UNIVERSIDADE DE ÉVORA
MESTRADO EM BIOLOGIA DA CONSERVAÇÃO

FACTORES DETERMINANTES NA MORTALIDADE DE VERTEBRADOS EM RODOVIAS

FACTORS DETERMINING VERTEBRATE ROADKILLS

Dissertação realizada por:
Filipe Granja de Carvalho

Orientador:
Prof. António Paulo Pereira Mira

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* "Fotografia da capa e contracapa, representa o pormenor de uma berma do troço estudado, tirada em 13-08-2005"

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Artigo científico

Factores determinantes na mortalidade de vertebrados em rodovias

Resumo

Nas últimas décadas, o incremento do número e extensão das rodovias tem contribuído para o declínio da biodiversidade em todo o mundo, principalmente devido à fragmentação dos habitats, perda de conectividade, e mortalidade da fauna selvagem por atropelamento.

No nosso estudo, foram registados 1922 atropelamentos de vertebrados em dois anos diferentes (1326 em 1996 e 596 em 2005) num troço de 26 km entre Portalegre e Monforte de um itinerário principal (IP2). Os dados foram agrupados em sete grupos faunísticos: anfíbios, répteis, carnívoros, "mamíferos presa" (pequenos mamíferos e lagomorfos), ouriços-cacheiros, corujas e passeriformes. A identificação dos factores que mais influenciam a mortalidade de vertebrados foi efectuada através de uma análise de redundância e partição da variância, agrupando-se as variáveis em três grupos: uso dos solos, métrica da paisagem e as coordenadas espaciais dos sectores onde foram registados os atropelamentos (localização). Entre os dois anos, avaliaram-se também possíveis diferenças nos padrões de mortalidade, considerando as mudanças ao nível climático e de intensidade de tráfego.

A variância explicada em 1996 (67.5%) foi maior do que em 2005 (48.7%). Os padrões de mortalidade foram diferentes entre os anos para o total dos vertebrados, anfíbios e ouriços. A variável mais importante, em ambos os anos, foi a distância ao Parque Natural da Serra de São Mamede (DPark). O número de atropelamentos foi gradualmente menor de norte para sul, coincidindo com um afastamento em relação aos limites do Parque Natural. A mortalidade rodoviária foi maior em áreas de montado e olivais, que estão concentradas na parte norte do troço. Em 2005, um dos anos mais secos em Portugal nas últimas décadas, outras variáveis como a distância a albufeiras (Ddam) foram significativas na explicação da mortalidade, o que sugere uma maior influência da disponibilidade hídrica nesse ano. A intensidade de tráfego foi cerca de 2.5 vezes maior em 2005, o que também pode explicar os aumentos de mortalidade de alguns dos grupos, como o ouriço-cacheiro, que foi quatro vezes superior em 2005. Por outro lado, o número de atropelamentos de anfíbios foi seis vezes menor.

Este estudo foi o primeiro que usou a partição da variância, para avaliar o contributo dos diversos grupos de variáveis nos padrões de atropelamento de vertebrados. Esta informação permitirá, no futuro aumentar a eficácia da implementação de medidas mitigadoras.

Palavras-chave: Mortalidade por atropelamento, análise de redundância, partição da variância, paisagem mediterrânea, vertebrados.

Factors determining vertebrate roadkills

Abstract

In the last decades, the sudden growth of road construction has threatened biodiversity all over the world, through habitat fragmentation, reduction in connectivity and wildlife roadkills.

In our study we detected 1922 vertebrate roadkills during two different years (1326 in 1996 and 596 in 2005) in a 26 km main road stretch (IP2) between Portalegre and Monforte. We grouped the data into seven ecological groups: amphibians, reptiles, carnivores, "prey mammals" (small mammals and lagomorphs), hedgehogs, owls and passerines. The factors determining vertebrate roadkills were evaluated through redundancy analysis and variance partitioning techniques. For the analysis, variables were grouped into three sets: land cover, landscape metrics and spatial coordinates (location). We also evaluated possible differences in roadkill patterns among years, considering climatic conditions and traffic intensity changes.

The variance explained in 1996 (67.5%) was greater than in 2005 (48.7%). The roadkills patterns were different between years for total vertebrates, amphibians and hedgehogs. The most important variable, on both years, was the distance to the Natural Park of Serra de São Mamede (DPark). The roadkills decrease as we move from north to south, departing from the Natural Park. Roadkills were higher on forested areas as "montado" and olive groves, concentrated in the north part of the road stretch. In 2005, one of the driest years in Portugal in the last decades, other variables such as distance to water reservoirs (Ddam) were significant in determining roadkills, suggesting a greater water dependence on this year. Traffic intensity was 2.5 times higher in 2005, which could explain the increase of roadkills for some vertebrate groups, as hedgehogs, for which number roadkilled were four times higher in 2005. On the other hand, amphibian's roadkills were six times lower.

To our knowledge, this study was the first one that used variance partitioning to evaluate the contribution of single variable sets on vertebrate roadkills patterns. That information, in the future, may be helpful to enhance the effectiveness of mitigation measures.

Key-words: Roadkills, redundancy analyses, variance partitioning, Mediterranean landscape, vertebrates.

Introdução

<u>Uma problemática globalizada</u>

A fragmentação de habitats e a mortalidade por atropelamento, devida à construção de infra-estruturas lineares de transporte é um dos maiores problemas ambientais à escala do planeta (Forman *et al.* 2003). O incessante crescimento económico e a necessidade de troca de bens entre as sociedades têm despoletado o aumento da rede rodoviária e o tráfego que nela circula a nível mundial. Em 2002, estimava-se que na Terra existiam cerca de 800.000.000 de veículos em circulação (Dargay *et al.* 2006). Estudos no EUA apontam para que os efeitos nefastos das estradas se estendam desde alguns metros até mais de um quilómetro em ambos os lados e que serão necessárias décadas para os avaliar (Forman & Deblinger 2000). As estradas constituem uma das maiores ameaças à biodiversidade, pondo em risco a conservação dos seres vivos em áreas por elas atravessadas e territórios adjacentes (Forman & Alexander, 1998; Trombulak & Frissel 2000; Sherwood *et al.* 2002; Coffin 2007). Estima-se que cerca de 5-7% de habitat das superfícies da Holanda, Bélgica e Alemanha se tenham perdido directamente devido à construção de estradas (Jaarsma *et al.* 2006). Na Suécia esse valor atinge os 1.5 % (Seiler 2003) e nos EUA o valor está cifrado em 0.45 % da superfície. Se contarmos com o "road effect zone" o valor chegará aos 20% da superfície dos EUA (Forman *et al.* 2003).
Consequentemente a conectividade tem vindo a desaparecer das paisagens, com especial destaque para os países desenvolvidos. A manutenção da conectividade, isto é, dos corredores ecológicos entre as diferentes parcelas numa paisagem, constituirá cada vez mais um importante desafio (Crooks & Sanjayan 2006).

<u>Efeitos na Biodiversidade</u>

As estradas podem afectar todas as formas de vida na Terra, desde pequenos animais como as lesmas até aos mamíferos de grande porte como o alce (*Alces alces*) e o urso pardo (*Ursus arctos*) (Mumme *et al*. 2000; Trombular & Frissell 2000, Iuell *et al.* 2003; Smith-Patten & Patten 2008).
Vários são os autores que têm estudado a natureza dos efeitos das estradas na biodiversidade, enumerando diversos tipos e origens. Destes destacam-se os trabalhos de revisão elaborados por Spellerberg (1998), Forman & Alexander (1998), Trombulak & Frissel (2000), Seiler (2003) e, mais recentemente, o trabalho de Coffin (2007). Apesar de nem todos os efeitos serem consensuais, neste trabalho destacam-se sete principais efeitos nefastos das estradas nos ecossistemas:

1. Mortalidade derivada da colisão com veículos;
2. Mortalidade derivada da construção das estradas;
3. Efeito barreira e alteração no comportamento dos animais;

4. Alteração do ambiente físico;
5. Alteração do ambiente químico;
6. Dispersão de espécies exóticas;
7. Aumento da utilização humana das áreas em que se inserem.

Dos efeitos enunciados, o efeito barreira e a colisão directa com veículos, serão os que terão maior impacte na fauna selvagem. Porém, a colisão com veículos é o mais visível para a população humana. Este afecta o efectivo populacional, quer de vertebrados quer de invertebrados. Várias medidas têm sido implementadas para reduzir os atropelamentos, no entanto, a sua eficácia está ainda longe de ser a melhor (para mais detalhes ver secção “Impacte nos vertebrados”).

A construção das estradas provoca a morte directa de organismos sésseis e de pequena vagilidade. Prejudica também os organismos que habitam as zonas envolventes à estrada e altera o seu ambiente físico (Spellerberg 1998; Trombulak & Frissel 2000).

O efeito de barreira diminui a capacidade de movimentação dos seres vivos na paisagem entre as parcelas, podendo levar à perda da persistência e viabilidade das populações e consequentemente à perda da biodiversidade (Forman & Alexander 1998). A presença de rodovias promove alterações de comportamento nos animais na medida em que destrói parte dos seus domínios vitais, altera as rotas de migração, diminuiu o sucesso reprodutor, compromete o estado fisiológico saudável dos animais e põe em causa a sua capacidade de fuga. Essas alterações de comportamento contribuem para o efeito de barreira, na medida em que os animais mostram alguma relutância em atravessar as estradas (Oxley *et al.* 1974; Mader 1984; Mcgregor *et al.* 2008). Esta situação é ainda mais evidente em estradas com um fluxo de tráfego muito elevado como as auto-estradas (Reijnen *et al.* 1995; Clevenger *et al.* 2003). Assim, a restrição nas movimentações dos animais, em busca de alimento ou em dispersão, conduzirá à diminuição da conectividade entre as populações de vertebrados. Como consequência, ocorre uma perda na diversidade genética, um aumento da consanguinidade e uma diminuição da resiliência das populações perante fenómenos estocásticos podendo levar a extinções locais (Van der Zande *et al.* 1980).

As estradas modificam a temperatura, a densidade e a capacidade de contenção do solo, os níveis de luminosidade, poeiras e gases, as águas superficiais e os padrões de escoamento e de sedimentação (Trombulak & Frissel 2000; Iuell *et al.* 2003). Por outro lado, contribuem para a contaminação das áreas adjacentes por metais pesados, sais, moléculas orgânicas, ozono e nutrientes (Trombulak & Frissel 2000; Iuell *et al.* 2003).

A construção destas infraestruturas promove também a dispersão de espécies exóticas porque alteram os habitats e causam *stress* às espécies nativas. Em contrapartida, as rodovias criam corredores ecológicos para as espécies exóticas e nativas aumentando o risco de atropelamento (para as espécies animais) (Spellerberg 1998; Trombulak & Frissel 2000).

Por fim, as estradas aumentam a pressão antrópica ao promoverem acessos a locais anteriormente recônditos, aumentando a perturbação passiva dos animais e promovendo mudanças ao nível da paisagem (Gratson & Whitman 2000).

<u>Impacte nos vertebrados</u>

O maior e mais visível impacte das estradas nos vertebrados, resulta da colisão de animais selvagens com os automóveis, principalmente quando daí resultam vítimas mortais em humanos e prejuízos avultados.

Quando a mortalidade incide em populações pequenas de espécies raras, esta pode ter consequências catastróficas na sua viabilidade (Forman & Alexander 1998). Actualmente, a mortalidade rodoviária da pantera da Florida (*Puma concolor cory*), atinge cerca de 49 % da população que se cifra em apenas 30-50 indivíduos (Taylor *et al.* 2002). A mortalidade rodoviária é também uma importante causa de morte para os ocelotes (*Leopardus pardalis*) nos EUA (Cain *et al.* 2003), o lince-Ibérico (*Lynx pardinus*) em Espanha (Ferreras *et al.* 1992), e o lobo (*Canis lupus*) em Itália (Lovari *et al.* 2007). Na Suécia, a colisão com veículos é também a principal causa de morte dos alces (*Alces alces*), provocando muitas mortes em humanos e elevados prejuízos materiais (Seiler 2003). No Reino Unido, os atropelamentos são a principal causa de morte de coruja-das-torres (*Tyto alba*) (Rasmden 2003). Os anfíbios são talvez o grupo mais sensível ao impacte das estradas devido à elevada mortalidade a que são sujeitos durante as migrações sazonais, aquando da reprodução (Fahrig *et al.* 1995). Hels & Buchwald (2001), estimaram que a mortalidade por atropelamento de anfíbios se situaria entre os 34 e 63% em estradas secundárias com um fluxo baixo de tráfego e que estes valores subiriam até aos 98% em estradas muito movimentadas como as auto-estradas. Assim, algumas estradas, consoante a largura e volume de tráfego, poderão ser praticamente impermeáveis aos anfíbios (Mazerolle *et al.* 2005). As cobras morrem atropeladas ao serem atraídas pelo asfalto quente, funcionando a estrada como uma armadilha e levando à morte a maioria dos juvenis de um determinado ano (Rudolph *et al.* 1999; Andrews & Gibbons 2005). Por fim, saliente-se que a mortalidade rodoviária constitui assim um problema, que vai para além das questões conservacionistas, englobando também uma forte necessidade de gestão das espécies selvagens, segurança rodoviária e saúde pública (Groot Bruinderink & Hazebroek 1996). Com efeito, a segurança rodoviária tem sido o principal motor no sentido de reunir esforços e pressionar para a elaboração e implementação de medidas mitigadoras para a fauna selvagem (Seiler 2003).

Atenuar o problema

Um dos maiores problemas, apontado anteriormente, é a mortalidade de vertebrados em rodovias, que tem também sido uma consequência e/ou causa do aumento do efeito barreira e da consequente perda da conectividade, potenciando a fragmentação da paisagem e das populações animais (Forman *et al.* 2003). Os efeitos nefastos das estradas e o despoletar gritante de rodovias à escala global tem alertado a comunidade científica para a necessidade de investir em estudos que visem a procura de soluções e que restabeleçam a conectividade na paisagem e nas populações. Desta forma, o risco de colisão no asfalto será também atenuado.

Na resolução desta problemática, o próximo passo poderá passar por identificar os principais factores determinantes na mortalidade de vertebrados e qual a sua importância relativa. Nos últimos anos têm-se realizado alguns estudos que abordaram esta questão, quer a uma escala da paisagem, quer a uma escala local em pequenos troços, procurando a identificação de pontos negros de mortalidade (Seiler 2003; Clevenger *et al.* 2003; Van Langvelde & Jaarsma 2004; Malo *et al.* 2004). Estes pontos negros correspondem a agregações de colisões (Clevenger *et al.* 2003; Malo *et al.* 2004; Ramp *et al.* 2006) que reflectem os habitats específicos, o uso dos solos e a topografia nas áreas adjacentes às rodovias (Forman & Alexander 1998; Caro *et al.* 2000; Gomes *et al.* 2008). Perceber também as variações sazonais na mortalidade, que poderão ocorrer entre os diversos grupos de vertebrados, poderá oferecer o conhecimento necessário para biólogos, engenheiros e gestores reduzirem as taxas de atropelamentos (Conrad & Gipson 2006).

Os fenómenos ecológicos resultam de numerosos factores que interagem a múltiplos níveis organizacionais em diversas escalas espaciais e temporais (Cushman & McGarigal 2002). A partição da variância é um método estatístico quantitativo, pelo qual a variabilidade nas variáveis resposta pode ser decomposta em componentes independentes. Estes reflectem a importância relativa dos diferentes grupos de descritores e os seus efeitos conjuntos (Borcard *et al.* 1992; Heikkinen *et al.* 2004). Esta técnica estatística é uma das que nos permitem modelar e encontrar os factores que mais contribuem para a mortalidade rodoviária de um ou mais grupos de vertebrados, consoante a hipótese a testar.

No futuro, com o apoio destas ferramentas, deveremos apurar de uma forma mais pragmática e fiável, quais as variáveis mais responsáveis pela morte de animais nas rodovias e assim implementar medidas mitigadoras (e.g. passagens para fauna) mais eficazes.

Objectivos

Com a realização desta tese de mestrado pretende-se determinar quais os principais factores que causam a mortalidade de vertebrados em rodovias. Em concreto os principais objectivos deste trabalho são: i) Descrever os padrões de mortalidade, durante os dois anos de estudo, num troço de um itinerário principal (IP2) entre Portalegre e Monforte na Região do Alto Alentejo, Portugal (Fig. 1); ii) Quantificar a importância relativa do uso dos solos, características da paisagem e localização espacial na determinação da mortalidade rodoviária e avaliar os factores individuais que a influenciam; e por fim, iii) Comparar os padrões de mortalidade nove anos depois, entre um ano normal em termos meteriológicos (1996) e um ano de seca extrema (2005), considerando variáveis climáticas e o aumento do fluxo de tráfego entre os dois anos estudados. Estes objectivos foram desenvolvidos num artigo “Factors influencing Vertebrate Roadkills in Mediterranean environment: a comparison nine years later” a ser submetido à revista “Biodiversity and Conservation”.

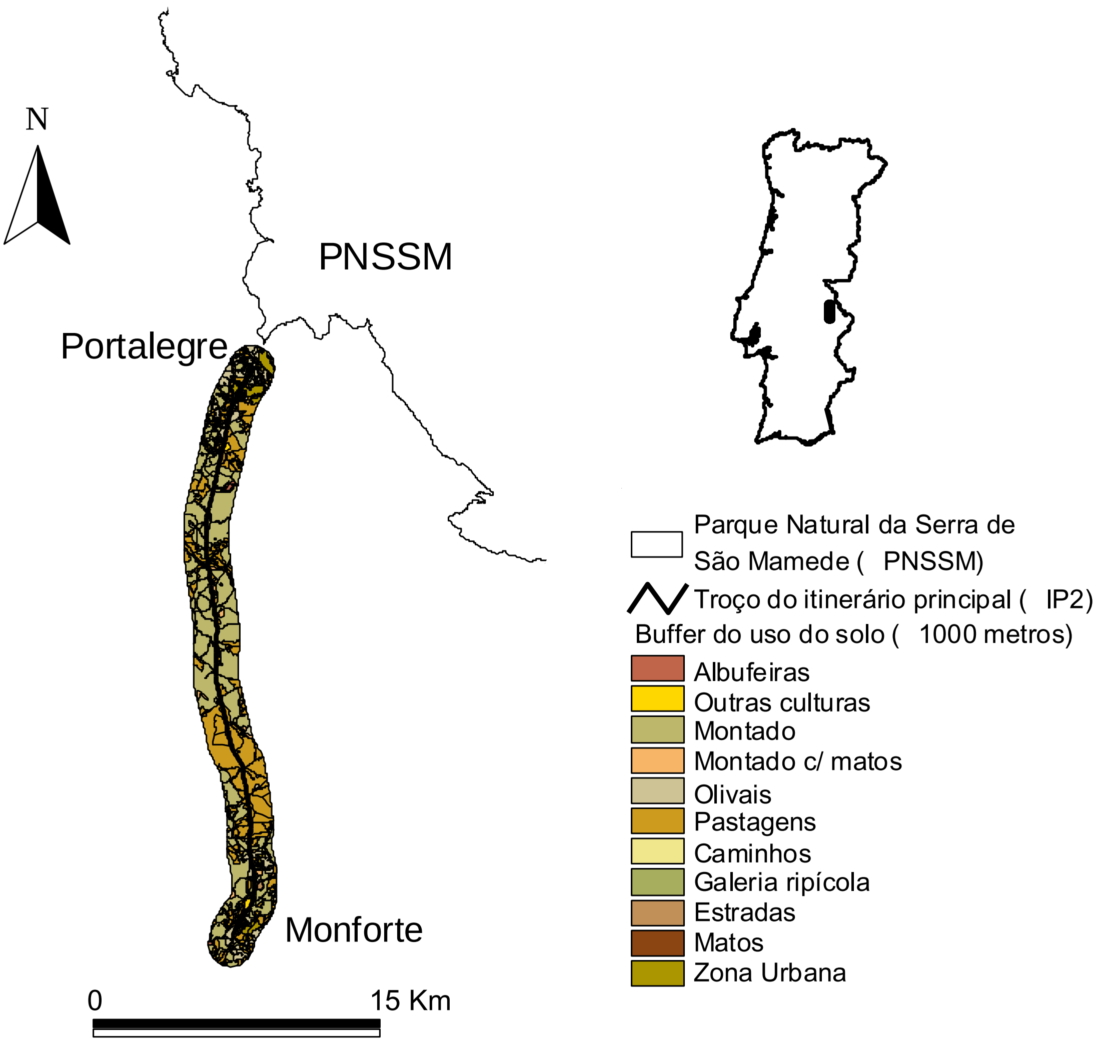

Figura 1 – Localização do troço de estrada estudado (IP2) próximo do Parque Natural da Serra de São Mamede (PNSSM), Portugal.

Artigo científico

FACTORS INFLUENCING VERTEBRATE ROADKILLS IN A MEDITERRANEAN ENVIRONMENT: A COMPARISON NINE YEARS LATER.

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Abstract

Roads, due to their direct relationship with habitat loss, fragmentation and degradation, and road fatalities are one of the major threats to wildlife persistence and survivorship. We surveyed roadkills, every two weeks, on a 26 km stretch of a main national road, in two different years (1996 and 2005). For analysis proposes we grouped the data in seven vertebrates groups: amphibians, reptiles, carnivores, prey mammals (small mammals and lagomorphs), hedgehogs, owls and passerines. The main factors determining vertebrate roadkills were evaluated through Redundancy Analyses (RDA) and variance partitioning techniques, using three sets of variables: land cover (LC), landscape metrics (LM) and spatial location (S) (coordinates). We compared the patterns of vertebrate roadkills between both years surveyed, taking into account meteorological conditions and changes in traffic intensity.

Roadkills patterns along the studied road were significantly different between years for hedgehogs, amphibians and all vertebrates when considered together. The variance explained by the explanatory variables in 1996 was (67.5 %) greater than in 2005 (48.7 %). Many variables determining roadkills were common on both years. The most significant descriptor was the distance to the Natural Park of Serra de São Mamede (NPSSM) (DPark). The roadkills decreased gradually as we move south, away from the NPSSM border. Moreover, the results show a prevalence of the roadkills in forested areas such as "montado" and traditional olive groves, which concentrated in the north part of the road stretch. In 2005, one of the driest years in the last decades in Portugal, other important variables were selected such as distance to water reservoirs (Ddam) suggesting a greater water availability influence in these conditions. Traffic flow was 2.5 times higher in 2005 when compared with 1996 values, which might explain the increase on roadkills in almost all the groups (e.g. four times more for hedgehogs) except for amphibians that decrease approximately six times.

As far as we know our study is the first one using variance partitioning techniques to quantify the relative contribution of different sets of variables to observed roadkills patterns. That information, in the future, may be helpful to enhance the effectiveness of mitigation measures.

Key words: Roadkills, vertebrates, Mediterranean landscape, redundancy analyses, variance partitioning.

Introduction

One of the most visible effects of roads on wildlife is road mortality, which is a major threat to biodiversity conservation (Forman 1998; Forman and Alexander 1998; Trombulak and Frissel 2000; Sherwood *et al.* 2002; Forman *et al.* 2003; Coffin 2007). Roads can affect all kinds of life forms on Earth, from small animals such as slugs to moose and brown bears (Smith-Patten and Patten 2008). Indeed, several authors point out that roads are one of the main cause of vertebrate population decline and decrease in viability across generations (Crooks and Sanjayan 2006; Ament *et al.* 2008). This may be especially true for small mammals where barrier effects lead to local extinctions because of their reluctance to cross roads (Rico *et al.* 2007; McGregor *et al.* 2008). In larger and rarer species such the Iberian lynx (*Lynx pardinus*), the roadkills are the principal cause of death in most of the cubs in Doñana populations (Ferreras *et al.* 1992). In Britain, carnivores such badgers may loose 66 % of all cubs pos-emergence and adults near urban zones (Clarke *et al.* 1998). The principal non-natural cause of death on otters in Britain is road fatalities, which contributed to the general decline of this species (Philcox *et al.* 1999). In Spain barn owls have decrease 70 % in ten years, being the roadkills the principal source of deaths (Fajardo 2001). Fahrig *et al.* (1995) indicates that roads and traffic intensity have contributed to the global amphibian decline. Several researches have revealed negative associations between anuran relative abundance and traffic density (Fahrig *et al.* 1995; Carr and Fahrig 2001; Hels and Buchwald 2001); anuran pond occupancy and road density (Vos and Chardon 1998); and amphibian species richness in breeding sites and paved road density (Findlay *et al.* 2001; Eigenbrod *et al.* 2008). Vertebrates are the most studied group in Road mortality not only for their size, but also because they comprise flagship species (Forman *et al.* 2003).

Understanding the explanatory factors of vertebrate roadkills, and the seasonal variations that might occur among vertebrate groups, will offer managers the knowledge to reduce collision rates and road impacts on wildlife (Saeki and Macdonald 2004; Ramp *et al.* 2005; Seiler 2005; Conrad and Gipson 2006). This will be accomplished, by determining and describing the location of roadkill aggregations (Clevenger *et al.* 2003; Ramp *et al.* 2006), which tend to be linked to specific habitats and landscape types in road vicinity (Forman and Alexander 1998; Caro *et al.* 2000; Gomes *et al.* 2008).

Like other ecological phenomena, roadkills are driven by numerous factors acting at multiple organizational levels, across multiple spatial and temporal scales (Cushman and McGarigal 2002). Variation partitioning is a quantitative statistical method by which the variation in the response variable(s) can be decomposed into independent components reflecting the relative importance of different groups of explanatory variables and their joint effects (Borcard *et al.* 1992; Heikkinen *et al.* 2004). We used this approach to understand the influence of land cover, landscape metrics and spatial location on vertebrate roadkills patterns in a Mediterranean context. The partitioning method is versatile, as it can be used in a variety of univariate and multivariate contexts, with linear or unimodal response methods (Cushman and McGarigal 2002).

The main goals of our study are: i) to describe vertebrate roadkill patterns, during the two year study, in a national main road section in central Portugal; ii) to quantify the relative importance of land cover, landscape characteristics and spatial location on determining roadkills and evaluate main single factors influencing it; iii) to compare roadkills patterns in a normal and dry year, taking into account climatic changes and the increase in traffic intensity between the two studied years.

Study area

This study took place in 26 km stretch of a main road (IP2) located at Portalegre district, central Portugal. The road has two paved lanes along its entire length and runs from the central-west border of Natural Park of Serra de São Mamede (NPSSM), in Portalegre, to a small village located south (Monforte), (Fig. 1). Recently, when our field work has already been concluded, the area surrounding Monforte was classified as Special Protection Zone (DR, 1ªsérie, nº40 de 26/02/2008).

This region located near the Spanish border is generally dominated by smooth areas, except for the mountain topography of the natural Park that reaches 1024 meters above the sea level. Road vicinity is composed by a characteristic Mediterranean agro-silvo-pastoral system of cork and holm oak trees stands (*Quercus suber* and *Quercus rotundifolia*), hereafter denominated as "montado", open land used as pasturelands, meadows, extensive agriculture, and olives groves. Road topography varies slightly along the 26 km being 30.8% levelled. 48.8% buried and 20.4 % raised, when comparing with the immediate adjacent area.

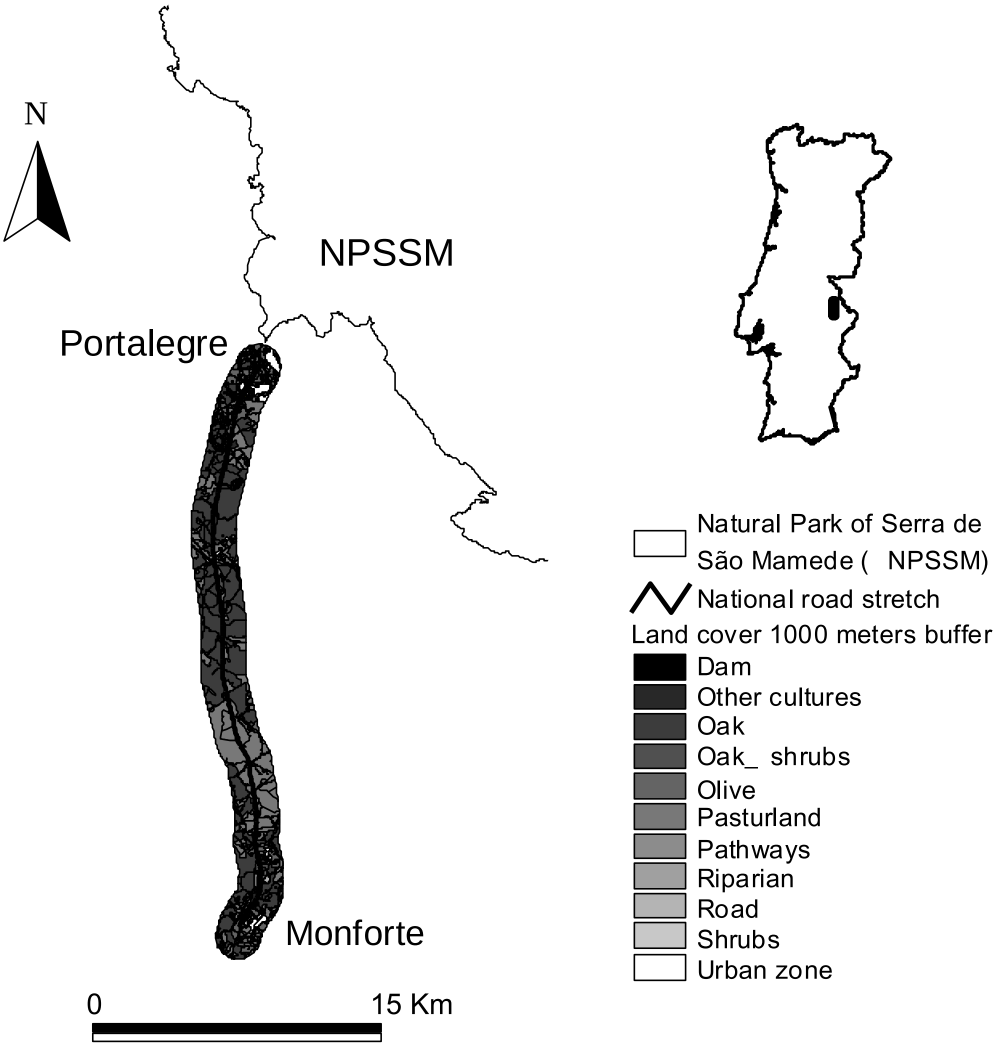

Figure 1 – Location of the studied stretch of IP2 road near the Natural Park of Serra de São Mamede (NPSSM), Portugal.

The climate is Mediterranean with warm and dry summers and cold and rainy winters. However, 2005 was an extremely dry year, with very low precipitation levels during all year around (Fig. 2 and 3). The NPSSM is considered an Atlantic biogeographic island embedded in a Mediterranean type matrix. This biogeographic cross-road enables the coexistence of several species from both regions which contribute to a great level of biodiversity inside the Park and surrounding areas.

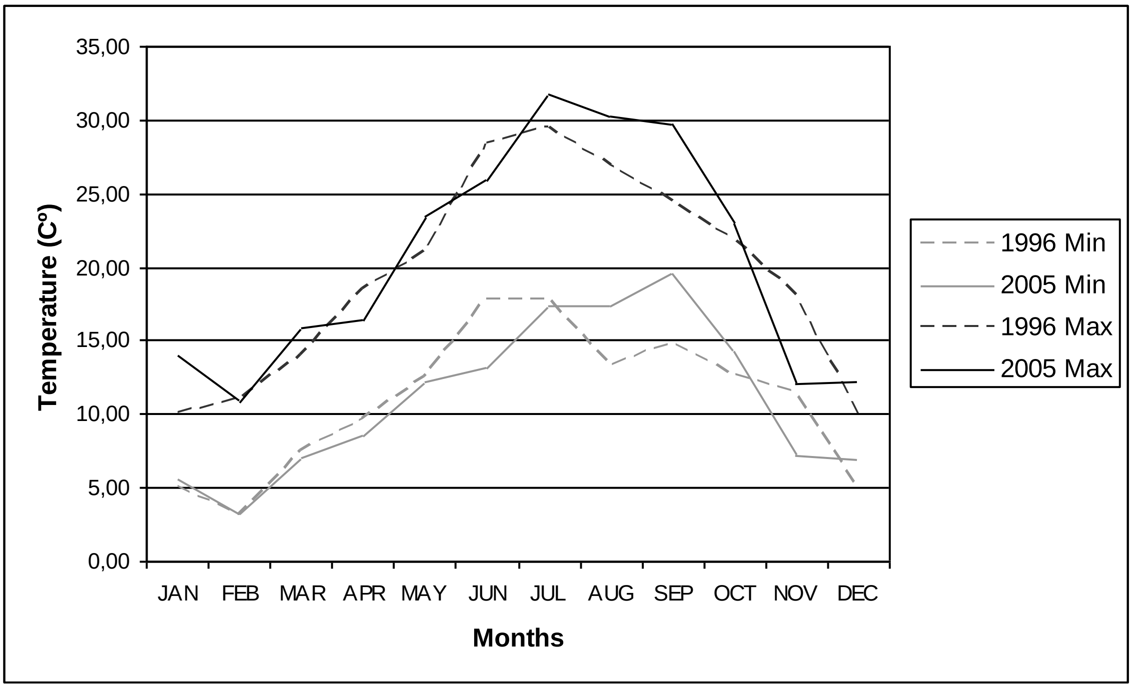

Figure 2 – Monthly average of minimum and maximum temperature for 1996 and 2005 in the study area (IM 2005). (The values presented correspond to the average of the 5 days before a road sampling on each month).

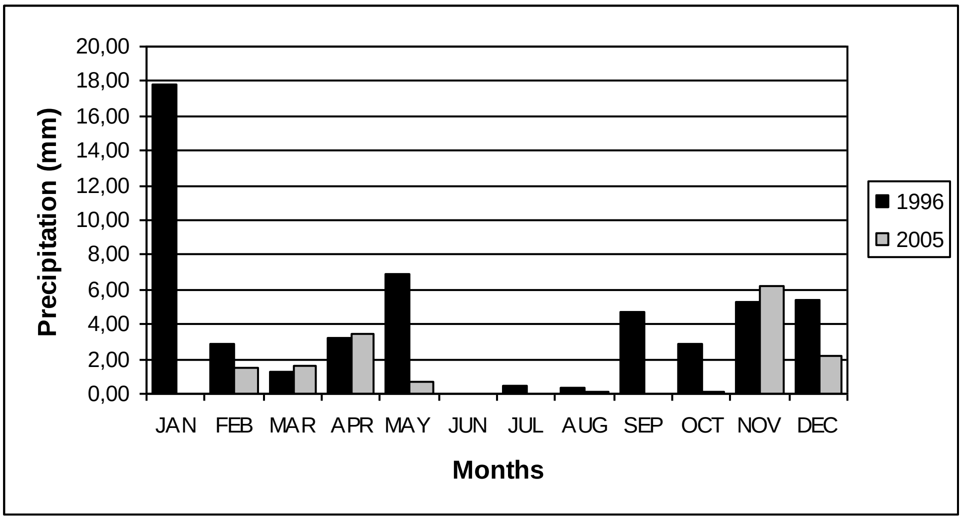

Figure 3 – Monthly average precipitation for 1996 and 2005 in the study area (IM 2005). (The values presented correspond to the average of the 5 days before a road sampling on each month).

The studied road stretch had in 1996, less than two years after the road has been enlarged, a moderate annual daily traffic volume of 2965 vehicles day^{-1}. In 2005 it reached high traffic intensity with an annual daily traffic volume of 6950 vehicles day^{-1}. At both years, traffic peaks are higher in August, when compared with the rest of the year (Fig. 4).

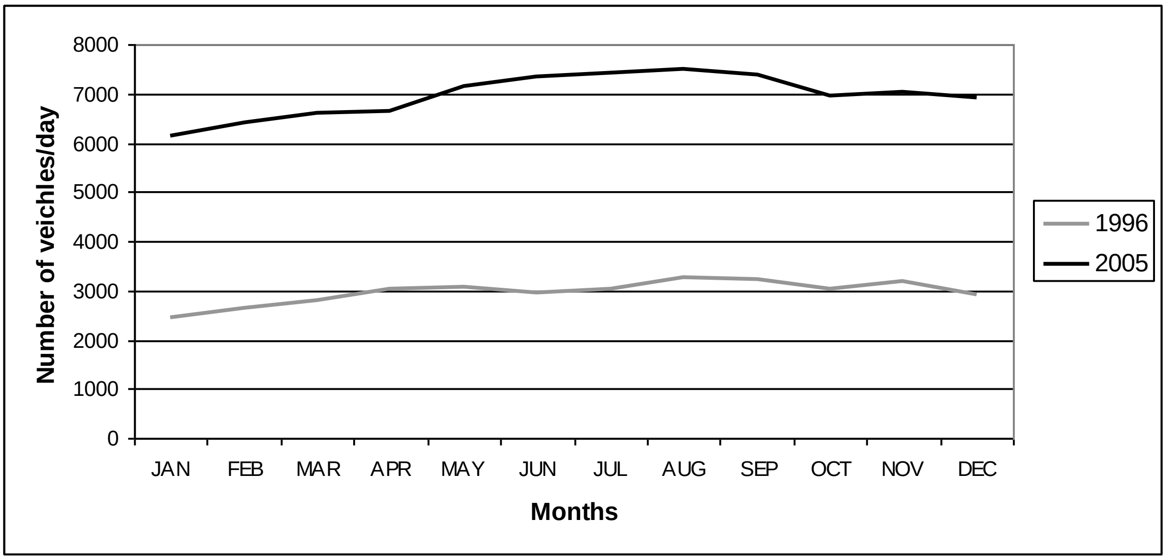

Figure 4 – Monthly average on the number of cars per day in 1996 and 2005 in the studied road stretch (IEP 2000; EPE 2005).

Methods

Roadkills survey

Roadkills were surveyed by car travelling at an average speed of 20 km/h every two weeks, from January through December, in 1996 and 2005 (26 road samplings per year). All vertebrates found were collected and identified to the species level *in loco*, whenever possible, or by analysis in the laboratory of skin, scales, feathers or hairs depending on the taxonomic group. We also obtained the geographic coordinate location of all roadkills using a global positioning system (GPS) unit combined with land cover maps and detailed maps (1:2000) of road profiles. Cadavers were removed from road to avoid double counting.

Sampling units

We created a buffer of 500 meters (m) around the surveyed road and divided this polygon in 52 segments with 500 m long each, obtaining 52 rectangular sampling units (≈50 hectares), hereafter referred as road sectors. We choose a 500 m buffer based on the average roads effects mentioned by Forman and Deblinger (2000) and Forman *et al.* (2002) concerning birds, Eigenbrod *et al.* (2008) for amphibians and Boarman and Sazaki (2006) for reptiles. We also believe that 500m segments along the road are a good length, for the implementation of roadkills mitigation measures.

Explanatory variables

Each of the 52 road segments was characterized for the 48 explanatory variables used in the present study. Variables were clustered into three groups: land cover (LC), landscape metrics (LM) and spatial coordinates (S) (table 1). Detailed Land cover maps where obtained through the interpretation of 2003 aerial photographs, complemented with fieldwork surveys. The comparison of 1995 aerial photographs with the 2003 ones revealed that only minor changes had occurred. Based on this evidence, we decided to use the same land cover map on both years. Land cover types include pasturelands, forests (comprising “montado”, old olive yards, pines and eucalyptus plantations), urban zones, aquatic areas (rivers and water reservoirs), shrublands and roads.

We used *Arcview 3.2* GIS program (ESRI 1999) and the *Patch analyst 2.2* (Eikie *et al.* 1999) extension to obtained the landscape metrics descriptors, for each segment (please see table 1 for details). Distances and all spatial descriptors were derived considering the midpoint of each 500 m road segment.

A very important landscape metric was the distance to the Natural Park of Serra de São Mamede (DPark). This variable should be interpreted as the distance to the central west limit of NPSSM which is an important natural area dominated by a mountain range NE-SW oriented. The Park is known for its high levels of humidity and rainfall and landscapes particularly well preserved. These landscapes are good examples of harmonious interactions between man and nature, maintaining high levels of biodiversity. Road topographic predictors, included in the landscape metrics set, were obtained through interpretation of detailed (1:2000) road profile maps furnished by Estradas de Portugal, SA.

The spatial set of explanatory variables (S) consisted of 10 spatial variables including a full third-order polynomial of x and y coordinates (9 spatial variables) and an autocovariate term (Borcard *et al.* 1992, Heikkinen *et al.* 2004) in order to account for nonlinear responses:

$$\hat{Z} = b_1x + b_2y + b_3x^2 + b_4xy + b_5y^2 + b_6x^3 + b_7x^2y + b_8xy^2 + b_9y^3$$

Before calculating each polynomial term, the x and y coordinates were centred to zero mean to reduce collinearity between the polynomial terms (Legendre and Legendre 1998). The existence of autocorrelation in all vertebrate groups’ roadkills was evaluated using Moran’s I. When autocorrelation was detected, further analysis took this into account, using an autocovariate term (Segurado *et al.* 2006).

Table 1 – List of the 38 variables (two sets LC and LM) used to describe vertebrate survey roadkills locations on the road section. * Variables selected by exploratory evaluation to further analysis.

Acronym	Units	Description
Land cover (LC)		
Road *	ha	Total area of paved roads per sector
Rip *	ha	Total area of riparian habitat per sector
Shrubs *	ha	Total area of shrubland per sector
Oak	ha	Total area of evergreen oak tree's forests per sector
Oak_sh *	ha	Total area of evergreen oak tree's forests with shrubland per sector
O_cul *	ha	Total area of orchards, vine yards and other cultures per sector
Olive	ha	Total area of olive grove per sector
Past *	ha	Total area of pasturelands, meadows and grasslands per sector
Urb *	ha	Total area of Urban zones per sector
Dam	ha	Total area of dam per sector
Landscape metrics (LM)		
Pway *	m	Total length of dirty roads per sector
Ddam *	m	Distance to the nearest dam
Dwat *	m	Distance to the nearest source of water
Dfor *	m	Distance to the nearest type of forest
Drip	m	Distance to the nearest riparian habitat
DPark *	m	Distance to the Natural Park of Serra de São Mamede
Dshrub *	m	Distance to the nearest patch with shrubland
Doak	m	Distance to the nearest evergreen oak tree's forests
Doak_sb *	m	Distance to the nearest evergreen oak tree's forests with shrubland
Doli *	m	Distance to the nearest olive groves
Durb	m	Distance to urban zones
AWMSI	none	Area-weighted mean shape index
ED *	m	Total length of edges per sector
MEDSP *	none	Mean patch size
MPAR	none	Mean perimeter-area ratio
MPE	m	Mean patch edge
MPFD	none	Mean patch fractal dimension
NUMP	none	Number of patches per sector
SDI	none	Shannon's diversity index
SEI *	none	Shannon's evenness index
TOPBUR *	%	Proportion of buried road on each segment
TOPDES	m	Difference between the null altitude and the minimum or maximum
TOPRAI	%	Proportion of raised road on each segment
TOPMAX	m	Maximum altitude on each segment
TOPAVE	m	Mean altitude on each segment
TOPMIN	m	Minimum altitude on each segment
TOPLEV *	%	Proportion of level road on each segment
TOPRAN *	m	Difference between the maximum and minimum altitude

The autocovariate term (AUTOCOV), considers the response at one road sector as a function of the responses at neighbouring sites. It was considered for each vertebrate roadkill data group and for all vertebrates taken together (Augustin *et al.* 1998; Knapp *et al*. 2003). This term was computed using the following equations:

a) $\sum_{j \neq i} w_{ij} - y_i$ and b) $\dfrac{d_{ij}^{-½}}{\sum_{j \neq i} d_{ij}^{-½}}$

Where w_{ij} is the **weighted distance** (meters) between the 500 meter road sector *i* centre and the centre of the neighbour segment *j*, and y_j is equal to the number of roadkills in the *i* segment. The weight distance was calculated by equation b), where d_{ij} is the **distance** (meters) between the 500m road segment *i* centre and the centre of the neighbour segment *j*. All the distances used were calculated over the road network (Knapp *et al.* 2003).

Statistical analysis

For analytical purposes and to avoid a great number of zeros in the final matrix, road fatalities were aggregated into seven ecological groups (Zuur *et al.* 2007): amphibians, reptiles, passerines, owls, carnivores, prey mammal and hedgehogs (table 2). The group "prey mammal" includes all small mammals and lagomorphs, due to small sample size, and their ecological affinities concerning habitat selection (both tend to concentrate on road verges) and importance in the trophic net. Hedgehogs were considered separately due to their spiny body cover, they remain on roads for a longer time than other small mammals and also because they are probably one of the most affected mammals, by road causalities all over the world (Huijser and Bergers 2000). We computed a roadkill index for each group and for all roadkills taken together, which shows the frequency of roadkills per 1000 km of road surveyed by year (Clevenger *et al.* 2003). For global roadkills results and for each ecological group, we tested: 1) the homogeneity (or heterogeneity) on the number of road causalities through space (road) and time (samples) on each year, using the chi-square test; 2) the significant differences between 1996 and 2005 in the road mortality pattern (peaks) along the road stretch and along the year (monthly samples), using the paired Wilcoxon test; 3) the significant differences between years, on the number of road causalities into the road stretch and samplings, using the Man-Whitney test (Sokal and Rohlf 1997). All comparisons were performed with *SPSS 16.0 TM* (SPSS Inc. 2008).
To reduced multicollinearity we removed from further analysis the variable with the lowest biological meaning from any pair of variables having a spearman correlation coefficient higher than ± 0.70 (Tabachnik and Fidell 2001). Original variables were transformed to approach normality. We used logarithmic transformation on continuous variables (including response variables) and angular transformation for proportion land cover data (Zar 1999).

Variance partitioning

To evaluate the effects of each explanatory variable set, on the seven ecological roadkills groups, we used the variation partitioning procedure proposed by Borcard *et al.* (1992) extended to the three sets of variables and adapted for Redundancy Analysis (RDA) (Liu 1997; Heikkinen *et al.* 2004). The choice between RDA and CCA (Canonical Correspondence Analysis) was decided after running a DCA (Detrended Correspondence Analysis) on the response matrix variables for the 52 road sectors for each year data. The length of the gradient for each year (1.497 and 2.261, for 1996 and 2005, respectively), suggests that a linear method (RDA) is more appropriate to deal with our data (Jongman *et al.* 1995; ter Braak and Smilauer 2002; Leps and Smilauer 2003).

In first step we ran a RDA on each set of explanatory variables using a manual selection option and Monte Carlo permutation tests (499 permutations) (ter Braak and Smilauer 2002). Only the predictor variables that contribute significantly ($P < 0.1$), and improve the fit of RDA models were retained in the following analysis (Borcard *et al.* 1992, Liu 1997; Heikinnen *et al.* 2004). At each RDA we also test the statistical significance of all axes and the sum of all canonical eigenvalues with a Monte Carlo permutation test (499 unrestricted permutations) (ter Braak and Smilauer 2002; Leps and Smilauer 2003).

For each year data, after developing single set models and identifying explanatory variables selected, we computed three joint models, one for each of the possible combinations of every two sets; and a global model including all the variables selected on each single set model.

This procedure allowed us to decompose the variance of the data into eight components: a) pure effect of land cover; b) pure effect of landscape metrics; c) pure effect of spatial component; ab) shared effect of habitat cover and landscape metrics; ac) shared effect of habitat cover and spatial component; bc) shared effect of landscape metrics and spatial components; abc) shared effect of the three groups of explanatory variables; and finally U) unexplained variation.

The variance partitioning procedures were done according the methodology explained in Heikkinen *et al.* (2004). All multivariate analysis was performed using the program *CANOCO version 4.5* (ter Braak and Smilauer 2002).

Results

Roadkills data

During the 52 road samplings (26 per year), a total of 1352 km of road were covered. We registered 2073 vertebrate roadkills belonging to 87 species (see appendix I). However, in data analysis, we only used 1922 vertebrate roadkilled belonging to 75 species, which we aggregated in previously explained seven ecological groups (table 2), after removing domestic animals and rare species that could not be include on any of the seven ecological groups considered.

In 1996 we found 63 mammals (12 species), 266 birds (29 species), 934 amphibians (10 species) and 63 reptiles (5 species) roadkilled. In 2005 we registered 95 mammals (15 species), 296 birds (32 species), 159 amphibians (8 species) and 43 reptiles (8 species).

Table 2 – Number of roadkills in each of the seven ecological groups considered, by year.

Vertebrate class	***Ecological group***	***Species*** **Scientific name / *Common name***		
Year			**1996**	**2005**
Mammals	**Carnivores**	*Genetta genetta*/ genet	3	3
		Herpestes ichneumon/ Egyptian mongoose	0	1
		Martes foina/ stone marten	0	3
		Meles meles/ badger	4	2
		Mustela nivalis/ weasel	0	1
		Mustela putorius/ polecat	1	3
		Vulpes vulpes/ red fox	6	4
		Subtotal	**14**	**17**
	Prey Mammals	*Apodemus sylvaticus*/ wood mouse	13	10
		Microtus Cabrera/ Cabrera's vole	1	0
		Mus spretus/ Algerian mouse	2	2
		Rattus norvegicus/ brown rat	1	0
		Rattus rattus/ black rat	0	1
		Rattus spp.	9	2
		Muridae NI	3	4
		Talpa occidentalis/ Iberian mole	1	7
		Crossidura russula/ greater white-toothed shrew	3	0
		Lepus granatensis/ Iberian hare	6	8
		Oryctolagus cuniculus/ rabbit	3	15
		Lagomorph NI	0	2
		Subtotal	**42**	**51**
	Erinacidae	*Erinaceus europaeus*/ western hedgehog	7	27
		Subtotal	**7**	**27**
	Total		**63**	**95**
Birds	**Passerines**	*Alauda arvensis*/ skylarq	0	3
		Anthus campestris/ tawny pipit	0	1
		Anthus sp.	1	0
		Carduelis cannabina/ linnet	1	0
		Carduelis carduelis/ goldfinch	1	9
		Carduelis chloris/ greenfinch	1	0
		Carduelis spinus/ siskin	1	0
		Cisticola juncidis/ fan-tailed warbler	1	2
		Delichon urbica/ house martin	0	1
		Erithacus rubecula/ robin	12	17
		Fringilla coelebs/ chaffinch	0	7
		Galerida cristata/ crested lark	1	0
		Galerida theklae/ thekla lark	0	1
		Galerida sp.	0	3
		Hirundo daurica/ red-rumped swallow	2	3
		Hirundo rustica/ barn swallow	0	1
		Hyppolais polyglotta/ melodious warbler	0	1
		Lanius meridionalis/ Southern grey shrike	1	0
		Lanius senator/ woodchat shrike	11	6
		Miliaria calandra/ corn bunting	18	13
		Motacilla alba/ white wagtail	0	2
		Muscicapa striata/ spotted flycatcher	1	0
		Oenanthe hispanica/ black-eared wheatear	0	2
		Parus caeruleus/ blue tit	11	14
		Parus major/ great tit	2	6
		Parus sp.	0	2
		Passer domesticus/ house sparrow	32	9
		Passer hispaniolensis/ spanish sparrow	1	2
		Passer sp.	4	42
		Petronia petronia/ rock sparrow	1	1
		Phylloscopus collybita/ chiffchaff	13	24
		Phylloscopus trochilus/ willow warbler	0	1
		Pica pica/ magpie	0	1
		Saxicola torquatus/ stonechat	14	23
		Serinus serinus/ serin	7	8
		Sylvia atricapilla/ black cap	26	5
		Sylvia melanocephala/ sardinian warbler	14	21
		Sylvia undata/ dartford warbler	7	1
		Sylvia sp.	5	4

Vertebrate class	***Ecological group***	***Species*** **Scientific name / *Common name***		
Year			**1996**	**2005**
		Troglodytes troglodytes/ wren	1	0
		Turdus merula/ blackbird	4	4
		Turdus philomelos/ song thrush	1	0
		Turdus sp.	1	0
		Passerines NI	56	36
		Subtotal	**253**	**275**
	Owls	*Athene noctua*/ little owl	5	7
		Strix aluco/ tawny owl	2	7
		Tyto alba/ barn owl	6	7
		Subtotal	**13**	**21**
	Total		**266**	**296**
Amphibians	**Anuran and Urodelos (amphibians)**	*Alytes cisternasii*/ Iberian midwife toad	2	1
		Bufo bufo/ common toad	100	11
		Bufo calamita/ natterjack toad	423	53
		Bufo sp.	1	0
		Discoglossus galganoi/ Iberian painted frog	13	1
		Hyla meridionalis/ stripless treefrog	7	0
		Pelobates cultripes/ Iberian spadefoot toad	224	35
		Rana perezi/ iberian green frog	5	3
		Anurean NI	5	2
		Subtotal	**780**	**106**
		Pleurodeles waltl/ sharp-ribbed newt	50	27
		Salamandra salamandra/ fire salamander	96	25
		Triturus marmoratus/ marbled newt	8	0
		Urodela NI	0	1
		Subtotal	**154**	**53**
	Total		**934**	**159**
Reptiles	**Lizards and Snakes (reptiles)**	*Lacerta lepida*/ ocellated lizard	9	3
		Psammodromus algirus/ large Psammodromus	0	4
		Subtotal	**9**	**7**
		Coronella girondica/ southern smooth snake	0	2
		Colluber hippocrepis/ horseshoe whip snake	3	2
		Macroprotodon cucullatus/ false smooth snake	0	1
		Malpolon monspessulanus/ Montpellier snake	10	6
		Natrix maura/ viperine snake	12	14
		Elaphe scalaris/ ladder snake	24	6
		Natrix sp.	0	3
		Snake NI	5	5
		Subtotal	**54**	**39**
	Total		**63**	**46**
Total Vertebrates			**1326**	**596**

The roadkills index (*RKI)* for amphibians in 1996 was the highest recorded during all the study (*RKI* = 690.828) (table 3). This result corresponding to 934 amphibians represents 70 % of all roadkills data in 1996 (table 3). On the other hand, in this year hedgehog had the lowest *RKI* (5.178), which reflects the small number of hedgehogs (only seven) found dead at this occasion (table 2). In 2005 the passerines had the highest roadkill index (*RKI* =203.402). In fact, 275 passerines were found dead (table 2), representing almost 46 % of the roadkills data in 2005.

Table 3 – Roadkills indexes (*RKI* – number of roadkills per 1000 km surveyed) for each ecological group in 1996, 2005 and total for vertebrate classes.

Year	**RKI *by Ecological group***							
	Total	**Amphibians**	**Reptiles**	**Passerines**	**Owls**	**Prey_mammals**	**Hedgehog**	**Carnivores**
1996	990.384	690.828	46.598	187.130	9.615	31.065	5.178	10.355
2005	449.704	117.603	34.023	203.402	15.532	37.722	19.970	12.574
Total	1533.000	808.431	79.142	415.680 (all birds)		116.864 (all mammals)		

Figure 5 shows the pattern of the distribution in roadkills per road kilometre, on each year. The majority of roadkills occurred in the northern part of the road section, nearest to the Natural Park, so the heterogeneity of roadkills along the stretch was significant in 1996 and 2005 (χ^2= 1077 and χ^2= 3071; df. = 25; p < 0.0001, respectively). In fact, the first ten kilometres of the road captured almost 61 % and 52 % of all road casualties and include the main peaks of mortality registered on each year.

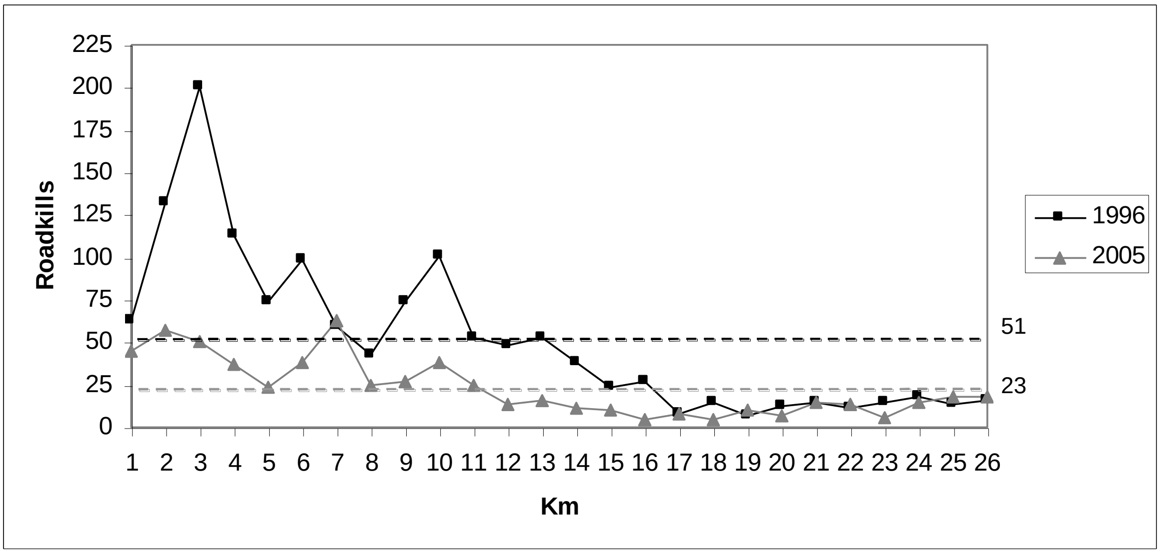

Figure 5 – Roadkills registered along the national road section (km) in each of the two studied years. Black and grey dashed lines correspond to the average roadkills per kilometre in 1996 and 2005, respectively.

The roadkills aggregation peaks were quite similar in both years along the road (*Z*= -1.067; N= 26 km; p= 0.286, Wilcoxon test; Fig. 5), and through time (*Z*= -0.229; N= 26 samples; p= 0.819, Wilcoxon test; Fig. 6).

Comparing now the number of casualties in the road stretch between years, we verified significant differences in all vertebrates roadkills (*Z*=-2.426; p=0.015, Mann-Whitney test), amphibians (*Z*=-4.244; p<0.0001, Mann-Whitney test) and hedgehogs (*Z*=-2.850; p=0.004, Mann-Whitney test) (Fig. 5). Hedgehogs were also the only ecological group which showed significant differences between samplings (*Z*=-2.956; p=0.003, Mann-Whitney test) (Fig. 6).

Concerning temporal variation, roadkills peaks on rainy months (usually in autumn) revealing the high number of fatalities of amphibians at these occasions. This pattern was particularly marked in 1996 (Fig. 6), the year with higher rainfall (Fig. 3). On both years, summer months were usually the ones with lower mortality. In drier months, when amphibians are scarcely roadkilled, roadkills were in general higher in 2005 when compared with 1996 values (Fig. 6).

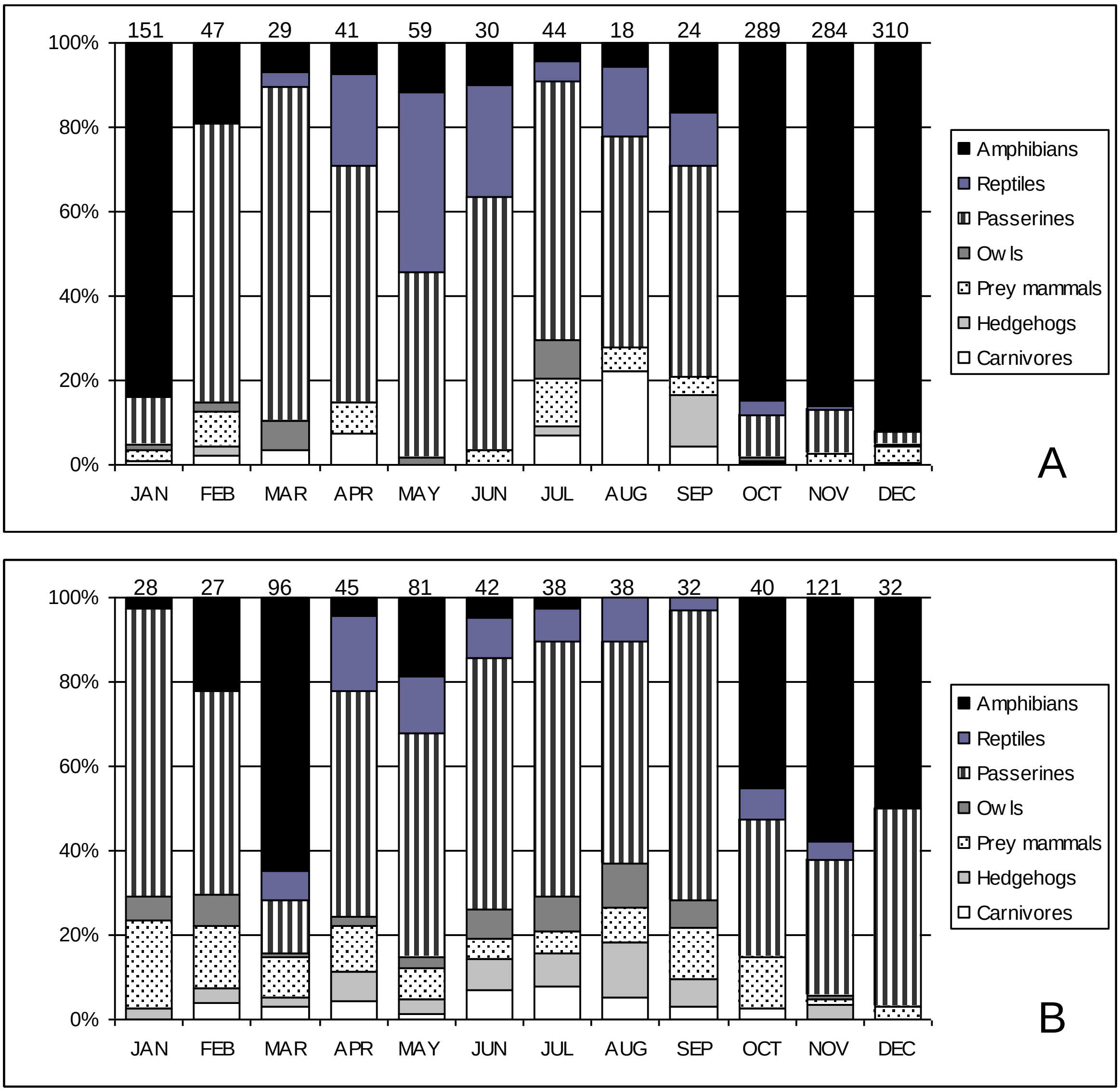

Figure 6 – Comparisons of the monthly roadkills for ecological groups, between years: A - 1996 and B - 2005. The total number of roadkills per month is on the top of each column.

Variation partitioning

From the 48 initial variables, 23 were used for further analysis, after removing collinear descriptors (see table 1). Initially we used the autocovariate term to account for the autocorrelation observed inside groups and in the overall mortality. However, this term was excluded in exploratory analysis due to the high correlation with DPark, which was easier to explain from the biological point of view.

Concerning 1996 RDA results, in the land cover set, the variables selected were: other cultures (O_cul), pasturelands (Past) and shrublands (Shrub) (table 4), altogether capturing 22.9 % of the explained variation on vertebrate roadkills (Fig. 7). From the Landscape metrics set, DPark, distance to shrublands (Dshrub), distance to forests (Dfor), length of pathways (Pway) and distance to “montado” with shrublands (Doak_sb) (table 4) were considered significant, explaining 51.3% of the variation (Fig. 7). In the spatial set, the variables selected were: longitude coordinates (X) and longitude and latitude coordinates interaction (XY), capturing 24.9 % of the variation in the data.

In 2005 O_cul, Past, and urban zones (Urb) were the variables selected on the habitat cover set RDA (table 5), capturing 14.6% of the explained variation on road killings. In landscape metrics set, four variables were considered significant, DPark, Dfor, Doak_sb and distance to water reservoirs (Ddam) (table 5), capturing 33.0 % of the variation. In this year, three variables of spatial group were included in the RDA: X, XY and squared longitude coordinate (X^2) (12.9 % of variation). When considered altogether the variables selected captured 67.5 % of the variance in 1996 and 48.7 % in 2005.

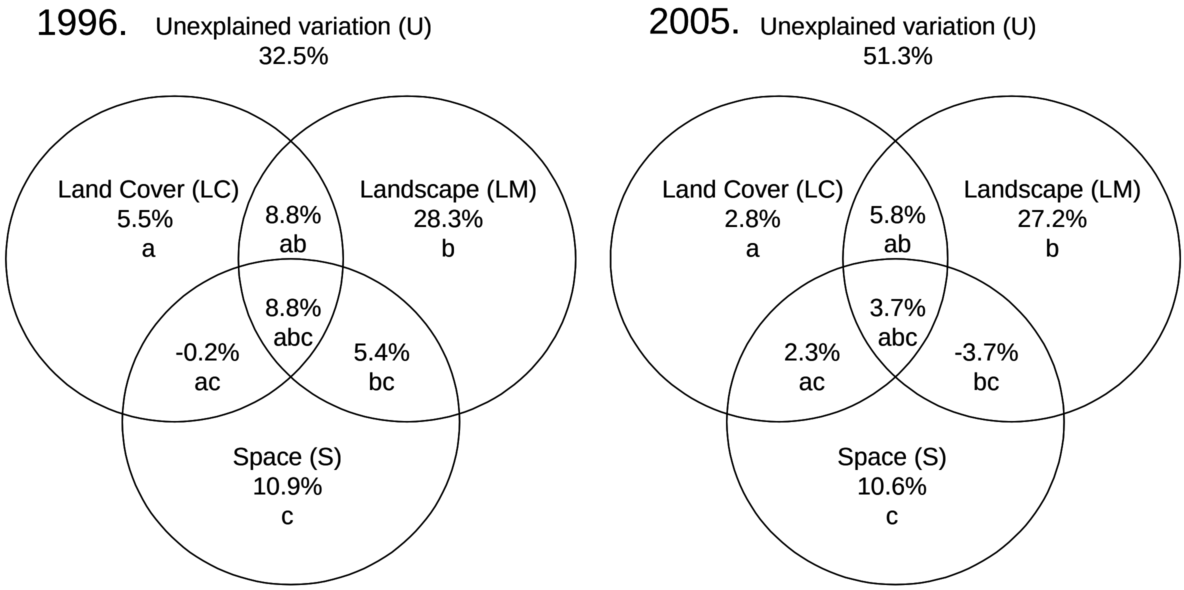

Figure 7 – Results of variation decomposition for the total vertebrate groups, showed as fractions of variation explained. Variation of the Ecological Vertebrate Groups (EVG) matrix is explained by three groups of explanatory variables: LC (Land cover), LM (Landscape metrics) and S (Spatial variables), and U is the unexplained variation; *a*, *b* and *c* are unique effects of habitat and landscape factors and spatial variables, respectively; while ab, ac, bc and abc are the components indicating their joint effects.

The land cover pure effect is meaningfully higher in 1996 (5.5%) than in 2005 (2.8%). Another interesting result is the small negative value of two fractions of variance, suggesting synergism between land cover and spatial coordinates in 1996 and landscape metrics and space in 2005 (Liu 1997; Legendre and Legendre 1998). The joints effects of the three groups of variables were higher in 1996 (8.8 %) compared with the one in 2005 (3.7 %).

RDA for 1996

The Monte Carlo test for the RDA of the vertebrate groups showed that the first canonical partial axis (F = 61.073, *P* = 0.002) and all canonical axes (F = 8.517, *P* = 0.002) were highly significant. Considering the vertebrate groups–environment relationship in 1996, the first two partial RDA axes captured 95.7 % of all the extracted variance (88.6% and 7.1%, respectively). The triplot graph (Fig. 8) shows that, in all groups, road mortality is negatively related to DPark, being this relationship particularly defined for prey mammals (Prey_ma), passerines (Passerin) and reptiles (Reptilia). Moreover, the higher mortality of Prey_ma, Reptilia and amphibians (Amphibia) is associated with the increasing of O_cul. Proximity to forests (decreasing on Dfor) and lower Past promotes mortality on Prey_ma, Passerin, Reptilia and Amphibia. Shrubs are highly related with owl and hedgehog fatalities.

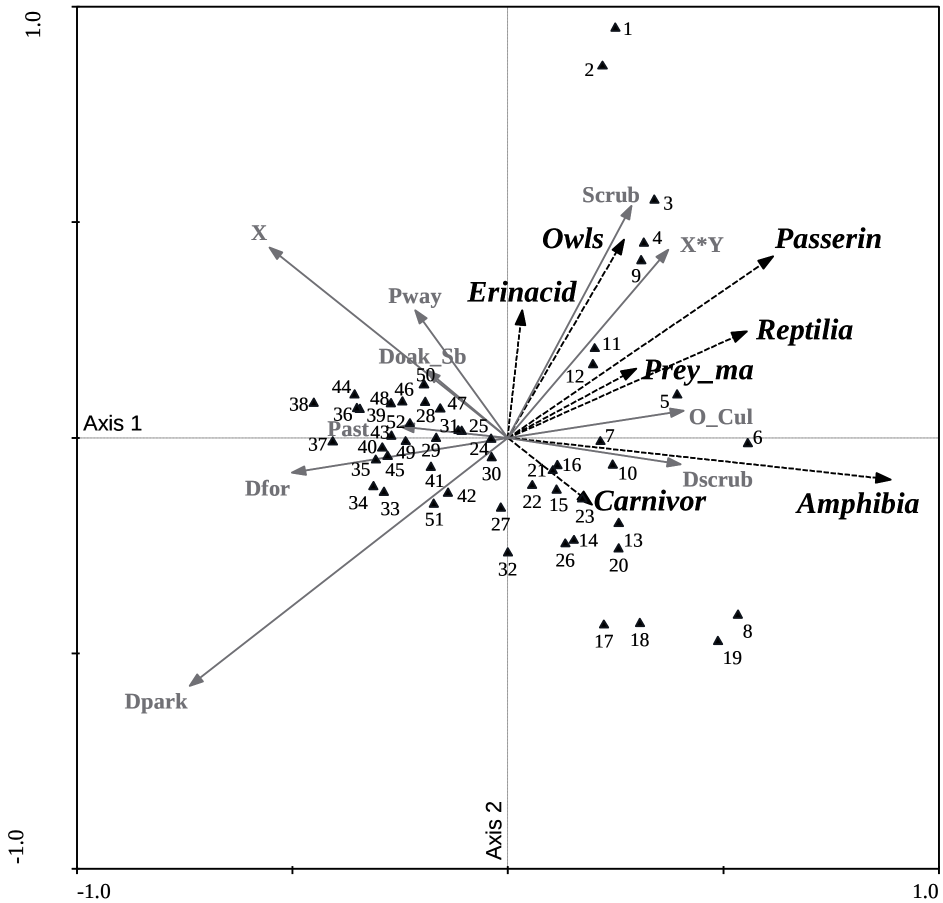

Figure 8 – Ordination triplot depicting the first two axes of the environmental (total) variables partial Redundancy Analysis of the species assemblages in 1996. Environmental variables (Land cover, Landscape metrics and Spatial) (grey colour) are represented by solid lines and their acronyms (see table 1). Ecological groups' (black colour) locations are represented by dashed arrows and their code (table 4). Samples are symbolized by black triangles. Prey_ma – prey mammals and Erinacid – hedgehogs.

Mortality of Carnivores (Carnivor) also seems to increase near forest patches (decreasing Dfor), and at lower Past. Wild carnivore mortality tends to increase in areas near "montado" with shrubs, with lower length of pathway and at lower longitude (X).

The table 4 shows every variable selected during RDA analysis and their conditional effects. DPark is the most important variable related to vertebrate mortality. This importance is also stressed in the triplot where it is the variable with the longest arrow.

Table 4 – Variables selected by the manual forward procedure for each set (LC – land cover, LM – landscape metrics and S – space) for 1996 data, for inclusion in the partial Redundancy Analysis of the vertebrate groups assemblages. The conditional effects (*λ - A*), the marginal effect of each variable (*λ - 1*), the statistics of the Monte Carlo significance test for the forward procedure (F) and the associated probability (P) are reported for each variable.

Set	Variable	*λ - A*	*λ - 1*	*F*	*P-value*
	O_cul	0.04	0.10	5.790	0.012
LC	***Past***	0.01	0.04	4.801	0.014
	Shrub	0.01	0.06	2.836	0.052
	DPark	0.34	0.34	26.036	0.002
	Dshrub	0.01	0.10	4.804	0.016
LM	***Dfor***	0.01	0.15	3.339	0.038
	Pway	0.03	0.03	3.486	0.028
	Doak_sb	0.04	0.03	3.242	0.038
S	***X***	0.13	0.19	12.094	0.020
	XY	0.06	0.10	3.554	0.028

RDA for 2005

The Monte Carlo test for the global RDA performed with all the descriptors included in the three single set models showed that the first canonical partial RDA axis and all canonical axes were highly significant (F = 21.002, P = 0.002 and F = 3.883; P = 0.002, respectively). The first two partial axes accounted for 88.4 % of all extracted variance (69.6 % and 18.7 %, respectively).

The triplot (Fig. 9) for the 2005 data also shows the strong negative association between DPark and mortality of Prey_ma, Passerin, Erinacid and Owls, as in 1996. On the other hand, wild carnivore causalities present a slightly positive relation with this variable. Reptilia mortality seems to be less related with DPark, that in 1996. For the Amphibia and Reptilia, the graph suggests an increase in causalities near water reservoirs and forest patches, lower pastureland land cover and lower longitudes (X). O_Cul land cover influences the mortality patterns in the same way described for 1996 data. In 2005, Owls present an increasing number of fatalities near "montado" with shrubs areas (lower Doak_sb). However, for this vertebrate group, and to a lesser extent for Erinacid, Urban areas and XY present now a strong positive association with roadkills.

In 2005, the variable more important was also DPark, with the greatest value for conditional effects (table 5).

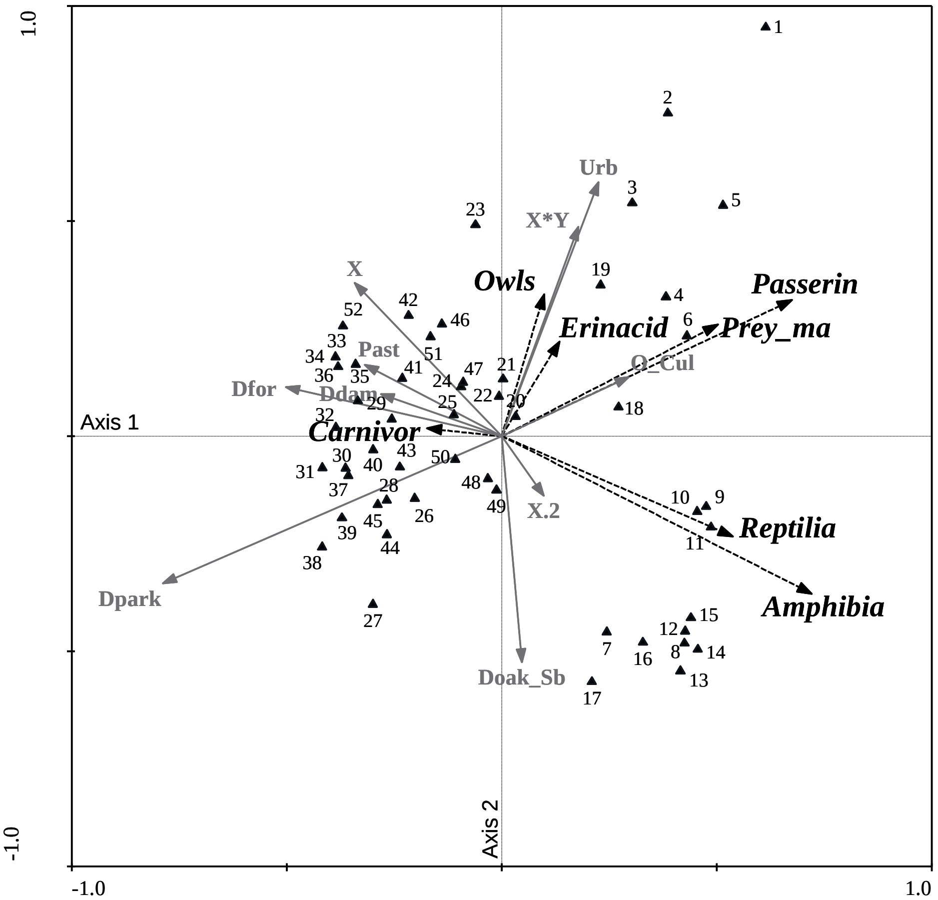

Figure 9 – Ordination triplot depicting the first two axes of the environmental (total) variables partial Redundancy Analysis of the species assemblages in 2005. See details in figure 8.

Table 5 – Variables selected by the manual forward procedure for 2005 data, for inclusion in the partial Redundancy Analysis of the vertebrate groups assemblages. See details in table 4.

Set	Variable	*λ - A*	*λ - 1*	*F*	*P-value*
LC	***Urb***	0.05	0.02	2.897	0.044
	Past	0.04	0.01	2.610	0.032
	O_cul	0.04	0.01	2.410	0.050
LM	***DPark***	0.22	0.22	14.292	0.002
	Dfor	0.09	0.01	2.624	0.024
	Doak_sb	0.03	0.03	2.574	0.024
	Ddam	0.04	0.03	2.113	0.052
S	***X***	0.05	0.06	2.805	0.036
	XY	0.03	0.10	2.060	0.098
	X^2	0.01	0.01	2.055	0.082

When comparing the results of both years we notice that in the global models, ten variables were selected in each case suggesting a good balance on the statistical analysis. Overall, from all the variables selected, six present strong differences in their relative importance on the results between the two years. Shrub, Dshrub and Pway were selected in 1996, but were non significant in 2005. On the other hand, Urb, Ddam, and X^2 only became significant in the later year. The other seven descriptors remain common to each year model.

Discussion

Global patterns of mortality

Information concerning overall vertebrate roadkills is scarce hence comparisons between our results and other studies are difficult to discuss. However, our roadkills numbers are higher - except for mammals- than the ones accounted by Lodé (2000) in Western France, who reports 294.1 amphibians, 11.1 reptiles, 266.6 birds and 434.9 mammals roadkills per 1000 km driven (see table 3 for results). Concerning studies that considered different vertebrates groups, Hell *et al.* (2005) in Slovenia report 60.3 mammals and 72.4 birds roadkills per 1000 km driven, which are values quite smaller when compared to ours, for the same groups (table 3). Finally, Clevenger *et al.* (2003) attained 4.8 mammals, 4.8 birds and 0.74 amphibians' roadkills per 1000 km driven in Canada. We believe that, besides differences on sampling protocols among the several studies reposted, our results may reflect the unusual high numbers of individuals and diversity of species occurring in the surveyed regions, which are embedded in a cross-road between an Atlantic and Mediterranean biogeographic regions located in a global and national biodiversity hotspot area (Myers *et al.* 2000).

Looking to the temporal distribution of the roadkills of each ecological group studied, results mainly reflect ecological demands and life history traits such as: breeding phenology and dispersal activity (Philcox *et al.* 1999; Clevenger *et al.* 2003; Erritzoe *et al.* 2003).

This is particularly true for the most killed vertebrates, the amphibians and passerines. Amphibians are extremely affected by road mortality during the breeding season which in Mediterranean regions takes place in autumn and spring (Fig. 6) when the rainy events are concentrated (Hels and Buchwald 2001). Passerine's roadkills occurred mostly from April to September corresponding to the breeding and dispersal periods of the juveniles in southern Europe (Erritzoe *et al.* 2003). Roadkills patterns of this group may also reflect the greater abundance of food near the roads during summer months because of agricultural crops. Nevertheless, at least part of passerine roadkills may also be associated to the higher traffic flow occurring during summer holidays (Fig. 4) (Erritzoe *et al.* 2003).

We have seen that spatial and temporal roadkills patterns were quite the same in both years (Fig. 5 and 6), besides the differences between years on roadkills. This may reflect that the locals where the vertebrates cross the road continue the same throughout the years, although the changes occurred between 1996 and 2005 (see further sections). Animals show some fidelity to corridors on landscape, especially inside their home ranges. Consequently roadkills aggregations persist along generations (Clevenger *et al.* 2003).

Land cover, landscape metrics and spatial effects on spatial roadkills patterns

Our study focussed on the effects of land cover, landscape metrics and spatial influence on roadkills. Until now, it was the first one that used the variance partitioning methodologies to evaluate the relative importance of these sets of descriptors on vertebrate roadkills patterns. Moreover, it is the first study comparing roadkills on the same road within a large time lag between sampling epochs (9 years).

The overall results of the variance partition analysis in the two years show the same tendencies, despite differences in the proportion of the total variance explained. Landscape metrics was always the most important set explaining vertebrate road fatalities followed by spatial features. In both years land cover was the set that less explains road fatalities patterns. This is a surprising result because we would expect a greater influence of the land cover set. Indeed, some studies point out the highest importance of habitat on roadkills (e.g. Clevenger *et al.* 2003) because animals tend to cross roads near optimum habitat patches. However, due to habitat fragmentation, the remaining patches become smaller and more isolated. These promotes longer movement among patches when looking for high quality habitats and increases the probability of being hit by a car outside optimum habitat (Forman *et al.* 2003; Alexander *et al.* 2005; Eigenbrod *et al.* 2008; Shepard *et al.* 2008).

Regarding to land cover variables, the O_cul land cover is associated with increased “prey mammals” causalities (Fig. 8 and 9) in both years. Small mammals living in Mediterranean areas such as rodents, moles and rabbits are often favoured by the presence of small orchards, vine yards and vegetable gardens which besides supplying water, also provide shelter and food. So, roadkills should be enhanced, if these cultures are in the road vicinities (Bennett 1990; Bellamy *et al.* 2000; Bautista *et al.* 2004; Rytwinski and Fahrig 2007). In a study that is now being conducted in another road in southern Portugal, Marques and colleagues have found a high concentration of rabbits and small mammals on road verges when compared with the surrounding areas (Helena Marques, pers. comm.).The prevalence of this kind of habitats in the Natural Park vicinity are also optimum habitats for passerines in the Mediterranean, explaining the higher number of fatalities occurring in the beginning of the studied road stretch (Fig. 8 and 9) (Erritzoe *et al.* 2003). The positive association between owls and “prey mammals” roadkills obtained in both years (Fig. 8 and 9) suggests that owls’ road fatalities may, at least, partially reflect an attraction to the road vicinity due to the higher prey availability. Here, they will be exposed to vehicles when flying from one perch to another (Hernandez 1988; Gomes *et al.* 2008).

DPark was the most important of all of the landscape metrics variables, being negatively correlated with the majority of the vertebrates’ fatalities in both years. This association may reflect the distance to a mountain range in Natural Park of Serra de São Mamede, where higher rainfall and humidity provide elevated water availability than in surrounding flat areas. In a Mediterranean context, water supply is critic, particularly in summer months and for some groups such as amphibians. Probably, elevated roadkills occurring in the road sectors closer to the Park are related to the expected southwards decreasing in water gradient on those environmental conditions (Fig. 8 and 9). These results must reveal the higher abundances of amphibians near the Park, as elevated numbers of these species are expected in moistly areas (Beja and Alcazar 2003; Mazerolle *et al.* 2005; Benayas *et al.* 2006; Eigenbrod *et al.* 2008). Carnivore roadkills were the ones least related with DPark. In 2005 this group showed a different pattern from the one registered in 1996. In fact, in 2005 most roadkills of wild carnivores took place in the final part of the road section, away from the Park but in the proximity of the largest stream (Ribeira Grande) in the study area, which has a well preserved riparian corridor. Virgós (2001) and Matos *et al.* (2008) emphasize the high importance of riparian woodlands for carnivores, especially when crossing open lands, as in our study area. This importance must be strengthened in dry years when animals look for water resources. We suggest that the difference in patterns between years are due to the extreme dry conditions registered in 2005 which prop up the use of riparian areas increasing the probability of getting killed near them. In 1996, as dams and little streams maintained water all over the year, the carnivores were able to explore a larger area, which is reflected in the dispersed distribution of the roadkills (Fig. 8).

Also worth to mention is that none of the descriptors related to the road profile included in landscape metrics set was selected as significant in any year. Clevenger *et al.* (2003) show that road side topography strongly influences small vertebrate roadkills, especially those with low vagility. These differences in the results may reflect the fact that our studied road crosses a flattened landscape and presents only small dissimilarities in the profile along the all the surveyed stretch.

The relatively high influence of the spatial set of variables on roadkills was in agreement with the spatial autocorrelation found in our data in both years. Despite the great influence of latitude revealed in the importance of DPark, longitude (X) and its interaction with the latitude (Y) were also important. Highest interaction (XY) values also occur near the NPSSM, (higher latitudes and longitudes) which may explain the high positive relation of this variable with mortality of many vertebrate groups (Fig. 8 and 9). This effect was slightly higher in 1996, revealing a greater spatial structure on roadkills in this year. Moreover, the spatial effects may also be a sign of other factors such as local resources, predation, competition that may affect species abundance and or space use, which may also define locations of vertebrate roadkills (Legendre 1993), but the relative importance of these factors was not possible to access with our data.

What makes the difference between 1996 and 2005?

The variance in the vertebrate road fatalities explained by almost the same explanatory variables decreased nine years later. Thus, it is important to analyse the factors which can contribute to the increase in unexplained variance. Two major changes occurred between the 1996 and 2005: i) a strong decrease in rainfall (Fig. 3); ii) a high increase in traffic volume (Fig. 4).

Most animals have a high physiological dependence on water, this being particularly strong for amphibians, as they entirely depend on it to live and complete their life cycles (Cushman 2006). Amphibians also show episodic massive road fatalities, usually coincident with migrations to and from spawning sites that can take place in only one night (Clevenger *et al.* 2003; Cushman 2006).

A dry year like 2005 affects amphibians' life cycle inhibiting their reproduction and therefore limiting their movements (Benayas *et al.* 2006; Cushman 2006) which in turns lowers roadkilling risk. Nevertheless, not only amphibians should be affected by weather. Extreme climatic conditions, such as the severe drought verified in Portugal in 2005, may have a large impact on the population density and space use of most species as a response to critical resource needs, particularly water (Vermeulena and Opdam 1995; Erritzoe *et al.* 2003; Araújo *et al.* 2006). The demand for water in dry conditions must explain the higher importance of distance to water reservoirs (Ddam) in structuring vertebrate road fatalities in 2005. This is particularly obvious for amphibians. However, carnivore mortality locations (road sections thirty and above in figure 9) seem also to confirm the importance of water availability in dry years. In fact they are aggregated in the southern part near the riparian zone where water, and simultaneously refuge are available.

In 1996, the abundance of water all over the study area was not a limiting factor, as the rivers and little streams kept water running throughout the year. The extreme dry conditions in 2005 may have also contributed to the higher mortality of most vertebrate groups, when compared to 1996 values, except for amphibians and reptiles. The moister conditions and higher vegetation growth on verges, when compared with the surrounding areas, translates in an increase in availability of cover and food, which attracts animals to the road side increasing the likelihood of being roadkilled (Bellamy *et al*. 2000; Sherwood *et al.* 2002; Erritzoe *et al.* 2003).

On the other hand, traffic load and speed also are important factors determining roadkills (Kacsenski *et al.* 2003; Baker *et al.* 2004; Jaeger *et al.* 2005) and they might have also played a role on the higher mortality of many vertebrate groups recorded in 2005. Indeed, in the studied road stretch, traffic increased about 2.5 times between the two studied years (Fig. 4). Most species or groups seem to be particularly vulnerable to the traffic increase. For instance, the hedgehogs' roadkills in 2005 were about four times higher than in 1996 (Huijser and Bergers 2000) and owl died about two times more (Fajardo 2001). Jaarsma *et al.* (2006) alert for the effect of higher speed which may increase the chance of an animal being hit by a car, while attempting to cross the road. This may explain the increase on bird and mammals roadkills verified in 2005 (Erritzoe *et al.* 2003; Van Langevelde and Jaarsma 2004). Additionally, in 1996 the road was previously enlarged less than two years before. This intervention on roadside with destruction of verges may have temporarily decreased the density of hedgehogs and rabbits on verges (Rondinini and Doncaster 2002; Bautista *et al.* 2004), thus lowering their probability of being killed in the first year of the study. Finally we also should consider that the traffic load should be greater near Portalegre due to the daily affluence of people from the surrounding villages to work there. This may also explain the higher roadkills for all groups in the first ten kilometres of the studied road stretch (Fig. 5). Originally our goal, concerning comparisons between 1996 and 2005, was to evaluate the effect of the traffic intensification on vertebrate roadkills. The large differences in rainfall between years confound the results and did not allow to the identification and discussion of the pure traffic effect on road fatalities.

Implications for conservation

This investigation gives the first results and discussion on the relative roles of land cover, landscape metrics and spatial effects on roadkills patterns in a Mediterranean context. It is also, to our knowledge one of the few who tries to evaluate the effect of traffic increase on road vertebrate mortality, on the same road stretch.

We have shown that landscape metrics is the most important group of factors influencing vertebrate roadkills. This means that when choosing road corridors, the landscape context must be taken into account as a hole, and not mainly land uses if we aim to reduce effectively the risk fauna fatalities. Our results also suggest that in drier years, water availability may be a main factor determining road killings of different vertebrate groups. So, also superficial water resources location must be considered when planning new roads.

Higher roadkill rates obtained by us, when compared with other studies, shows that when crossing biodiversity hotspots or other important natural areas, particular care must be taken in planning and deciding road locations. In fact, in these areas large increases in mortality rates may take place and specific actions must be taken to minimize them. The particularly higher values of road fatalities found in our study also enhance the urgency of implementing mitigation measures in existing roads. In this road, hotspots of mortality must be identified on the basis of field surveys and modelling, where mitigation measures must take place for each vertebrate group, and be defined specifically on the basis of their behaviour and ecological needs (Caro *et al.* 2000; Underhill and Angold 2000; Iuell *et al.* 2003; Grilo *et al.* 2008). However, amphibians due to particularly high number of fatalities recorded must be prioritized as suggested by other authors (Benayas *et al.* 2006; Cushman 2006).

For the implementation of effective and objective mitigation measures further studies are needed in order to disentangle the pure effects of different kinds (road characteristics, landscape features, traffic volume and speed, weather conditions, etc.) on fauna road mortality patterns. We hope that our results will give some insights for prioritizing and choosing the best directions when several options are available.

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Appendix I. List of all road-kills by species found in the national road section in 1996 and 2005. ICNB – status following Portuguese red list data book (Cabral *et al.* 2005). IUCN – status following international red list data (IUCN 2007). NI – not determined.

Class	**Species**		**Status**		**Roadkills**		
Years	**Scientific name**	**Common name**	**ICNB**	**IUCN**	**1996**	**2005**	**Total**
Amphibians	*Alytes cisternasii*	iberian midwife toad	LC	NT	2	1	3
	Amphibian NI	-	-	-	39	4	43
	Anurean NI	-	-	-	5	2	7
	Bufo bufo	common toad	LC	LC	100	11	111
	Bufo calamita	natterjack toad	LC	LC	423	53	476
	Bufo sp.	-	-	-	1	0	1
	Discoglossus galganoi	iberian painted frog	NT	LC	13	1	14
	Hyla meridionalis	stripless treefrog	LC	LC	7	0	7
	Pelobates cultripes	iberian spadefoot toad	LC	LC	224	35	259
	Pleurodeles waltl	sharp-ribbed newt	LC	LC	50	27	77
	Rana perezi	iberian green frog	LC	LC	5	3	8
	Salamandra salamandra	fire salamander	LC	LC	96	25	121
	Triturus marmoratus	marbled newt	LC	LC	8	0	8
	Urodelo NI	-	-	-	0	1	1
Birds	*Alauda arvensis*	skylarq	LC	LC	0	3	3
	Alcedo atthis	kingfisher	LC	LC	1	0	1
	Alectoris rufa	red-legged patridge	LC	LC	2	1	3
	Anthus campestris	tawny pipit	LC	LC	0	1	1
	Anthus sp.	-	-	-	1	0	1
	Athene noctua	litle owl	LC	LC	5	7	12
	Bird NI	-	-	-	1	3	4
	Bubulcus ibis	cattle egret	LC	LC	0	1	1
	Buteo buteo	buzzard	LC	LC	0	2	2
	Carduelis cannabina	linnet	LC	LC	1	0	1
	Carduelis carduelis	goldfinch	LC	LC	1	9	10
	Carduelis chloris	greenfinch	LC	LC	1	0	1
	Carduelis spinus	siskin	LC	LC	1	0	1
	Ciconia ciconia	white stork	LC	LC	1	0	1
	Cisticola juncidis	fan-tailed warbler	LC	LC	2	1	3
	Columba livia	rock dove	DD	LC	2	6	8
	Coturnix coturnix	quail	LC	LC	0	1	1
	Delichon urbica	house martin	LC	LC	0	1	1
	Emberiza calandra	corn bunting	LC	LC	18	13	31
	Erithacus rubecula	robin	LC	LC	12	17	29
	Falco tinnunculus	kestrel	LC	LC	0	2	2
	Fringilla coelebs	chaffinch	LC	LC	0	7	7
	Galerida cristata	crested lark	LC	LC	1	0	1
	Galerida sp.	-	-	-	0	3	3
	Galerida theklae	thekla lark	LC	LC	0	1	1
	Hirundo daurica	red-rumped swallow	LC	LC	2	3	5
	Hirundo rustica	barn swallow	LC	LC	0	1	1
	Hyppolais polyglotta	melodious warbler	LC	LC	0	1	1
	Lanius meridionalis	Southern grey shrike	LC	LC	1	0	1
	Lanius senator	woodchat shrike	NT	LC	11	6	17
	Motacilla alba	white wagtail	LC	LC	0	2	2
	Muscicapa striata	spotted flycatcher	NT	LC	1	0	1
	Oenanthe hispanica	black-eared wheatear	VU	LC	0	2	2
	Parus caeruleus	blue tit	LC	LC	11	14	25
	Parus major	great tit	LC	LC	2	6	8
	Parus sp.	-	-	-	0	2	2
	Passer domesticus	house sparrow	LC	LC	32	9	41
	Passer hispaniolensis	spanish sparrow	LC	LC	1	2	3
	Passer sp.	-	-	-	4	42	46
	Passerin NI	-	-	-	56	36	92

Class	Species		Status		Roadkills		
Years	**Scientific name**	**Common name**	**ICNB**	**IUCN**	**1996**	**2005**	**Total**
	Petronia petronia	rock sparrow	LC	LC	1	1	2
	Phylloscopus collybita	chiffchaff	LC	LC	13	24	37
	Phylloscopus trochilus	melodious warbler	LC	LC	0	1	1
	Pica pica	magpie	LC	LC	0	1	1
	Saxicola torquatus	stonechat	LC	LC	14	23	37
	Serinus serinus	serin	LC	LC	7	8	15
	Strix aluco	tawny owl	LC	LC	2	7	9
	Sylvia atricapilla	black cap	LC	LC	26	5	31
	Sylvia melanocephala	sardinian warbler	LC	LC	14	21	35
	Sylvia sp.	-	-	-	5	4	9
	Sylvia undata	dartford warbler	LC	LC	7	1	8
	Troglodytes troglodytes	wren	LC	LC	1	0	1
	Turdus merula	blackbird	LC	LC	4	4	8
	Turdus philomelos	song thrush	LC	LC	1	0	1
	Turdus sp.	-	-	-	1	0	1
	Tyto alba	barn owl	LC	LC	6	7	13
	Upupa epops	hoopoe	LC	LC	0	1	1
Mammals	*Apodemus sylvaticus*	wood mouse	LC	LC	13	10	23
	Canis familiaris	domestic dog	NA	NA	11	3	14
	Chiroptera NI	-	-	-	3	2	5
	Crossidura russula	greater white-toothed shrew	LC	LC	3	0	3
	Erinaceus europaeus	western hedgehog	LC	LC	7	27	34
	Felis catus	domestic cat	NA	NA	8	12	20
	Genetta genetta	genet	LC	LC	3	3	6
	Herpestes ichneumon	Egyptian mongoose	LC	LC	0	1	1
	Lagomorph NI	-	-	-	0	2	2
	Lepus granatensis	iberian hare	LC	NA	6	8	14
	Mammal NI	-	-	-	1	8	9
	Martes foina	stone marten	LC	LC	0	3	3
	Meles meles	badger	LC	LC	4	2	6
	Microtus cabrerae	Cabrera’s vole	VU	NT	1	0	1
	Muridae NI	-	-	-	3	4	7
	Mus spretus	Algerian mouse	LC	LC	2	2	4
	Mustela nivalis	weasel	LC	LC	0	1	1
	Mustela putorius	polecat	DD	LC	1	3	4
	Oryctolagus cuniculus	rabitt	NT	LC	3	15	18
	Pipistrellus kuhli	Kuhl's pipistrelle	LC	LC	4	0	4
	Pipistrellus sp.	-	-	-	5	10	15
	Rattus norvegicus	brown rat	NA	NA	1	0	1
	Rattus rattus	black rat	LC	LC	0	1	1
	Rattus sp.	-	-	-	9	2	11
	Rhinolophus ferrumequinum	greater horseshoe bat	VU	NT	1	0	1
	Talpa occidentalis	iberian mole	LC	LC	1	7	8
	Vulpes vulpes	red fox	LC	LC	6	4	10
Reptiles	*Coronella girondica*	southern smooth snake	LC	NE	3	2	5
	Colluber hippocrepis	horseshoe whip snake	LC	NE	0	2	2
	Lacerta lepida	ocellated lizard	LC	NE	24	6	30
	Macroprotodon cucullatus	false smooth snake	LC	NE	9	3	12
	Malpolon monspessulanus	Montpellier snake	LC	NE	0	1	1
	Mauremys leprosa	Mediterranean pond turtle	LC	NE	10	6	16
	Natrix maura	viperine snake	LC	NE	8	8	16
	Natrix sp.	-	-	-	12	14	26
	Snake NI	-	-	-	0	3	3
	Psammodromus algirus	large Psammodromus	LC	LC	5	5	10
	Elaphe scalaris	ladder snake	LC	NE	0	4	4
Total					1413	660	2073

Considerações finais

Desde 1945, a rede rodoviária em Portugal tem apresentado um elevado crescimento e expansão, cifrando-se em aproximadamente 15,505 km de estradas alcatroadas (INE 2007). Desta forma os impactes das rodovias na biodiversidade em Portugal subiram exponencialmente. Todavia, pouca atenção foi sendo dedicada a esta problemática. Os trabalhos realizados são poucos e apenas despontaram na última década. Destacam-se as publicações de Brito & Alvares (2004) que se focou nos padrões de mortalidade de duas espécies de víboras. O trabalho de Petronilho & Dias (2005) que comparou o impacte de duas estradas florestais, uma alcatroada na totalidade e outra parcialmente alcatroada, nos padrões espaciais e temporais dos vertebrados. Ascensão & Mira (2007) e mais recentemente o estudo de Grilo *et al.* (2008) testaram o uso de passagens hidráulicas por carnívoros e outros pequenos mamíferos. Por fim um novo estudo de Grilo *et al.* (2009) que estudou os padrões espaciais e temporais da mortalidade de carnívoros no Alentejo.

A investigação sobre os efeitos das estradas na Biodiversidade e as respectivas medidas mitigadoras para os combater, surge assim como fulcral no contexto nacional, sendo de extrema importância promover linhas de investigação que visam diagnosticar correctamente o problema e permitam encontrar respostas para a implementação de medidas de mitigação mais eficientes. Foi neste contexto que se enquadrou o presente trabalho.

O estudo apresentado foi um dos poucos que tentou identificar os factores determinantes na mortalidade em rodovias num ambiente Mediterrâneo, recorrendo a técnicas de partição da variância. Assim, avaliou-se a influência relativa de descritores relacionados com o uso dos solos, métrica da paisagem e localizações espaciais dos atropelamentos. Este trabalho foi também o primeiro a apresentar dados comparativos na mortalidade rodoviária para um mesmo troço, entre dois anos separados temporalmente por período relativamente longo (nove anos). Acresce o facto de 2005 ter sido um ano de seca extrema. Este facto permitiu uma primeira avaliação do efeito que, estas condições climáticas particulares teriam nos padrões de atropelamento de vertebrados. Finalmente, o aumento da intensidade de tráfego no troço estudado, nove anos depois, permitiu-nos também tecer algumas considerações acerca do impacte do tráfego na mortalidade.

O número de atropelamentos foi elevado em ambos os anos, principalmente em 1996, comparativamente o registado noutros estudos. Estes resultados podem reflectir o facto da área de estudo numa região de fronteira entre duas regiões biogeográficas: Atlântica e Mediterrânea. Este facto promove a presença de uma abundância e número de espécies elevada, estando esta área incluída num dos “hotspots” de biodiversidade a nível mundial (Myers *et al.* 2000).

Os factores que melhor explicaram a mortalidade de vertebrados foram, na sua maioria, coincidentes em ambos os anos. O grupo de variáveis com mais importância foi a métrica da paisagem, seguindo-se os efeitos da localização espacial. Pontualmente alguns factores relativamente ao uso dos solos foram também importantes para alguns grupos de vertebrados. Os aspectos seguintes resumem os principais resultados do corrente estudo:

- ***O Parque Natural da Serra de São Mamede (PNSSM).***

A distância ao PNSSM (DPark) foi a variável mais importante, correlacionando-se negativamente com a mortalidade de quase todos os grupos de vertebrados, em ambos os anos. Sugere-se que à medida que nos afastamos do PNSSM e nos movemos para sul os índices de biodiversidade e abundância diminuem, reflectindo um menor número de atropelamentos. Junto ao PNSSM, factores como a existência de pequenas vinhas, hortas e pomares (culturas mistas), os maiores níveis de humidade e a elevada extensão de áreas florestais deverão, em conjunto contribuir para a explicação destes resultados;

  - ***As culturas mistas.***

Os locais, com outras culturas (O_cul), foram uma variável importante, dentro do uso dos solos em ambos os anos, predominando nos locais onde se concentrou a mortalidade de pequenos mamíferos, coelhos, ouriços e também de passeriformes. Estas área, para estas espécies podem constituir um bom recurso na medida em que proporcionam abundância e diversidade de alimento, alguns abrigos e em geral grande disponibilidade de água, mesmo quando próximas de zonas urbanas (Rondinini & Doncaster 2002; Erritzoe et al. 2003);

  - ***Os níveis de humidade.***

Em 1996 os níveis de humidade foram elevados, resultando num elevado número de anfíbios atropelados na zona norte da estrada em estudo, junto ao PNSSM.

Em 2005 a água poderá ter sido um factor limitante, que restringiu os movimentos dos vertebrados, nomeadamente anfíbios, tornando-os menos susceptíveis à mortalidade rodoviária.

  - ***As áreas florestais.***

Em ambos os anos a mortalidade, nomeadamente de passeriformes e anfíbios, concentrou-se, maioritariamente, em zonas próximas de povoamentos florestais de montado e/ou olival localizadas na parte norte do troço. Muitas espécies de passeriformes são particularmente abundantes nestas zonas (Erritzoe et al. 2003). Por outro lado, as áreas florestais apresentam uma cobertura de vegetação mais elevada onde os níveis de humidade são superiores, o que favorece a presença de anfíbios (Findlay et al. 2001);

- ***As galerias ripícolas.***

Em 2005 os atropelamentos de carnívoros concentraram-se mais a sul do troço, junto à Ribeira Grande, os habitats ripícolas parecem ser refúgios particularmente importantes em tempos de seca, particularmente em áreas abertas (Virgós 2001; Matos et al. 2008);

- ***O tráfego.***

  O aumento da intensidade de tráfego em 2005 poderá explicar, pelo menos parcialmente os aumentos consideráveis na mortalidade de ouriços e corujas, grupos que são particularmente susceptíveis ao atropelamento (Huijser & Bergers 2000; Fajardo 2001). O aumento da velocidade poderá justificar o aumento da mortalidade de passeriformes em 2005 (Erritzoe et al. 2003; Van Langevelde & Jaarsma 2004);

Os níveis de mortalidade rodoviária obtidos foram elevados e alertam para a necessidade de implementação de medidas mitigadoras neste troço. A identificação de pontos-negros de mortalidade dever ser efectuada com base na bio-eologia das espécies, devendo ser consideradas de forma agregada, espécies com necessidades idênticas (Caro *et al.* 2000; Underhill & Angold 2000; Iuell *et al.* 2003). Os anfíbios devido às elevadas taxas de mortalidade que normalmente apresentam, deverão ser prioritários neste tipo de estudos (Benayas *et al.* 2006; Cushman 2006). Esta informação, quando aliada à identificação dos factores que promovem a mortalidade por atropelamento constitui a base para a implementação de medidas mitigadoras eficazes. Esperamos que o presente estudo seja um primeiro contributo para a compreensão dos fenómenos em análise e que ajude na definição de linhas orientadoras para a investigação que visa encontrar mais e melhores soluções para a minimização do problema da mortalidade que é transversal a toda a fauna terrestre.

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