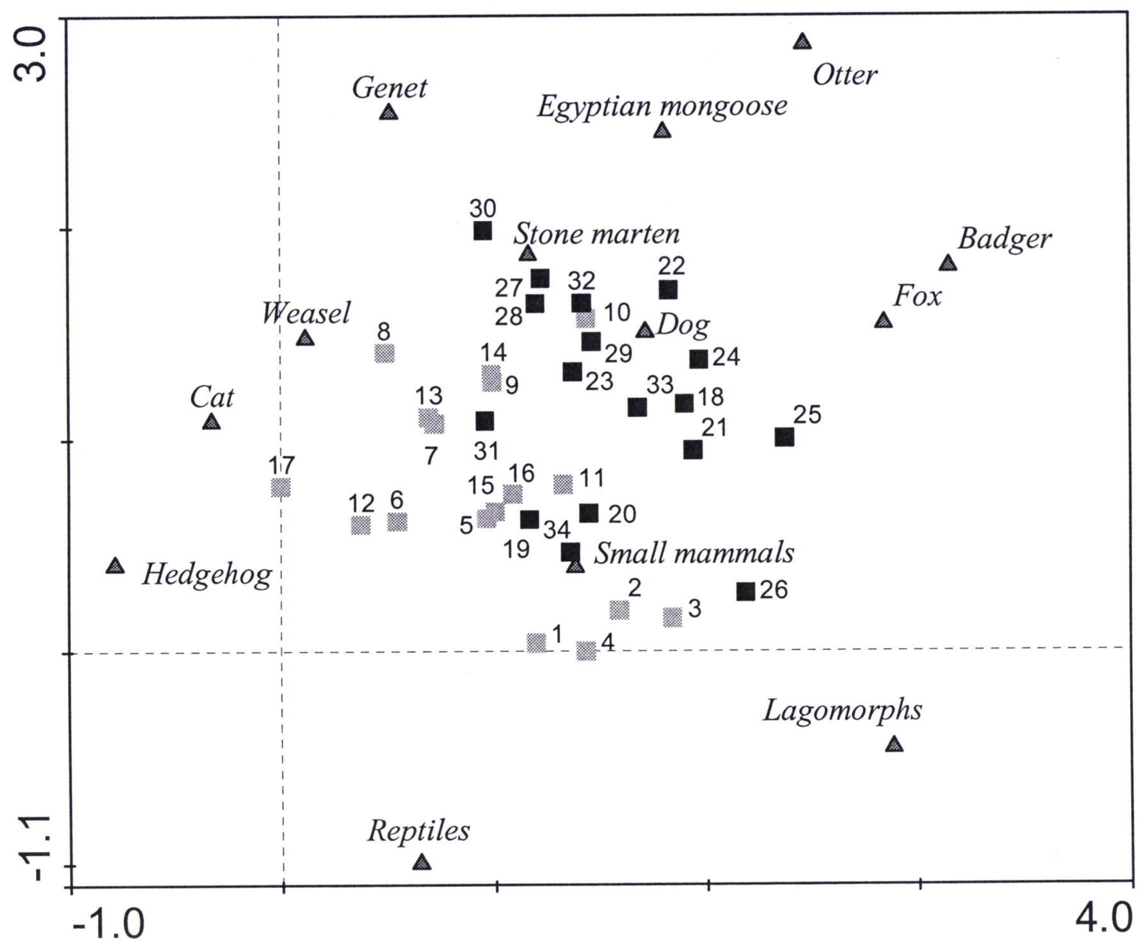



Figure 1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Badger
Fox
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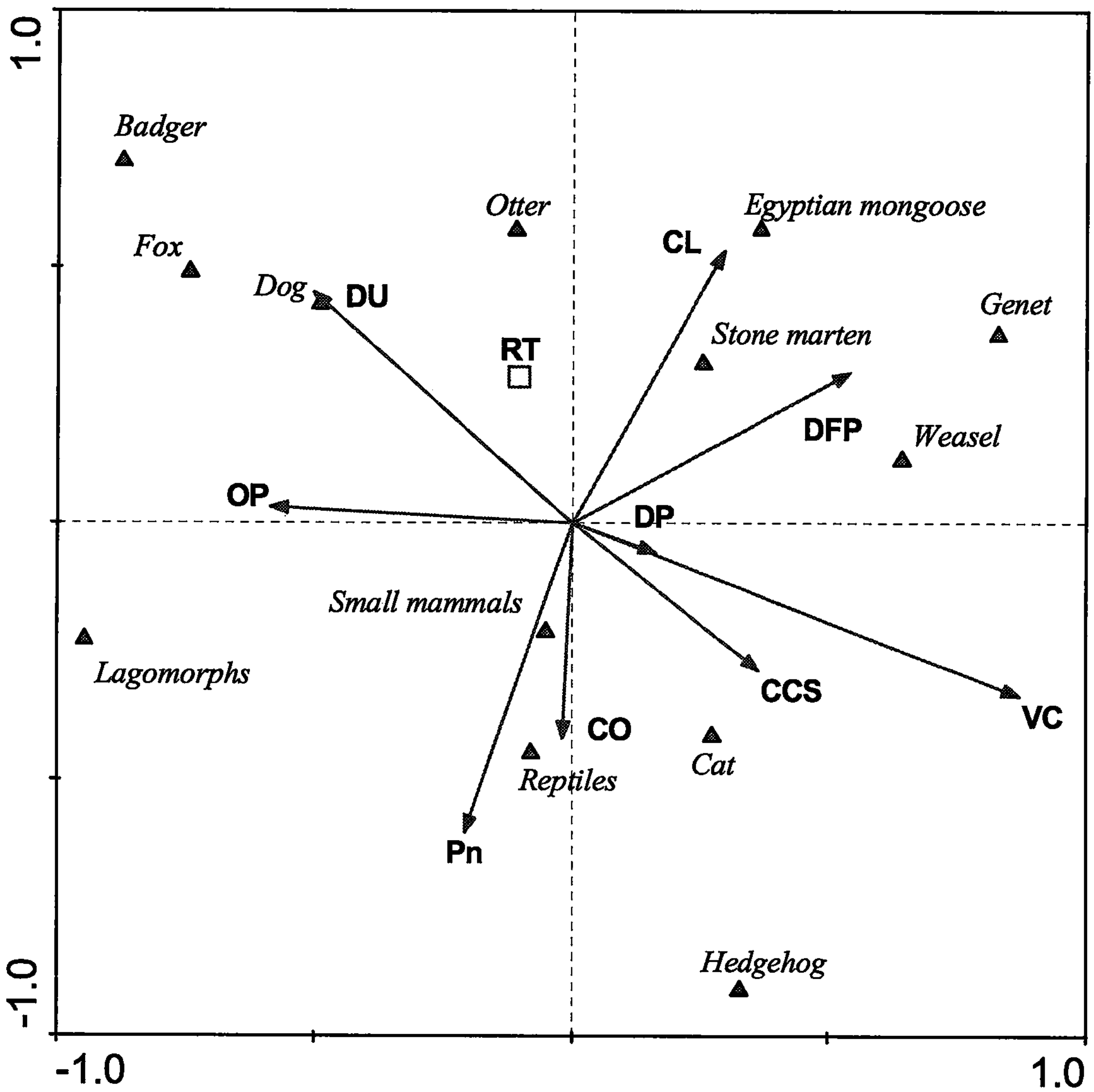
Figure 2



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Badger
Fox
Dog
DU
Otter
RT
Egyptian mongoose
CL
Genet
Stone marten
DFP
Weasel
OP
DP
Small mammals
Lagomorphs
CCS
VC
CO
Cat
Reptiles
Pn
Hedgehog

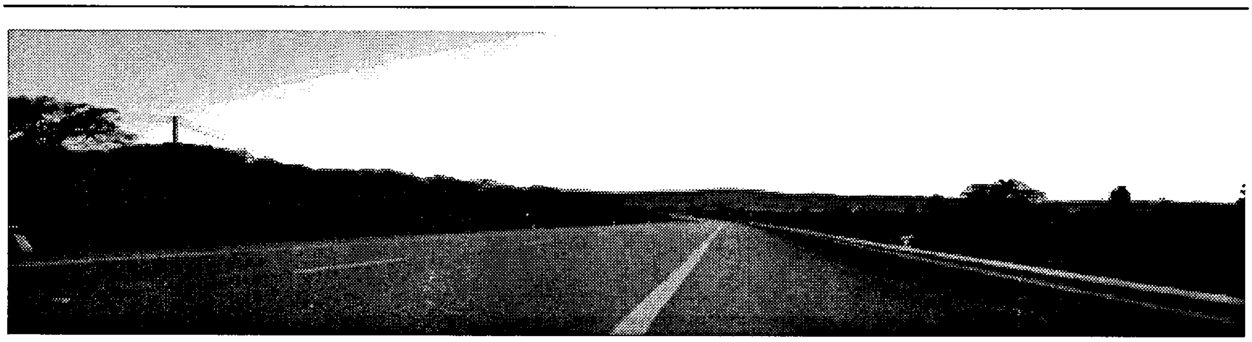

Figure 3

Appendix 1: Features of sampled culverts on EN370 (passages 1 to 17) and IP2 (passages 18 to 34): CL – culvert length, CCS – culvert cross section area, CO – openness, DFP – distance from pavement; DP – detritus pits, VC- vegetation cover, OP – open land, MNT – montado with no shrubs, MSh – Montado with shrubs, OL – olive groves, DU – distance to urban areas, Pn – probability of use. See text for details on variables. Y – attribute present, N – attribute not present.

| Culvert ID | CL (m) | CCS ($m^2$) | CO (m) | DFP (m) | DP | VC | OP (ha) | OL (ha) | MNT (ha) | MSh (ha) | DU (m) | Pn (x100) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 8 | 0.33 | 4.1 | 1.0 | N | N | 12.9 | 15.2 | 0.0 | 0.0 | 4116 | 0.97 |
| 2 | 9 | 0.48 | 5.3 | 1.5 | N | N | 11.8 | 10.4 | 5.9 | 0.0 | 3628 | 1.43 |
| 3 | 9 | 0.24 | 2.7 | 1.8 | N | N | 13.4 | 3.5 | 11.1 | 0.0 | 3485 | 5.22 |
| 4 | 8 | 0.39 | 4.9 | 1.0 | N | Y | 15.6 | 0.0 | 12.5 | 0.0 | 2154 | 5.21 |
| 5 | 10 | 1.00 | 10.0 | 1.3 | N | Y | 8.8 | 2.1 | 0.0 | 17.2 | 275 | 1.22 |
| 6 | 9 | 1.00 | 11.1 | 2.5 | N | Y | 3.9 | 0.0 | 0.0 | 24.2 | 778 | 1.43 |
| 7 | 11 | 0.64 | 5.8 | 3.3 | Y | Y | 2.8 | 0.0 | 1.6 | 14.6 | 261 | 2.02 |
| 8 | 16 | 0.64 | 4.0 | 5.0 | N | Y | 8.2 | 0.0 | 4.5 | 14.1 | 551 | 2.14 |
| 9 | 9 | 1.00 | 11.1 | 3.0 | N | Y | 0.0 | 0.0 | 0.0 | 28.1 | 555 | 1.29 |
| 10 | 9 | 0.75 | 8.3 | 3.5 | N | Y | 0.8 | 0.0 | 0.0 | 27.3 | 295 | 2.59 |
| 11 | 9 | 0.36 | 4.0 | 2.0 | Y | Y | 13.7 | 0.0 | 0.0 | 14.4 | 1285 | 1.91 |
| 12 | 13 | 1.00 | 7.7 | 5.0 | N | Y | 9.8 | 0.0 | 1.9 | 16.4 | 1536 | 3.12 |
| 13 | 9 | 0.64 | 7.1 | 3.8 | N | Y | 10.5 | 4.3 | 0.0 | 13.3 | 1246 | 2.14 |
| 14 | 21 | 1.00 | 4.8 | 7.0 | N | Y | 10.0 | 1.6 | 0.0 | 16.5 | 1130 | 1.40 |
| 15 | 13 | 1.00 | 7.7 | 3.3 | N | Y | 16.9 | 1.3 | 0.0 | 10.0 | 901 | 1.30 |
| 16 | 9 | 0.68 | 7.6 | 2.5 | N | Y | 20.8 | 5.8 | 0.7 | 0.8 | 561 | 2.09 |
| 17 | 9 | 1.20 | 13.3 | 2.5 | N | Y | 4.8 | 0.0 | 12.5 | 0.0 | 90 | 2.69 |
| 18 | 18 | 0.50 | 2.8 | 3.8 | N | N | 28.1 | 0.0 | 0.0 | 0.0 | 1780 | 2.28 |
| 19 | 16 | 0.79 | 4.9 | 1.7 | N | N | 26.0 | 0.9 | 1.2 | 0.0 | 1400 | 1.64 |
| 20 | 19 | 0.50 | 2.7 | 2.5 | N | N | 22.2 | 0.0 | 5.9 | 0.0 | 1600 | 0.95 |
| 21 | 8 | 0.79 | 13.1 | 2.0 | N | N | 1.8 | 0.0 | 13.7 | 0.0 | 2310 | 1.92 |
| 22 | 9 | 0.50 | 5.6 | 2.5 | N | N | 13.4 | 0.0 | 13.8 | 0.0 | 3490 | 1.40 |
| 23 | 23 | 0.50 | 2.2 | 2.5 | N | N | 24.6 | 0.0 | 1.9 | 0.0 | 3284 | 0.72 |
| 24 | 25 | 0.50 | 2.0 | 2.3 | N | N | 26.4 | 0.0 | 0.0 | 0.0 | 3127 | 0.72 |

| Culvert ID | CL (m) | CCS ($m^2$) | CO (m) | DFP (m) | DP | VC | OP (ha) | OL (ha) | MNT (ha) | MSh (ha) | DU (m) | Pn (x100) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 25 | 12 | 0.79 | 6.5 | 2.3 | N | N | 26.4 | 0.0 | 0.0 | 0.0 | 2940 | 0.91 |
| 26 | 28 | 0.38 | 1.4 | 3.5 | N | N | 28.1 | 0.0 | 0.0 | 0.0 | 1366 | 2.16 |
| 27 | 26 | 0.50 | 1.9 | 3.5 | N | Y | 8.0 | 0.0 | 20.1 | 0.0 | 1647 | 0.73 |
| 28 | 28 | 0.50 | 1.8 | 3.5 | N | Y | 5.1 | 0.0 | 23.0 | 0.0 | 1731 | 0.53 |
| 29 | 35 | 0.79 | 2.2 | 10.0 | Y | Y | 8.3 | 0.8 | 0.0 | 10.8 | 2950 | 0.55 |
| 30 | 37 | 0.79 | 2.2 | 15.0 | N | Y | 7.1 | 4.4 | 0.5 | 14.1 | 1494 | 1.00 |
| 31 | 20 | 0.50 | 2.6 | 2.8 | Y | Y | 0.0 | 25.5 | 0.0 | 2.6 | 657 | 1.55 |
| 32 | 25 | 0.79 | 3.1 | 5.5 | N | Y | 0.0 | 0.0 | 28.1 | 0.0 | 4265 | 2.29 |
| 33 | 18 | 0.50 | 2.8 | 2.0 | N | N | 0.0 | 9.0 | 5.2 | 13.9 | 4080 | 1.07 |
| 34 | 16 | 0.50 | 3.1 | 2.5 | Y | Y | 18.6 | 0.0 | 0.0 | 9.5 | 2588 | 1.74 |

# DISCUSSÃO FINAL



# DISCUSSÃO FINAL

Nos últimos séculos a destruição e fragmentação da paisagem resultantes da acção humana provocaram o desaparecimento de vastas áreas naturais e, consequentemente, diversas espécies sofreram uma quebra populacional bastante acentuada. De acordo com Daugherty & Allendorf (2002), a população humana atingiu recentemente os 6 biliões; em 2050 seremos 7.9 a 10.9 biliões nas projecções da ONU; 25% dos 50.000 vertebrados conhecidos estão em risco de extinção, e estes números definem o nosso tempo. Acrescente-se algumas estatísticas respeitantes às vias rodoviárias: na União Europeia, entre 1990 e 1998, cerca de 33,000 ha (10 ha por dia), foram convertidos em vias rodoviárias; o tamanho médio de paisagem contínua, sem ser atravessada por vias rodoviárias importantes, é de 130 $km^2$, variando de 20 $km^2$ na Bélgica a na 600 $km^2$ Finlândia (EEA 2001). Nos Estados Unidos da América, 6.2 milhões de quilómetros de estradas públicas são cruzadas por cerca de 200 milhões de veículos, atingindo uma densidade de 1.2 $km/km^2$ (Forman & Alexander 1998). Em Portugal, e de acordo com o Plano Rodoviário 2000, a rede rodoviária experimentou um acréscimo de 65%, atingindo 16,500km de estradas pavimentadas.

Esta expansão generalizada da rede rodoviária tem com consequência um incremento exponencial dos impactes na biodiversidade (Langevelde & Jaarsma 2005). Deste modo, o estudo do impacte do tráfego e das vias rodoviárias nas populações animais reveste-se de uma importância global, onde a grande necessidade de conhecimento e de soluções contrasta com os trabalhos e investimentos realizados. Mais, em Portugal foram escassos os trabalhos realizados sobre esta temática, particularmente em mortalidade de vertebrados por atropelamento, conhecendo-se apenas o trabalho de Brito & Álvares (2004) como publicação científica.

No presente trabalho foi possível reunir diferentes estudos referentes a oito estradas, onde se pôde observar que um elevado número de espécies é afectado pela mortalidade por atropelamento, incluindo espécies de elevado interesse para a conservação. A comparação dos rankings das estradas usando o índice de mortalidade e os índices de conservação mostrou diferenças acentuadas, sugerindo que as estradas onde morrem mais vertebrados não são necessariamente aquelas onde o impacte em termos de valor conservacionista é maior. Como resultado, a aplicação de medidas mitigadoras poderão ser mais urgentes em estradas onde aparentemente se observa menores índices de mortalidade.

Por outro lado, embora os dois índices de conservação tenham seleccionado as mesmas estradas como as mais importantes, as diferenças encontradas quanto às espécies seleccionadas como prioritárias sugerem um certo desfasamento entre os critérios de origem política (Livro Vermelho, Directivas Habitats e Aves) e as características biológicas e geográficas das espécies. No entanto, saliente-se que cerca de 50% das espécies são comuns aos dois índices. Deste modo, a conjugação dos dois índices poderá fornecer uma resposta mais robusta sobre quais os troços mais importantes para a aplicação de medidas de mitigação.

As espécies mais abundantes e amplamente distribuídas foram as que sofreram um número superior de atropelamentos. De facto, espécies como sapo-comum (*Bufo bufo*), cobra-rateira (*Malpolon monspessulanus*), pardal-comum (*Passer domesticus*) ou ouriço-cacheiro (*Erinaceus europaeus*), cuja distribuição é ampla a nível nacional, foram detectadas em praticamente todas as estradas e, em geral, em maior número, dentro da respectiva classe. No entanto, deve ser dado um especial ênfase às espécies que surgem simultaneamente no índice de mortalidade e num dos índices de conservação, como sejam coelho-bravo (*Oryctolagus cuniculus*), rã-de-focinho-pontiagudo (*Discoglossus galganoi*), cobra-rateira (*Malpolon monspessulanus*), cobra-de-escada (*Elaphe scalaris*) e Coruja-das-torres (*Tyto alba*). Esta dupla ocorrência sugere que elevados níveis de mortalidade poderão ocorrer nestas espécies com interesse para a conservação, devendo por isso ser alvo de medidas e programas específicos para a diminuição dos atropelamentos.

De salientar também que espécies com elevado interesse para a conservação foram ocasionalmente detectadas, como salamandra-lusitânica (*Chioglossa lusitanica*), víbora-cornuda (*Vipera seoanei*), peneireiro-cinzento (*Elanus caeruleus*) e gato-bravo (*Felis silvestris*). Embora tenham uma contribuição mínima para o índice de mortalidade, o seu valor para a conservação eleva estas espécies para os lugares de topo nos restantes índices, e consequentemente os troços das estradas onde foram detectados.

Ainda relativamente ao primeiro artigo, é de salientar que o troço de Portalegre surgiu como um dos mais importantes quer a nível da *quantidade* dos dados, quer pela *qualidade* das espécies detectadas. Como é discutido, este facto talvez seja resultado da sua localização, nas imediações do Parque Natural da Serra de S. Mamede (PNSSM), um local com uma diversidade e riqueza específica singular. De facto, as suas características edafo-climáticas fazem deste espaço uma ilha atlântica num meio mediterrânico, reunindo assim espécies características destas duas regiões biogeográficas. Assim, e tendo em conta a reconhecida importância destas áreas de cruzamento biogeográfico para a conservação da biodiversidade (Spector 2002), estas áreas poderiam ser um alvo preferencial para o estudo e aplicação de medidas mitigadoras da mortalidade por atropelamento. Para além do PNSSM, encontram-se em Portugal outras áreas com estas características como o Sítio da Serra de Monfurado ou a Serra de Monchique.

Uma vez localizados os troços de estradas com elevado impacte de mortalidade, seja a nível de número de atropelamentos, como pontos de passagem de migração de anfíbios; seja pelo valor de conservação dos animais detectados, interessa promover a conectividade entre as bermas das estradas, afim de reduzir os riscos de colisão entre os animais e os veículos. Como foi referido, existem inúmeras soluções para a colocação de passagens para a fauna. Este trabalho debruçou-se, no segundo artigo, sobre a possibilidade de as passagens hidráulicas (Ph's) poderem promover o contacto entre as bermas. Os resultados demonstraram que, no seguimento da bibliografia consultada, diversas espécies usam frequentemente estas estruturas. Mais, os resultados sugerem que o uso das Ph's pelos carnívoros não se deve tanto a uma busca activa duma alternativa ao cruzamento da rodovia pelo asfalto, mas talvez pelos seus hábitos de prospecção e busca de alimento. Isto porque apesar dos níveis extremamente baixos de tráfego na EN370 (observações pessoais), o uso das Ph's aqui estudadas era frequente, ainda que não existam estruturas que possam conduzir ou encaminhar os animais em direcção às passagens, como é o caso no IP2. De facto, no IP2 a presença de vedações nas bermas ao longo de todo o seu trajecto pode ser uma causa dum maior uso das passagens pelos carnívoros (e.g.: Clevenger et al. 2001). Embora não tenha sido possível avaliar o efeito da densidade das populações na proximidade das estradas no índice de uso das passagens, é provável que a vedação e a maior intensidade de tráfego no IP2 encaminhem os animais para as passagens. No entanto, um grande número de cadáveres foi detectado ao longo do IP2, pelo que a presença de Ph's não parecem ser suficientes para evitar a ocorrência de atropelamentos.

Quanto aos factores que parecem influenciar o uso destas estruturas pelos vertebrados, e relativamente às características das passagens, a presença de vegetação envolvendo as aberturas das manilhas parece favorecer o uso de espécies florestais como a geneta (*Genetta genetta*), provavelmente porque permite a continuidade do seu habitat preferêncial, nomeadamente áreas de montado com matos; as passagens mais longas foram aparentemente evitadas por animais de pequeno porte como os micromamíferos, talvez pela inibição de algumas espécies em cruzarem estruturas artificiais mais longas (Goosem 2001); resultado também relacionado com a distância das aberturas ao asfalto, uma vez que as passagens mais longas tendem a ser aquelas cuja distância ao asfalto é maior; também a presença de caixas colectoras parece inibir o uso das passagens para a quase totalidade das espécies e grupos identificados. Este facto salienta a necessidade em melhorar o *design* das passagens, devendo ser incluídos melhoramentos de ordem biofísica de modo a permitir e incentivar o seu uso crescente e pelo maior número de espécies.

Relativamente às características relacionadas com a localização da passagem, as Ph's afastadas das áreas urbanas tiveram um uso superior, provavelmente devido à menor perturbação humana aqui existente; a presença de áreas abertas parece ter incentivado o uso das passagens por lagomorfos, provavelmente pelo abrigo que lhe pode providenciar enquanto se alimentam nas bermas; e por último, o uso por espécies de menor porte poderá ser influenciado pela densidade de passagens, pelo que devem ser colocadas passagens com uma proximidade suficiente que incentive o seu uso por estes animais.

Em suma, no futuro as estratégias de conservação devem integrar informação relativa às Ph's, nomeadamente considerações acerca do *design* e da sua localização: as estradas devem ser equipadas com estruturas que encaminhem os animais para as passagens existentes; devem ser colocadas diversas passagens com diferentes tamanhos de modo a permitir uma variabilidade de escolha, de acordo com os diferentes requisitos biológicos; devem ser evitadas as caixas colectoras nas extremidades das Ph's; e o melhoramento das passagens deve ser feito preferencialmente longe das áreas urbanas.

Por último, deve ser realçado que estas estruturas poderão ser inadequadas para diversas espécies, nomeadamente para javali (*Sus scrofa*), uma vez que em trabalhos de prospecção nas zonas adjacentes às estradas foram detectados diversos indícios de presença desta espécie, e em nenhuma estrutura se observou o seu cruzamento. Desta forma, embora as Ph's possam diminuir o efeito de barreira e risco de atropelamento, deve ser considerado a aplicação de estruturas de passagem específicas, em algos pontos mais sensíveis.

A criação de modelos sobre a distribuição espacial da mortalidade por atropelamento devem integrar tanto a vertente da *quantidade* das observações como a vertente da *qualidade*, de modo a definir importantes pontos de passagem dos animais ou de mortalidade por atropelamento. Nestes pontos poderão ser criadas estruturas que encaminhem os animais para passagens, que poderão ser passagens hidráulicas melhoradas ou passagens específicas. Desta forma, a ecologia de estradas terá dado um contributo significativo à coexistência *pacífica* entre o Homem e a Natureza e à conservação da biodiversidade.

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