AVALIAÇÃO DOS EFEITOS DAS ESTRADAS NOS MORCEGOS: A IMPORTÂNCIA DA PAISAGEM, DAS CARACTERÍSTICAS DAS ESTRADAS E DA ACTIVIDADE NOS ATROPELAMENTOS.

ASSESSING ROAD EFFECTS ON BATS: THE ROLE OF LANDSCAPE, ROAD FEATURES AND BAT ACTIVITY ON ROAD KILLS.

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"Versão Corrigida"

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Artigo Científico

"Assessing road effects on bats: the role of landscape, road features and bat activity on road kills."

Resumo:

Estudos recentes sugerem que as estradas podem ter um impacto significativo nas populações de morcegos. Este é um dos grupos de vertebrados europeus mais ameaçado, porém continua a faltar informação sobre quais os principais factores que determinam este impacto e quais as possíveis medidas de mitigação. Entre Março e Outubro de 2009, foram amostrados diariamente 51 km de três tipos de rodovias. Recolhemos 154 morcegos atropelados, pertencentes a 11 espécies, as mais frequentes foram *Pipistrellus kuhlii* e *P. pygmaeus*, representando cerca de 64% da amostra. Foram também recolhidas espécies com estatuto de ameaça ou pouco conhecidas, tal como, *Rhinolophus ferrumequinum e Barbastella barbastellus* A maioria da mortalidade ocorreu entre o final do Verão e o princípio do Outono. A actividade dos morcegos foi também monitorizada na área de estudo e encontrou-se uma relação positiva forte entre as áreas com maior actividade e os *hotspots* de mortalidade de morcegos. As características da paisagem foram o conjunto mais importante na explicação do padrão da mortalidade, tendo sido registado um maior número de atropelamentos em locais onde a estrada atravessa habitats de grande qualidade. Os resultados indicam também que o volume do tráfego e a proximidade aos abrigos contribui para o incremento da mortalidade. Relativamente à actividade dos morcegos, não encontramos uma clara evidência de que os morcegos evitem a proximidade das estradas.

Palavra-chave: Morcegos, Mortalidade por atropelamento, Paisagem Mediterrânica, Actividade de morcegos, Partição de variância.

Abstract:

Recent studies suggest that roads can significantly impact bat populations. Thought bats are one of the most threatened groups of European vertebrates, studies aiming to quantify bat mortality and determine the main factors driving it are still scarce. Between March and October 2009, we daily surveyed road killed bats in a transect of 51 km including different types of roads, in southern Portugal. Bat activity was also evaluated on roads and their surroundings. We found 154 road-killed bats of 11 species. *Pipistrellus kuhlii* and *P. pygmaeus* represented 64% of total specimens collected. We also found threatened and poorly known species like *Barbastella barbastellus* and *Rhinolophus ferrumequinum*. A peak of mortality occurred mostly in late summer and early autumn. Spatial analysis revealed a strong positive relationship between core activity areas and the bat mortality hotspots. Landscape features were the most important variable set in explaining bat casualties. Nevertheless, bat activity, distance to known roosts and traffic volume also had a significant influence on it. Concerning activity data, we found no clear evidence of road avoidance by bats.

Key words: Bats; Road kills; Mediterranean landscape; Bat activity; Variance partitioning.

Introdução:

A biodiversidade global está a ser alterada a um ritmo improcedente como resultado de diversas alterações induzidas pelo Homem no meio ambiente (MEA, 2005; Pimm et al., 1995, Sala et al., 2000). Os factores principais que afectam a biodiversidade devem-se às mudanças no uso e na cobertura do solo, alterações climáticas, fragmentação e desenvolvimento de infra-estruturas (Alkemade et al., 2009; Sala et al., 2000; Sanderson et al., 2002). A ubiquidade das redes rodoviárias e o crescimento do conhecimento sobre o conjunto de evidências dos efeitos negativos que estas infra-estruturas têm na vida selvagem e nos ecossistemas, sugerem que estas podem ser o factor fundamental na perda de biodiversidade (Alkemade et al., 2009). Durante os últimos séculos a paisagem tem sofrido alterações marcantes, das quais a rede rodoviária constitui um dos exemplos mais visíveis e radicais (Forman, 2000; Trocmé et al., 2003). Estas redes de transporte são uma componente intrínseca do quotidiano, facilitando o movimento e o desempenho das trocas comerciais e potenciando inúmeras interacções sociais (Forman et al., 2003). Um variado conjunto de factores socioeconómicos tem propiciado que as estradas e outras infra-estruturas lineares se tornassem uma componente cada vez mais conspícua da nossa paisagem, aumentando consequentemente o número de veículos que nelas circulam (Giullio et al., 2009, EEA, 2002). Dargay et al. (2006) concluíu que no início do actual milénio existiam mais de 800 milhões de veículos em circulação, grande parte destes concentrados em paisagem já altamente modificadas pelo Homem.

Os efeitos das estradas vão para além da zona asfaltada e das zonas imediatamente em volta. Segundo alguns estudos, estes podem mesmo chegar a várias centenas de metros para além da zona pavimentada (e.g. Benítez-López et al., 2010; Forman & Deblinger, 2000). Na generalidade, a presença de uma estrada está altamente correlacionada com mudanças na composição específica local (Forman & Alexander, 1998), no tamanho da população (Benítez-López et al., 2010; Fahrig & Rytwinski, 2009), nas áreas vitais (Bissonette & Rosa, 2009), nos padrões de movimento (Clevenger et al., 2003), no sucesso reprodutivo (Trombulak & Frissel, 2000), na capacidade de resposta (Jaeger et al., 2005) e no estado fisiológico das espécies (Coffin, 2007; Spellerberg, 1998). Possivelmente um dos impactos principais e mais preocupantes dos efeitos das estradas é a fragmentação de habitat e o consequente isolamento das populações, criado pelo efeito barreira ou de repulsa que estas estruturas têm nalgumas espécies. Portanto, a manutenção da conectividade entre as várias parcelas de habitat numa determinada paisagem, constitui cada vez mais um importante desafio para os agentes implicados na conservação da natureza e na gestão do território (Crooks & Sanjayan, 2006; Lookingbill et al., 2010). Porém, o efeito negativo mais visível das estradas é a mortalidade, causada pela colisão com os veículos, com particular importância para as espécies com maiores domínios vitais ou no caso de populações pequenas de espécies raras (Ascensão & Mira, 2006; Ferreras et al., 1992; Lovari et al., 2007). As rodovias podem actuar como barreiras ou filtros nos movimentos durante os períodos de alimentação ou dispersão, impedindo o uso de habitats óptimos para as populações locais.

Devido à crescente exigência de mais e melhores vias de comunicação pela sociedade, o conflito entre as estradas e a natureza está cada vez mais intenso. Estamos assim perante o desafio de adaptar as actuais e futuras rodovias a um sistema sustentável, que permita mitigar e corrigir os efeitos nefastos destas infra-estruturas. Nos últimos anos foi surgindo muita informação focada na mortalidade de vários mamíferos (Barthelmess and Brooks, 2010; Carvalho & Mira, 2010; Grilo et al., 2008, 2009; Ramp & Roger, 2009; Rico et al., 2007), aves (Erritzoe et al., 2003; Gomes et al., 2009), anfíbios (Eigenbrod et al., 2008; Goosem et al., 2007; Fahrig et al., 1995; Mazerolle, 2004) e répteis (Woltz et al., 2008; Vijayakumer et al., 2001), porém existem poucos estudos que se foquem nos impactos das estradas nas comunidades de morcegos (Bafaluy, 2000; Gaisler et al., 2009; Kerth & Melber, 2009; Lesiński et al., 2007, 2010; Limpens et al., 2005; Russell et al., 2009).

O estudo dos efeitos destas estruturas neste grupo faunístico é relativamente recente e a maioria da informação vem do Nordeste e Centro da Europa (e.g. Gaisler et al., 2009; Haensel and Rackow, 1996; Kerth and Melber, 2009; Kiefer et al., 1995; Lesiński et al., 2007, 2010; Limpens et al., 2005), porém para o Sul da Europa e na América do Norte este conhecimento é ainda escasso (Bafaluy, 2000; González-Prieto et al., 1993; Keeley, no date; Russell et al., 2009). As estradas podem estar a contribuir para agravar as consequências das ameaças externas à conservação destes animais, ainda para mais quando este grupo faunístico tem associado alguns factores intrínsecos, como a baixa taxa de reprodução ou requisitos ecológicos muito especializados, que dificultam a viabilização das populações. Atendendo às evidências de declínio das populações a um nível global, bem como à diminuição da área de distribuição de muitas espécies e consequentemente dos seus estatutos de conservação, é peremptório que haja maior e melhor informação sobre como podemos mitigar os efeitos das estradas neste grupo. Os estudos acima referidos reportam que os impactos das rodovias neste grupo podem ser bastante significativos, podendo mesmo ser um dos grupos de mamíferos mais afectados por esta problemática (Haensel & Rackow, 1996; Lesiński, 2007). Porém as espécies podem ser diferentemente afectadas, dependendo especialmente da altura de voo e da estratégia de alimentação (Lesiński et al., 2010; Stratman, 2006).

Brinkmann et al. (2008) referem que as espécies mais vulneráveis aos atropelamentos são aquelas que têm um voo baixo e lento, enquanto que as mais susceptíveis à fragmentação do habitat são aquelas que preferem voar ao longo de estruturas na paisagem, incluindo as espécies que caçam maioritariamente sobre massas de água. Muitas espécies tendem a orientar-se através das características da paisagem (marcas da paisagem), sobretudo com a ajuda de elementos bem definidos que se encontram na mesma, são exemplo disso as estruturas lineares, tais como linhas de árvores, sebes, orlas de floresta, estradas, entre outras marcas (Grodzinski et al., 2010; Holderied et al., 2006; Limpens and Kapteyn, 1991). Estas estruturas providenciam protecção do vento e, dos predadores, disponibilidade de abrigo e, nalguns casos, são zonas com grande abundância de insectos (Verboom & Huitema, 1999).

O alto nível de ruído perto das estradas pode reduzir a distância e a área de cobertura dos sinais acústicos emitidas pelos animais, podendo ser especialmente perturbador para os grupos que comunicam e caçam através da emissão de ecolocalização (Laiolo, 2010; Barber et al., 2009). Um estudo recente de Schaub et al. (2008) mostrou como o ruído do tráfego pode influenciar o comportamento de animais que procuram o seu alimento pelo barulho que as presas produzem ao deslocar-se na vegetação.

Muitos estudos referem também que as comunidades locais de morcegos e a sua distribuição, estão associadas com o tipo de paisagem, condicionadas pela diversidade de abrigos de maternidade, pela proximidade de abrigos de hibernação ou mesmo pela topografia, latitude e condições climáticas, para além de causas antropogénicas, como a gestão do uso do solo, urbanização e desflorestação (Duchamp et al., 2004; Furlonger et al., 1987; Ghert and Chelsvig, 2003, 2004; Graham, 1983; Humphrey, 1975; Owen et al., 2004; Patten, 2004; Sparks et al., 2005). Contudo, muito pouco se conhece sobre a resposta das comunidades de morcegos à presença e à distância das estradas. Alguns estudos recentes forneceram modelos preditivos da ocorrência de mortalidade (Ascensão & Mira, 2006; Barrientos & Bolonio, 2009; Grilo et al., 2009; Malo et al., 2004; Ramp et al., 2005), porém poucos utilizam variáveis de uso do espaço, que segundo Roger & Ramp (2009) têm um elevado poder preditivo, aumentando assim a capacidade explicativa do modelo.

A crescente consciencialização da opinião pública sobre os efeitos das estradas, associada a uma crescente necessidade de gestão da fauna cinegética, e a uma urgência nas medidas da segurança rodoviária, bem como a uma preocupação crescente sobre a saúde pública, tem obrigado a que os agentes responsáveis pela administração das redes viárias reúnam esforços no sentido da elaboração e implementação de medidas de mitigação mais eficazes (Bruinderink & Hazebroek, 1996; Seiler, 2003). Desta forma, têm vindo a ser publicados alguns trabalhos que abordam a questão de onde se deve intervir, de forma a reduzir o risco de colisão entre os veículos e os animais (Gomes et al., 2009; Ramp et al., 2006). Estes estudos têm como objectivos a definição e a localização de pontos negros de mortalidade (*hotspots*), ou seja, locais onde há uma agregação ou uma distribuição anómala da mortalidade de uma espécie ou de um grupo de animais (Clevenger et al., 2003; Malo et al., 2004).

Muitos dos padrões de mortalidade descritos na bibliografia estão intrinsecamente ligados às características da paisagem nas zonas adjacentes à estrada. Por exemplo, na vizinhança de uma mancha de vegetação autóctone ou na proximidade de uma massa de água, existe nestes locais um acréscimo da probabilidade de atropelamento dos animais. No caso específico dos morcegos, existe pouca informação sobre quais as características da paisagem, as características da estrada ou mesmo os requisitos ecológicos do grupo que aumentam o risco de colisão com os veículos. Para isso é necessária mais informação, de forma a completar esta lacuna e aumentar a eficácia na implementação de medidas de mitigação dos efeitos das estradas.

Objectivos:

O presente estudo foi desenvolvido para tentar esclarecer a relação entre as estradas e as comunidades de morcegos numa paisagem Mediterrânica, com o propósito de contribuir para um melhor planeamento e gestão da rede rodoviária. Os principais objectivos deste estudo são: i) descrever o padrão espacial e temporal da mortalidade de morcegos vítima de colisão com veículos, identificando a localização dos pontos negros de mortalidade; ii) quantificar o efeito da distância à estrada no nível da actividade de morcegos e na riqueza específica; e por fim iii) identificar quais os factores mais importantes na mortalidade de morcegos e quantificar a importância relativa das características da paisagem, especificidades da estrada e requisitos ecológicos dos morcegos na distribuição dos atropelamentos.

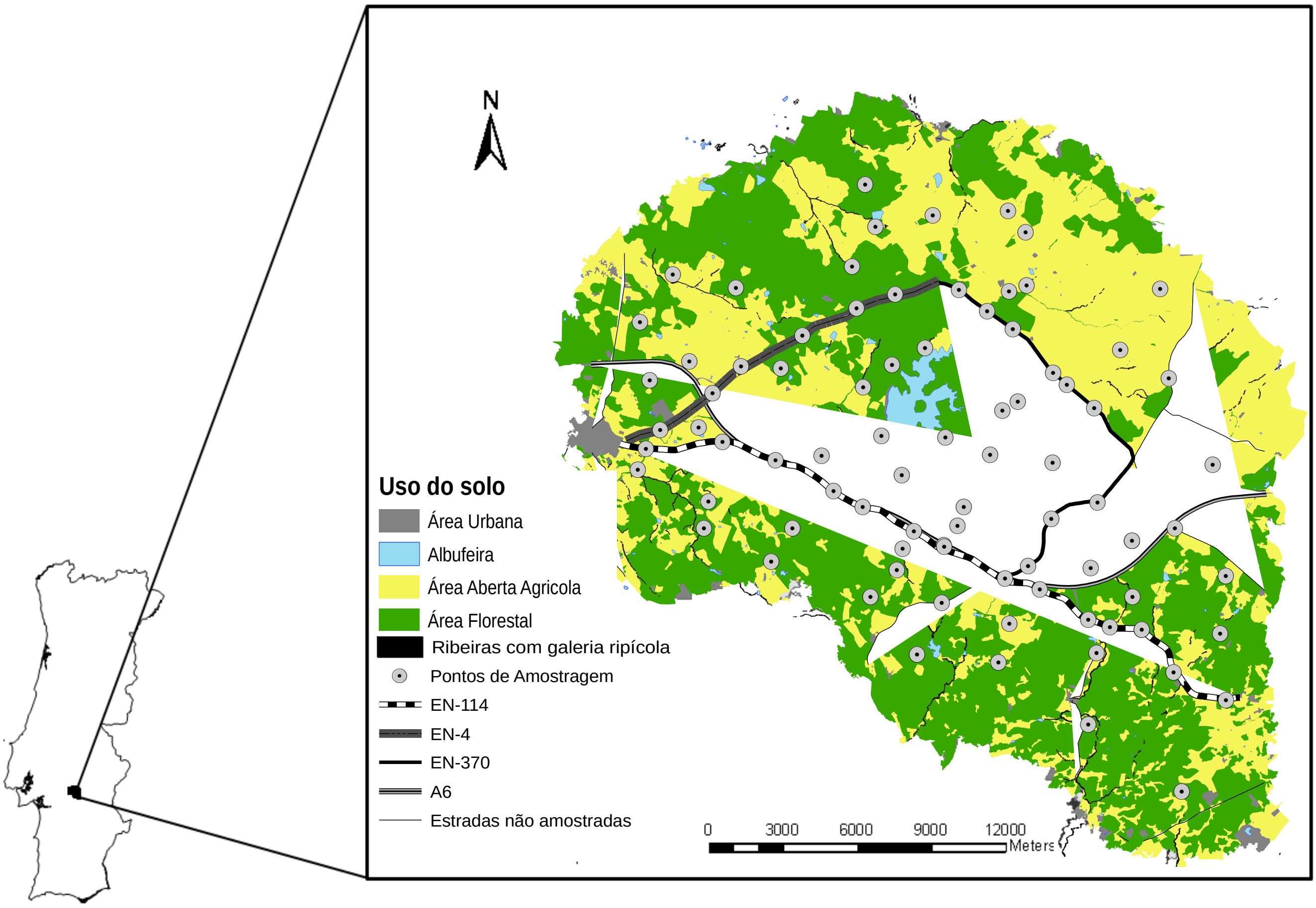

Figura 1. Localização da área de estudo, estradas amostradas e pontos de amostragem da actividade de morcegos

Artigo Científico:

ASSESSING ROAD EFFECTS ON BATS: THE ROLE OF LANDSCAPE, ROAD FEATURES AND BAT ACTIVITY ON ROAD KILLS.

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Abstract: Recent studies suggest that roads can significantly impact bat populations. Thought bats are one of the most threatened groups of European vertebrates, studies aiming to quantify bat mortality and determine the main factors driving it are still scarce. Between March and October 2009, we daily surveyed road killed bats in a transect of 51 km including different types of roads, in southern Portugal. Bat activity was also evaluated on roads and their surroundings. We found 154 road killed bats of 11 species. *Pipistrellus kuhlii* and *P. pygmaeus* represented 64% of total specimens collected. We also found threatened and poorly known species like *Barbastella barbastellus* and *Rhinolophus ferrumequinum*. A peak of mortality occurred mostly in late summer and early autumn. Spatial analysis revealed a strong positive relationship between core activity areas and the bat mortality hotspots. Landscape features were the most important variable set in explaining bat casualties. Nevertheless, bat activity, distance to known roosts and traffic volume also had a significant influence on it. Concerning activity data, we found no clear evidence of road avoidance by bats.

Key words: Bats; Road kills; Mediterranean landscape; Bat activity; Variance partitioning.

1. Introduction:

Roads are widespread and increasingly present in most world landscapes. In fact, transport infrastructure is a conspicuous component of recent land-use, and roads and traffic densities are usually high in a human-dominated landscape (EEA, 2002; Giullio et al., 2009). They are associated with negative effects on biotic integrity in ecosystems and play a substantial role in defining landscape pattern (Hawbaker et al., 2004; Trombulak and Frissel, 2000). The ubiquity of road networks represents a major driving factor of biodiversity loss (Alkemade et al., 2009; Benitez-López et al., 2010; Forman et al., 2003; Spellerberg, 1998). Overall, the presence of roads is highly correlated with changes in species composition (Forman and Alexander, 1998), population size (Benítez-López et al., 2010; Fahrig and Rytwinski, 2009), home range (Bissonette and Rosa, 2009), movement patterns (Clevenger et al., 2003), reproductive success (Trombulak and Frissel, 2000), escape response (Jaeger et al., 2005), and physiological state (Coffin, 2007; Spellerberg, 1998) but species and ecosystems are not equally affected by them. The most evident effect of roads on wildlife is the mortality caused by collisions with motor vehicles, with particular importance for species with large home ranges (Ascensão and Mira, 2006; Forman and Alexander, 1998; Grilo et al., 2008; Sherwood et al., 2002;). Roads can also act as barriers or filters to movement during foraging and dispersal. Thus, local animal population may be prevented from accessing a formerly used habitat which can lead to population decline (Clark et al., 2010; Eigenbrod, 2008; Murcia, 1995). This is supported by reports that small and recently constructed roads can have a significant impact on the population genetic structure of some species (Clark et al., 2010).

While much documentation of road kills has focused on terrestrial mammals (Barthelmess and Brooks, 2010; Benítez-López et al., 2010; Carvalho and Mira, 2010; Grilo et al., 2008, 2009; Ramp and Roger, 2009; Rico et al., 2007), birds (Erritzoe et al., 2003; Gomes et al., 2009), amphibians (Eigenbrod et al., 2008; Fahrig et al., 1995; Goosem et al., 2007; Mazerolle, 2004) and reptiles (Vijayakumar et al. 2001; Woltz et al., 2008), a few studies focused on bats (Bafaluy, 2000; Gaisler et al., 2009; Kerth and Melber, 2009; Lesiński et al., 2007, 2010; Limpens et al., 2005; Russell et al., 2009).

The study of the impacts of roads on bat populations is a relatively new field in conservation, with most information coming from eastern and central Europe (e.g. Gaisler et al., 2009; Haensel and Rackow, 1996; Kerth and Melber, 2009; Kiefer et al., 1995; Lesiński et al., 2007, 2010; Limpens et al., 2005). On the other hand, in North America and southern Europe information is still scarce (Bafaluy, 2000; González-Prieto et al., 1993; Keeley, no date; Russell et al., 2009). Road mortality may pose additional conservation problems to bats due to their characteristic low reproductive rate which further increases the extinction risk. However, bat species may be differently affected by roads, depending on their species specific behaviour, in particular their flight height and foraging strategies (Brinkmann et al., 2008). There is mounting evidence that species usually flying at a lower height are more susceptible to road casualties (Haensel and Rackow, 1996; Lesiński, 2007; Stratman, 2006) but, for fast and high flying species, the risk of collision seems to be negligible because they can easily cross open air spaces (Limpens et al., 2005). Furthermore, roads can cause habitat fragmentation for bat species that use landscape structures for guidance, including species that regularly fly over water (Limpens et al., 2005).

Many bat species commute along well-defined linear landscape features, such as hedgerows, treelines, waterways, woodland edge and roads (Grodzinski et al., 2010; Holderied et al., 2006; Limpens and Kapteyn, 1991). These routes provide shelter from wind and avian predators. They also act as acoustic landmarks or guiding structures, allowing bats to navigate using short-range echolocation (Verboom and Huitema, 1999). The higher noise levels near roads can reduce the distance and area over which acoustic signals can be perceived by animals, particularly those that use echolocation (Barber et al., 2009; Laiolo, 2010). A new study by Schaub et al. (2008) shows how traffic noise can influence foraging behaviour in animals that find their food by listening for the sounds that their prey produce. Differences in bat community composition and distribution across different types of landscapes, controlling for summer roosts diversity, proximity to winter hibernacula, topography, latitude, and climate conditions, as well as human-induced land use changes, such as urbanization and deforestation, have been evaluated (Duchamp et al., 2004; Furlonger et al., 1987; Ghert and Chelsvig, 2003, 2004; Graham, 1983; Humphrey, 1975; Owen et al., 2004; Patten, 2004; Sparks et al., 2005). However, to our knowledge, no studies compared the response of bat species with different foraging ecology to the presence and distances to roads.

Recently, many quantitative models of animal-vehicle collision have been developed (Macdonald, 2004; Malo et al., 2004; Jaeger et al., 2005; Orlowski and Nowak, 2006; Saeki and Gaines et al., 2005). Ramp and Roger (2009) reported the importance of space use patterns and population density variables when modelling wildlife road mortality. In fact, when this information is included in road fatality models it improves their predictive capacity (Baker et al., 2004; Grilo et al., 2009; Jaarsma et al., 2007; Mikusinski et al., 2007; Seiler, 2005) and the models will be more effective and efficient in the identification of mortality hotspots of the collisions between animals and vehicles (Gomes et al., 2008; Malo et al., 2004; Ramp and Roger, 2009). Results of previous studies on bats do not provide much information regarding factors increasing the risk of vehicle-collision, although some authors state that habitat surrounding the road plays an important role in the frequency of bat collisions (Lesiński, 2007). For example, linear landscape elements and wetlands promote the risk of casualties (Gaisler, 2009; Lesiński, 2007, 2008). However, particularly in Mediterranean landscapes, more information is needed to properly evaluate areas where high bat vehicle-collision risk is predicted if we aim to implement efficient mitigation measures. Therefore, the main goals of our study are: i) to describe the spatial and temporal pattern of bat vehicle-collisions, identifying hotspot locations; ii) to quantify the effect of distance to roads on bat activity level and species richness, and iii) to identify the most important factors influencing bat casualties and to quantify the relative importance of landscape characteristics, road features and bat ecological traits on road kills.

2. Methods

2.1 Study area

The study area, with 39400 ha is located in central Alentejo, southern Portugal ($38^{o}66'$N, $8^{0}07'$W) on the main terrestrial transportation corridor linking Lisbon to Madrid. The corridor is crossed by different types of roads including a major motorway (A6), two National Roads with a medium/high traffic volume (EN4 and EN 114) and several other National or Municipal paved roads with low traffic volume (less than 3000 vehicles/day) (Figure 1). Topography is plain, with altitude ranging from 100 to 400m. The landscape is dominated by human-altered habitats, including "*montado*", a Mediterranean agro-forestry system of evergreen tree stands of cork (*Quercus suber*) and/or holm (*Q. rotundifolia*) oaks intermixed with extensive agricultural areas including cereal crops, grasslands and meadows, vineyards and olive groves. The area partially includes the Natura 2000 Site *Serra de Monfurado* (PTCON0031). The climate is Mediterranean, characterized by a dry and hot season (Rivas-Martínez and Loidi, 1999) from June to October, with low rainfall and monthly average temperatures ranging from 20 to 23^{o}C and a colder and wetter season in the other months, when monthly mean rainfall is above 70mm and monthly mean temperatures range from 10 to 15^{o}C.

2.2 Bat road kills survey

Between 15th March to 31thOctober 2009, we daily surveyed bat road kills on a 51 km road transect including stretches of different types of roads: high, medium and very low traffic national roads (EN-114, EN-4 and EN-370, respectively) a low traffic regional road (EN-529, hereafter called EN-370). All surveyed roads encompassed two also planned this sampling period based on a preliminary vertebrate mortality study that took place in 2005 and lasted for 365 days. Most of the bats were found road killed between April and mid-October (Mira, A. unpublished data.)

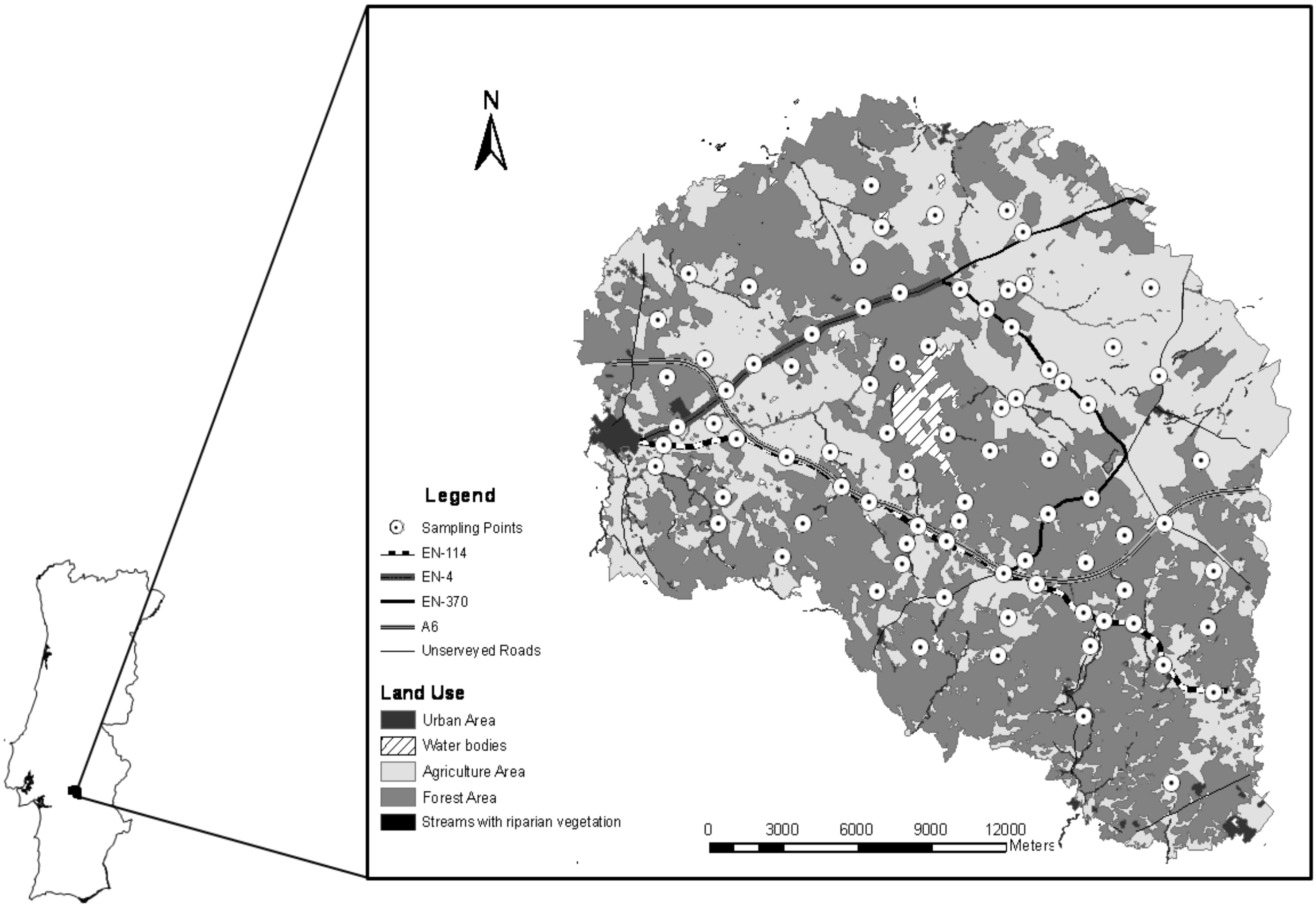

Figure 1. Study area location, surveyed roads and bat activity sampling points.

Bat casualties were searched at early hours of each day to reduce the probability of removal by scavengers and avoid peak traffic volume (Slater, 2002). Daily searches are the recommended sampling technique for recording small vertebrates road kills (Barthelmess and Brooks, 2010; Slater, 2002). In fact, mean bat carcass retention time on these roads is about 1.3 days (Mira, A. unpublished data). Despite this short time of retention we removed all bat carcasses from the road to avoid double-counting and to confirm specific identification. Bats were identified to species level using identification keys (Dietz and von Helversen, 2004; Palmeirim, 1990) and, when possible, sexed and aged. Age was determined by the degree of ossification of the carpal joints and development of nipples and testis (Baagøe, 1977). Bat carcasses that were mere remnants and thus not identifiable, or if their age or sex could not be determined, were excluded from the respective analyses.

2.3 Bat activity survey

We monitored bat activity on 87 sampling points located inside the study area. We surveyed each point for three times between April and September, with a two month interval between surveys. At each point sampling lasted 15 minutes and was done using an ultrasound detector D240x (Pettersson Elektronik AB) and a digital recorder (Archos AV 500 Mobile Digital Video Recorder). Sampling was done on the representative habitats, including streams and at several distances to roads. No surveys were made on windy or rainy nights (Berkova and Zukal, 2006). Sounds were analyzed using Audacity 1.3.10 Beta (http://audacity.sourceforge.net/). We used a sampling frequency of 44.1 kHz, 16bits/sample, and automatic fast Fourier transform size 1024 pt with a Hanning window. We consider a bat pass as a sequence of three or more echolocation pulses in the microphone sampling cone (Fenton, 1999). All passages recorded were screened for six call parameters: frequency of maximum energy (FMaxE), start frequency (SF), end frequency (EF), bandwith (BW), inter-pulse interval (IPI) and pulse duration (D). Bat species were identified using published data on echolocation calls (Obrist and Flückiger, 2004; Papadatou et al., 2008; Parsons and Jones, 2000; Preatoni et al., 2005; Russo and Jones, 2002). Doubtful calls were noted as unidentified bat passes to reduce identification error.

To assess the effects of roads on bat activity, sampling points were grouped into five categories defined on the basis of their distance to the nearest paved road as follows: 1 – 0-100 m; 2 – 100-500 m; 3 – 500-1500 m; 4 – 1500-2500 m; 5 – 2500-3500 m. To diminish the effect of distance to roost only were considered sampling points more than 1km away from known roosts.

2.4 Data analysis

2.4.1 Explanatory variables

For analysis purposes, the four stretches of surveyed roads were subdivided into 500m segments (n=101). At each segment, the number of total bat casualties during the sampling period was the response variable in the mortality model. We used twenty three explanatory variables included in three sets: land use and landscape metrics (LAND); road features (ROAD) and bat activity related variables (BAT) (Table 1). Most LAND explanatory variables were derived from a digital land use map using a 500 m buffer around each road segment. A buffer of 500m has been reported as being meaningful to vertebrate road kills and was a fit length for the implementation of road kill mitigation measures (Boarman and Sazaki 2006; Carvalho and Mira, 2010; Eigenbrod et al.. 2008; Forman and Deblinger, 2000; Grilo et al., 2009).

Main land uses were obtained through interpretation of aerial photos (year 2005), as well as detailed cartography of linear structures (e.g. rivers, streams with and without riparian gallery, paved roads). Several road features were also mapped like culverts, viaducts and fences. Later, we grouped areas with structural resemblance and reclassified the land use data into five classes: (1) “open agricultural-urban area”, which includes cereal crops, fallow and *montados*, with less than 10% tree cover, (2) “sparse *montado* areas”, areas with evergreen oak tree´s cover between 10 - 30%, (3) “dense montado areas”, areas with more than 30% *montado* cover, (4) “forest areas”, areas with more than 30% tree cover of any type, excluding tree riparian galleries, and finally (5) “urban areas”, represented by cities, small villages, and isolated farmhouses. Measurements relative to edge density forestry and mean patch size forestry, as well as others landscape metrics descriptors were computed for each buffer, using the Patch Analyst 4 extension (Elkie et al., 1999) for ArcGis 9.3 GIS program (ESRI, 2008). The location and length of verge with fences on each side of segments were also considered. Minimum distances to others roads, streams, villages and known roosts were assessed from the central point of each segment, using Hawth´s Analysis Tools 3.27 (Beyer, 2004).

Table 1. Description and summary statistics of explanatory variables (three sets LAND, ROAD and BAT) used to describe survey bat casualties locations on the road segment

Variable (unit)	**Description**	**Transformation**	**Mean±S.E.**	**Range**
Landscape characteristics (LAND)				
PSMT (%)	Proportion of Area with Evergreen Oak Tree´s Forest with cover between 10% - 30%	Angular	19.2 ± 2.3	0 – 14.7
PDMT (%)	Proportion of Area with Evergreen Oak Tree´s Forest with more than 30% cover	Angular	41.2 ± 4.6	0 – 15.7
PAA (%)	Proportion of Area with Agricultural-Urban Area (arable fields, pasturelands and <10% of *montado*)	Angular	83.4 ± 5.2	0 – 15.7
PFA (%)	Proportion of Forest Area with more than 30% tree cover	Angular	42.7 ± 4.7	0 – 15.7
PSA (%)	Proportion of Urban Area	Angular	11.4 ± 0.4	0 – 42.9
PWB (%)	Proportion of Water Bodies	Angular	0.5 ± 0.2	0 – 15.1
POLIV (%)	Proportion of Area with Olive groves	Angular	12.5 ± 0.5	0 – 35.8
PVIN (%)	Proportion of Area with Vineyards	Angular	2.3 ± 0.8	0 – 48.6
PTE (%)	Proportion of Area with Pine or Eucalyptus Plantation	Angular	1.1 ±0.3	0 – 34.8
ED_FA (m/ha)	Edge density relative to the forest area	Logarithmic	8.2 ± 2.7	1.3 – 240
MPS_FA (hectares)	Mean Patch Size for forest patches	Logarithmic	16.7 ±1.2	4.5 – 50.0
AWMSI (square meters)	Area weighted Mean Shape Index	-	1.5 ±0.2	1.2 – 2.2
D_SWG (meters)	Minimum distance to stream with riparian gallery	Logarithmic	2.7 ± 4.1	0.6 – 3.3
D_SNG (meters)	Minimum distance to stream without riparian gallery	Logarithmic	2.9 ±0.3	1.8 – 3.4
D_WB (meters)	Minimum distance to water bodies	Logarithmic	2.6 ± 0.3	1.4 – 3.2
D_URB (meters)	Minimum distance to urban areas (> 10 ha)	Logarithmic	3.5 ±0.4	1.2 – 3.9
D_A6 (meters)	Minimum distance to Motorway – A6	Logarithmic	3.1 ± 0.7	1.6 – 3.9
Road features (ROAD)				
TRAF (category)	Traffic intensity estimated in number of vehicles per night (TRAF1) < 150 (TRAF2) 150 - 1200 (TRAF3) > 1200	-	2.15 ± 0.9	1 – 3
L_Fence2 (meters)	Length of road with fences on both sides	Logarithmic	0 ± 4.1	1.2 – 1.8
N_Culv (category)	Number of Culverts in road segment (0) 0 (1) 1 (2) 2 (3) 3	-	0.7 ± 0.8	0 – 3
PA_Viad (category)	Presence (1) or Absence (0) of Viaducts or Bridges in the road segment	-	1.0 ± 0.2	0 – 1
Bat Ecology				
ACT (index)	Average of bat activity level on segments measured from Inverse Distance Weighted interpolation	-	0.9 ± 0.2	0.4 – 1.0
D_Roost (kilometers)	Nearest distance to known roost closest to the road segment	Logarithmic	3.5 ± 0.4	2.1 – 4.0

The bat activity for each road segment was estimated from Inverse Distance Weighted interpolation map based on the mean bat activity data from each sampling point. We used *Zonal Statistics auto* to characterize mean activity level on each segment of roads (Soeller, 2004). Traffic counts were carried out on several segments of the three roads during the same period of bat activity monitoring. Traffic data was later classified into three categories complying with classification of Estradas de Portugal, the Portuguese Road Institute. Accordingly, EN-114 has high/moderate traffic volume during the night (≈1210 vehicles/night), EN-4 has moderate traffic (≈277 vehicles/night) and EN-370 has low traffic (less than 100 vehicles/night).

2.4.2 Statistical analysis:

Prior to statistical analysis, we used Moran´s I to evaluate the presence of spatial autocorrelation on bat fatality data. The distances of bat mortality locations among segments were obtained using the distance along the road "network" with Crimestat III software (Levine et al., 2005). To investigate the locations of fatality hotspots, we compared the spatial pattern of casualties presence with that expected in a random situation in which case the likelihood of collisions for each road section would show a Poisson distribution (Boots and Getis, 1988; Malo et al., 2004). This process is known as Malo´s method and with the application of this equation it is possible to estimate the number of casualties on a random situation. For each segment, the probability of having χ number of casualties is calculated by a) where λ is the mean number of fatalities for 500m road segments.

$$\text{a)} \quad p\,(x) = \frac{\lambda^{x}}{(x!\,e^{\lambda}\,)}$$

For our data, the mean probability of collision was λ=1.3 casualties 500m^{-1}, and therefore with simple computations it was possible to calculate that 93% of all road kills were not in a clustering situation. Therefore, segments with more than 5 casualties were defined as a bat mortality hotspot those. We used the chi-square test to check differences in casualties by sex and age. We also used chi-squared test to determine differences on road kill numbers by road section (Sokal and Rohlf, 1997). The Kruskal-Walis test (Zar, 1999) was carried out to evaluate the significance of proximity to roads on bat activity. Pairwise comparisons of activity in water courses near and away from road were undertaken with Wilcoxon test (Zar, 1999). These computations were done on SPSS 17.0 for Windows software (SPSS 2008, Inc.).

2.4.3 Model building

We used Generalized Linear Methods (GLM) with a Poisson error structure to model the number of bat road kills per road segment. Logarithmic $[\log(x+1)]$ and angular (arcsine $\sqrt{p}$) transformations were applied to continuous variables and proportions, respectively, in order to achieve normality and soften the effects of extreme values (Gotteli and Ellison, 2004; Zuur et al., 2007). To avoid collinearity, prior to analysis, we performed pairwise Pearson rank correlations between all non-categorical explanatory variables. From pairs of variables showing correlation values higher than 0.7, only the most biological meaningful variable was retained for further analysis (Tabanick and Fidel, 2001). Preliminary reduction of variables was done through univariate Poisson regression. We tested the linear response of each variable by introducing quadratic terms ($X + X^2$) into the univariate GLMs and the best fitting curve was carried forward to subsequent analysis, using Akaike weights (w_i) as the model selection criteria (Burnham and Anderson, 2002; Bustamante, 1997; Pita et al*.,* 2009). Only significant ($P<0.05$) and nearly significant ($0.05<P<0.1$) predictors were considered in further model building. The nearly significant predictors were also considered to shrink the influence of Type II errors and avoid rejecting ecologically relevant effects at an early stage (e.g. Buhl, 1996; Underwood, 1997).

Multivariate models were developed for each set of explanatory variables (LAND, ROAD, BAT) and the best regression model was selected, using the Akaike Information Criterion (AIC). To evaluate the differential effects of each set of predictors on bat road kills we used a variance partition approach as suggested by Borcard et al. (1992). An extension of the method to three sets of explanatory variables was used to decompose the variation in eight components (Galantinho and Mira, 2009; Heikkinen et al., 2004).

To estimate the percentage contribution of each set of predictors and their shared effect on bat road kills, three joint models, one for each pair of predictor sets (LAND+ROAD; LAND+BAT; ROAD+BAT) and a full model with all the variables selected in each variable set (LAND+ROAD+BAT) were developed. These allowed us to account for eight fractions of variation: a) pure LAND effect; b) pure ROAD effect; c) pure BAT effect; ab) combined LAND and ROAD effects; ac) combined LAND and BAT effects; bc) combined ROAD and BAT effects; abc) combined LAND, ROAD and BAT effects; and finally U) unexplained variation.

Temporal autocorrelation on fatality data was evaluated with the standardized residuals and plotting the autocorrelation function with 15 days lag (Zuur et al*.*, 2009). All modelling was done using R 2.10.1 statistical package (R Development Core Team, 2009).

3. RESULTS:

3.1 Species composition and temporal pattern of road kills

We sampled a total of 11 839 km during 229 successive days and recorded 154 road killed bats belonging to 11 species (Table 2). The majority of fatalities were recorded for three species, *Pipistrellus kuhlii* (n=61), *P. pygmaeus* (n=38), followed by *P. pipistrellus* (n=13), representing over 73% of the total specimens found. We also recorded bat species with threatened or poorly known conservation status: e.g. *Rhinolophus hipposideros* (n=5); *Barbastella barbastellus* (n=3).

The majority of carcasses were adults (Figure 2). Juveniles were 19% of the total specimens found and were only recorded from June onwards. Males outnumbered females, except in July, but differences were not significant (χ^2 =1.29, *p*=0.26). The number of casualties varied strongly throughout the survey period (Figure 2), being higher from late July to the beginning of September, where 66% of the casualties were concentrated. Moreover, the last two weeks of August present a peak of mortality corresponding to about 27% of the bats collected. The number of road killed species also changed throughout the survey. A higher number of species, many of them threatened, was found during the mating season and migration of bats to swarming roosts. *Rhinolophus* spp., *Nyctalus leisleri* and *Miniopterus schereibersii* fatalities were recorded only in this period.

Table 2. Number of bats road killed per species and sex. Recorded of bat passages. Status of species on Portuguese red data Portugal. * considered phonic group with *Nyctalus leisleri/Eptesicus serotinus/E. isabellinus* (n=192); ***N. noctula /N. lasiopterus*; ****Pipistrellus* sp./ *Miniopterus schreibersii* and *Plecotus* sp.. ^ *Pipistrellus* sp. specimens casualties.

Species	Road Kills				Ultrasound Recording (n= 1735)	Red List Categories (Portugal)
	Total	Male	Female	Undeterm -ined		
Rhinolophus ferrumequinum	**1**	0	1	0	0	VU
Rhinolophus hipposideros	**5**	3	0	2	0	VU
Myotis daubentonii	**3**	2	1	0	11	LC
Myotis escalerai	**1**	1	0	0	1	VU
Myotis myotis	**0**	0	0	0	9	VU
*Eptesicus serotinus/E. isabellinus**	**3**	1	0	2	*	LC
*Nyctalus leisleri**	**1**	0	1	0	*	DD/DD
*Nyctalus lasiopterus/N. noctula***	**0**	0	0	0	6	DD
Pipistrellus kuhlii	**61**	19	17	25	419	LC
Pipistrellus pipistrellus	**13**	3	4	6	141	LC
Pipistrellus pygmaeus	**38**	14	10	14	416	LC
Pipistrellus **sp.^ /***M. schreibersii****	**11^**	0	0	11	307	
***Plecotus* sp.**	**0**	0	0	0	1	DD/LC
Barbastella barbastellus	**3**	0	1	2	2	DD
Miniopterus schreibersii	**1**	1	0	0	15	VU
Tadarida teniotis	**0**	0	0	0	16	DD
Not detemined	**13**	0	0	13	296	

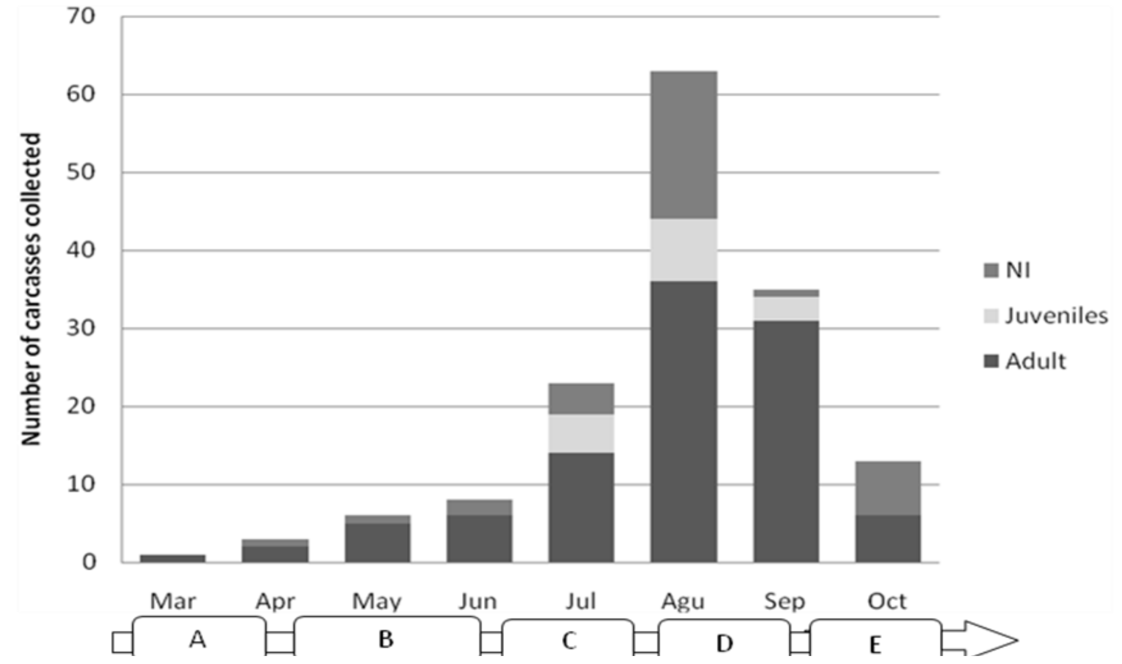

Figure 2. Total number of bat casualties per month found during the survey period. Bat annual cycle phases are shown beneath the X axis: A-Clustering and nursery roosts; B – Pregnancy; C - Births; D - Young first flights; E – Mating and Swarming.

3.2 Spatial pattern of road kills and influence of roads on bat activity level

The number of killed bats on each surveyed road per each kilometer is shown in Figure 3. On average, about three bats were road killed per kilometer during the survey period. The average of bat casualties was highest on EN-114 (3.99 ± 0.83), representing about 60% of all mortality, followed by EN-4 (3.60 ± 0.89). The lowest mortality was recorded on EN-370 (1.00 ± 0.30). The number of casualties differed significantly between road types (χ^2 = 331.87, *p*<0.001), as well as along the segments (χ^2 = 244.58, p<0.0001), with bat road kills concentrated on road segments crossing dense *montado* areas and water courses (Figure 3). However, there was no significant spatial autocorrelation in our data along the road, Moran´s I. = 0.011 (Z = -0.02, *p*<0.001).

Concerning Malo´s method, we identified eight bat mortality hotspots (BMH), mostly located in the proximity of habitats with high quality, inside of core activity areas and proximity of known roosts (Figure 4).

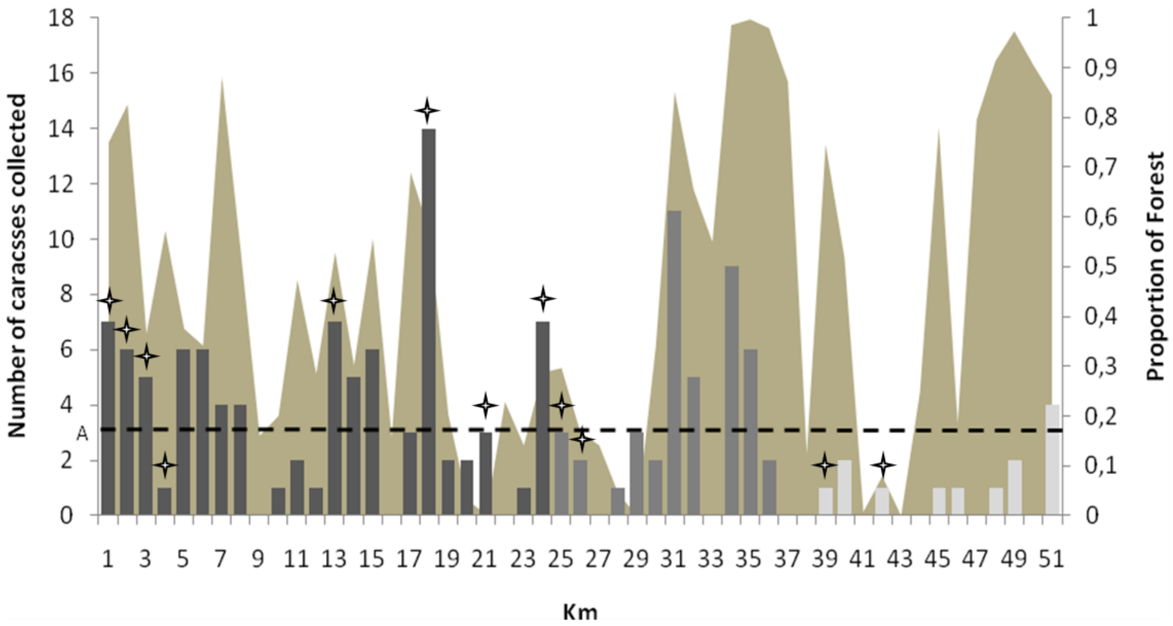

Figure 3. Bat mortality (bars) and proportion of forest (shaded light brown area) per 1000m road section. Traffic volume in each road section is characterized by the bar filling color: dark grey for high traffic and light grey for low traffic sections. Stars in black show road sections crossed by streams with developed riparian gallery. Black dashed line (A) represents the mean of road kill per kilometer.

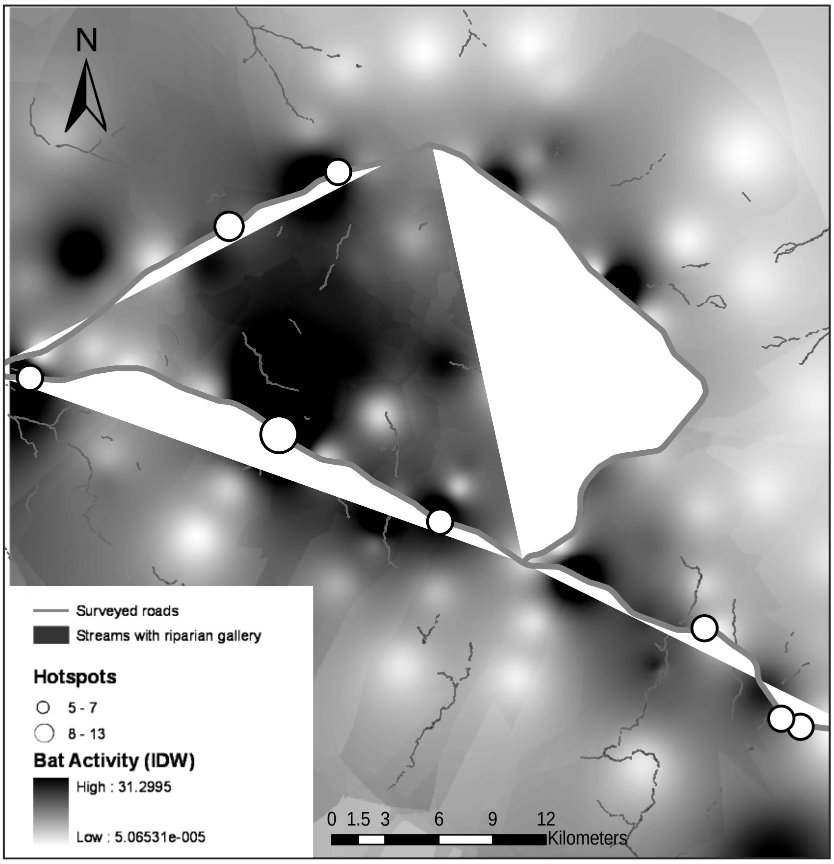

Figure 4. Bat activity and road mortality map. Color map represents bat activity estimates, areas with high activity are shown in black and low activity areas are light grey. Bat activity estimates were obtained by Inverse Distance Weighted interpolation. Road segments with bat casualties are identified by graduated circles, and white filled circles show mortality hotspots (> 5 individuals in a 500m road segment - Malo´s method). Stream with developed riparian vegetation is characterized by dark grey lines.

During the 64 hours of ultrasonic sampling we recorded 1735 bat-passes, and species could be identified in 1195 cases. The remaining 540 bat-passes were classified in phonic groups (e.g. *Eptesicus serotinus*/*Nyctalus leisleri* = 192; *Pipistrellus* sp./*Miniopterus schreibersii* = 307) or not determined (296) (Table 2). In total, 12 species were detected and identified while foraging and/or commuting. Pipistrelle bats are the most common species among the Mediterranean bat fauna, and consequently species more commonly found were *P. kuhlii* and *P. pygmaeus,* with 4.81±0.72 (mean ± standard error) and 4.78±0.82 contacts per 15 minutes, respectively (Table 2). Two species and two phonic groups were recorded using bat detectors but had no road kills: *Myotis myotis* and *Tadarida teniotis and Plecotus* sp. and *N. lasiopterus* / *N. noctula*, respectively. Bat passes of these species, as well of *B. barbastellus* and *M. escalerai* were only recorded at least one kilometre away from roads. Bat activity was higher near water places, but several species perhaps foraged and commuted often in oak woodlands, thus the most abundant species made use of almost all habitats.

Bat activity level of was not different between areas near and far from the road (F= 1.59, *p*= 0.21), neither was species richness (F=0.21; *p*=0.81) (Figure 5). Specifically, activity on streams with riparian vegetation was higher near the roads when compared to sites distant from roads, though the difference was not significant (t= 1.62, *p*=0.16).

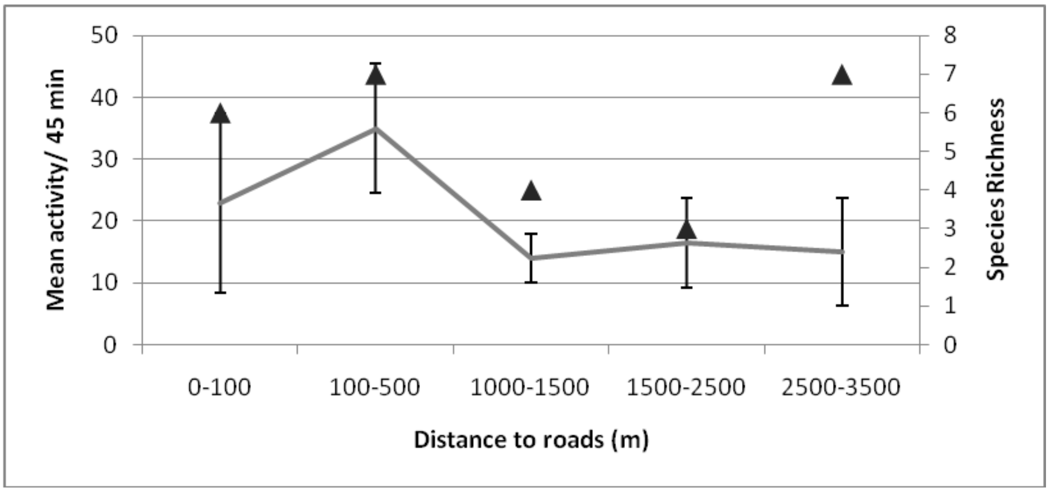

Figure 5. Grey line show mean bat activity *per* each category of distance to roads. Bars represent mean standard error. Triangle shows species richness *per* each category of distance to roads.

3. 3 Factors influencing bat road kills

Table 3 shows the results of the multivariate GLM models for each of the variable sets analysed and for the full model. LAND is the most important of the three variable subsets, explaining 26.5% of the total variance in bat road kills (Figure 6). The casualties occurred mainly on segments with high quality habitats: dense *montado* areas; near water courses with riparian vegetation; and in the proximity of water reservoirs. ROAD partial model explained 20.9% of variance, shows that road kills occurred preferentially on segments with higher traffic volume and with viaducts. BAT partial model accounted for 20.6% of variance and suggested that road kills occur mostly when roads cross areas of high bat activity and in the vicinity of known bat roosts. LAND showed the strongest pure effect (11.5%), whereas the pure effect of ROAD and BAT, explained 4.5% and 4.2% of variance, respectively. The full model accounted for 42.8% of explained variation on fatalities data. The shared effects among the three subsets were 22.4%, suggesting the existence of a strong interaction among them.

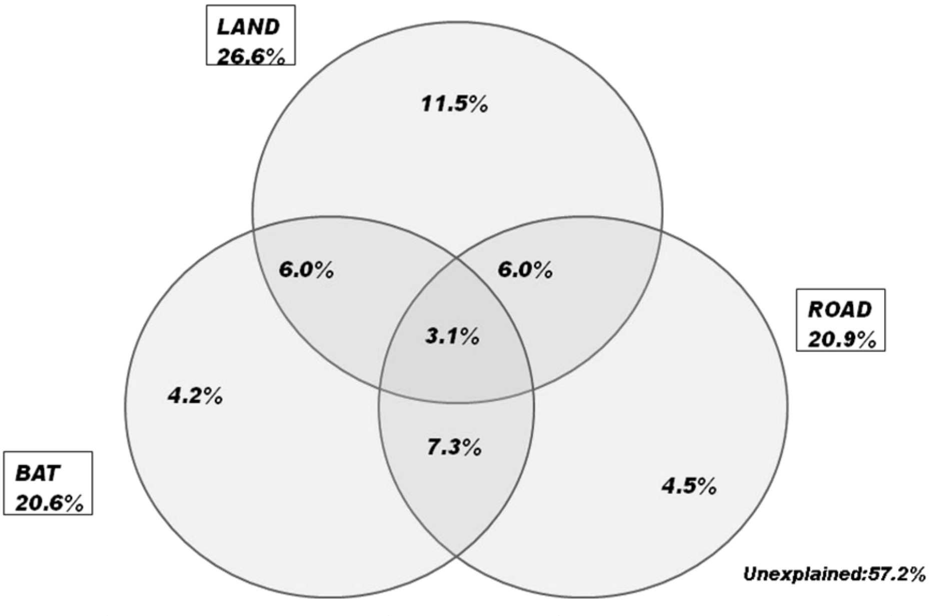

Figure 6. Results of variance decomposition for the model of total bat road kills, shown as fraction of variation explained. Variation of the data is explained by three sets of explanatory variables: landscape features (LAND), road characteristics (ROAD), and bat ecology (BAT), and unexplained variation.

Table 3. Partial models for the landscape features, road characteristics, and bat ecology sets and full model used in variation partitioning.

	Partial Models		Full model	
Variables[1]	B	*p*	B	*p*
LAND				
PDMT	0.52	0.02*	0.82	0.002**
PSMT	-0.78	0.04*	-0.36	0.43
PTE	2.57	0.07	2.25	0.22
PWB	12.74	<0.001***	-1.30	0.73
D_SWG	-0.54	<0.001***	-0.15	0.44
Intercept	1.69	<0.001***		
ROAD				
TRAF1				**Indicator**
TRAF2	1.30	<0.0001***	1.04	<0.001***
TRAF3	1.29	<0.0001***	0.72	0.032*
N_Viad	1.01	<0.0001***	0.92	0.001**
Intercept	-0.76	0.003**		
BAT				
ACT	1.63	<0.0001***	0.53	0.281
D_Roost	-0.75	<0.0001***	-0.93	0.004**
Intercept	1.46	0.075		

4. Discussion:

4.1 Species, sex and age composition of road kills

The mean density of bat casualties found in our study (2.96 individuals/km/year) is similar to the values found in studies carried out on Mediterranean landscapes, 4.3 individuals/km/year (González-Prieto et al., 1993), and 4.9 individuals/km/year (Saint-Girons, 1981), and in Poland 3.6 ind/km/year (Lesiński et al., 2010) and it is twice higher than the density previously recorded by Lesiński (2007) also in Poland. However, it is relatively low when compared to other studies in central Europe and North America, that have reported 7.4 individuals/km/year (Gaisler et al., 2009) and 6.5 individuals/km/year (Russell et al., 2008).

Bat casualties are rarely reported in vertebrate road kill studies, because research is often concentrates on larger vertebrates. The percentage of bats in our road killed data (about 4.0%) is, to our knowledge, the highest reported for similar studies where it is often lower than 1%: Great Britain 0.2-0.3% (Hodson, 1960; Slater, 2002); Poland 0.1-0.2% (Bartoszewicz, 1997; Gryz and Krauze, 2008) USA - 0.1-0.5% (Glista and De Vault, 2008; Seibert and Conover 1991; Smith and Dodd, 2003). We believe that the discrepancy of these results may reflect differences in local bat assemblages' composition and abundances, as well as differences in sampling protocols and road traffic volume of the sampled roads. In fact, different methodologies and sampling frequency have been used, and discussions among different studies results must be done carefully and should include analysis of sampling protocols. Other studies report casualties searched by foot while in our study, due to constrains related with the need to survey other vertebrates, surveys were done by car, driving at a low speed (<30km/h). Slater (2002) found that searching road killed bats by foot was more efficient at detecting the carcasses than from a moving vehicle and that even sampling by foot, road kills rate can be 12-16 times higher than the estimate by simple counts of casualties. However, Barthelmess and Brooks (2010), estimate this bias as about 4.8 times higher. For small sized species (<150 g) underdetection is inherent in road surveys, because small carcasses remain on paved road for less time than larger ones (Ford and Fahrig, 2007; Jaarsma et al., 2006; Langen et al. 2007; Slater, 2002; Orlowski, 2008). In fact, when smashed, cadavers of small species are easily destroyed by cars or removed by scavengers such as crows, cats, wasps and ants, and may be also thrown into roadside vegetation (Russell et al., 2008; Prosser et al., 2008; Antworth et al., 2005; Erritzoe et al., 2003; Slater, 2002; Pickles, 1942). Nevertheless, in a preliminary essay searching by foot road killed bats near a mortality hotspot in the surveyed road, we did not found any new carcass, despite the low sampling effort (4 km during 3 days, during the peak of bat mortality season).

Moreover, the length of the road surveyed and the period of survey may also play an important role on the road killed results. Clevenger et al. (2003) report that sampling of shorter road stretches, during shorter periods, may record a higher number of casualties.

A large number of species described for the area (about 65%) (Marques and Rainho, 2006) were found road killed, though with very different numbers. Brinkmann et al. (2008) stated that vulnerability of each species to vehicle collision may be related with bat specific flight and foraging behavior. Bat species with a slow flight that forage close to vegetation or ground, and that fly at limited distances along guiding landscape structures have a higher risk of collision with vehicles (Brinkmann et al., 2008; Gaisler et al., 2009; Jones et al., 2003; Kerth and Melber, 2009; Limpens et al., 2005; Schoeber and Grimmberger, 1996). In our fatality data these species (horseshoe and barbastelle bats) are poorly represented, probably because they are rare in the region and they avoid foraging over the road (Lesiński et al., 2010; Marques and Rainho, 2006).

P. kuhlii was the most road killed species, followed by *P. pygmaeus*. Concerning activity data, these species are also the most common in the study area and their activity level is very similar. Nevertheless Kuhl´s pipistrelle casualties were twice as higher than fatalities of the soprano pipistrelle. We believe that our results, besides showing differences in foraging behaviour (flying time, number of foraging bouts and distances flown per foraging bout) may also reflect differences on prey selection and flight agility (Bartonicka and Rehak, 2004; Davidson-Watts and Jones, 2006; Goiti et al., 2003; Grodzinski et al., 2009; Nichols and Racey, 2006, Russo and Jones, 2003). Kuhl´s pipistrelle may be more susceptible to road killing because it flies at a lower altitude and with less sinuous routes (Grodzinski et al., 2009). According to the Norberg´s model (Norberg and Rayner, 1987), *P. kuhlii* is a faster hawker than *P. pygmaeus*, therefore it is restricted to a relatively straighter flight with less maneuverability (Goiti et al., 2003; Grodzinski et al., 2009). Lesiński et al. (2010) reported that *P. pygmaeus* flies higher and rarely uses forest lanes (e.g. tree lines on roadsides). This species often forages under the forest canopy, immediately after their emergence, perhaps to reduce predation and taking advantage of the large concentrations of small and weak flying insects occurring at these places (Nichols and

Racey, 2006; Verboom and Huitema, 1997), which reduces the risk of being road killed. The fact that Kuhl´s pipistrelles often hunts jointly in groups may also explain its higher vulnerability (Barak and Yom-Tov, 1989; Bartonicka and Rehak, 2004; Davidson-Watts and Jones, 2006; Goiti et al., 2003; Grodzinski et al., 2009; Nichols and Racey, 2006). Moreover, time of evening emergence of *P. kuhlii* is earlier than the one of *P. pygmaeus* and coincides with an increased traffic volume period, at dusk, thus increasing the likelihood of collision with a vehicle (Kalko, 1995). The wider range of movements of *P. kuhlii* in habitat fragmented areas like our study site may also force them to a higher number of road crossings.

Nevertheless, contrasting patterns of the most common road killed species has been described for different parts of Europe. In the Iberian Peninsula (Bafaluy, 2000; González-Prieto et al., 1993) and France (Capo et al., 2006) , *P. kuhlii* and *P. pipistrellus* are referred as the most road killed species, while *Pipistrellus nathusii*, *M. daubentonii* and *Plecotus auritus* were the most commonly found species in central Europe (Dietz et al., 2009; Lesiński, 2007). Therefore, differences in the bat community structure of each region may be the main determinant of the composition of bat casualties within species that forage close to the ground.

Our results support the hypothesis that individuals flying higher are killed less frequently (Stratman, 2006; Lesiński, 2007). Aerial feeding insectivores bats that fly high (e.g. *N. lasiopterus* - usually flying over 10 m above the ground) and easily cross open air spaces may have a lower risk of collision because they spend most of their active time flying above vehicle height (Gebhard and Bogdanowicz, 2004; Schoeber and Grimmberger, 1996; Norberg and Rayner, 1987). In fact, we found very low numbers of bat carcasses belonging to species that forage above tree canopies such as *N. leisleri*, *E. serotinus,* and *M. schreibersii*. However, in a few studies in Germany and Poland noctule bats and *E. serotinus* casualities were often recorded (Haensel and Rackow, 1996; Lesiński et al., 2010; Rackow and Schlegel, 1994).

Night weather may also influence fatalities of some species (Lesiński, 2007). Probably after rain some individuals take smooth road surface for water surface. This curious fact could explain why most *M. daubentonii* casualties, a species that forages mainly on water body’s surfaces, were recorded after rainy nights.

A peak of bat mortality occurred in late summer and early autumn. Other studies reported a similar increase on the bat mortality in this period (Bafaluy, 2000; Gaisler et al., 2009; Lesiński, 2007, 2008; Lesiński et al., 2010; Kiefer, 1995). This result may be explained by the higher number of bats flying during this period due to the emergence of the fledged young and the higher intensity of foraging activity related to mating and migration which can increase flying time and distances flown by bats (Davidson-Watts and Jones, 2005). Other vertebrate groups showed a similar summer peak of road kills in this period (mammals - Clevenger et al., 2003; Ramp and Roger, 2008; carnivores - Carvalho and Mira, 2010; badgers - Aaris-Soresen, 1995; birds - Erritzoe et al., 2003; owls - Gomes et al., 2009). These authors argue that besides life cycle factors, part of the road kills in this period may be associated to the higher summer traffic volume (Moshe and Mayer, 1999; Underhill and Angold, 2000).

Female seem more vulnerable to road casualties in early summer; its mortality is particularly high during the period of births and lactation, a critical phase in their life cycle because newborns are totally dependent on their presence to survive (Dietz et al., 2009). Perhaps females' higher body mass during this season interferes with their flight performance, making them less maneuverable (Rayner et al., 1989). Studies on genus *Pipistrellus* and other bats revealed that during the period of late pregnancy and lactation females emerge earlier from roosts, which may expose them to the late afternoon and early evening peak traffic. Racey and Swift (1985) and Nichols and Racey (2006) reported that during the breeding period females, choose to hunt close to the colony shelter and return several times to the roost to suckle their young. In our study site many known roosts are on road bridges or close to roads. However, Kerth and Melber (2009) did not found evidence that females in close proximity to the highway presented a reduced reproductive success. On late summer, males call particularly above open spaces, along roads and vegetation edges (better sound transmission), thus increasing the probability of contact with females (Cunnington and Fahrig, submitted). Hence, males could be more susceptible to road kills during the mating period.

Gaisler et al. (2009), Lesiński (2007, 2008) and Russell et al. (2008) showed that young bats are more vulnerable to traffic than adults. However, in our study, young bats constitute a small percentage of the total road killed individuals and we only recorded young of the two most common species, *P. kuhlii* and *P. pygmaeus*, were recorded. This is a surprising result because juvenile bats are inexperienced flyers and are expected to be more susceptible to road mortality. However, young bats often have shorter flight distances and/or road avoidance behaviour because they are unfamiliar with this habitat, like has been suggested for others groups of animals (Grilo et al., 2009; Knowlton et al., 2010). Lesiński et al. (2010) suggest that the low prevalence of young bats in road casualties' surveys may happen if sampled roads do not cross important routes of bat dispersal.

It is worth noting that recorded fatalities of threatened or poorly known species, as *R. hipposideros*, *R. ferrumequinum*, *M. schreibersii*, *N. leisleri* and *B. barbastellus*, are concentrated in the period of mating and migration to swarming and winter roosts. Despite of the low numbers of casualties recorded for these species and, the non-natural mortality due to road kills may have a significant impact, due to their usually small local populations and rarity nationwide.

4.2 Influence of roads on spatial pattern of activity

Data from the present study do not suggest that areas close to roads could be degraded in their suitability as commuting and foraging areas for the bat community. However, our sample consists mainly of pipistrelle bat passes which are known to be tolerant to anthropogenic disturbance (Russo and Jones, 2003). Further analysis showed a species specific response of bat activity to the presence of roads. We recorded a higher activity of *Eptesicus serotinus/isabellinus,* and *Nyctalus* spp. near roads compared to areas far from the road, a likely indication of road attraction behaviour in these species. Serotine bats are commonly found in urban areas and can be attracted by the high prey abundance associated with streetlights alongside roads or car headlights (Avilla-Flores and Fenton, 2005; Arletaz et al., 2000; Brinkmann et al. 2008). Verboom and Huitema (1997) also reported high activity of Serotines close to linear landscape structures and tree lines are often associated with road verges. *Nyctalus* sp. bats are high altitude flyers and probably are not affected by the presence of roads. As mentioned before, some bat species often exploit swarms of insects that concentrate around streetlamps alongside roads (Stone et al., 2009), but other species avoid foraging and commuting close to illuminated areas (Russo and Jones, 2003; Stone et al., 2009). Some of the bat activity sampling points were located in periurban and rural areas, our data set could be potentially used to test the effect of street lighting on bat activity. However, due to the low number of streetlamps along the sampled roads we were not able to do so.

We only recorded species belonging to the group of "passive listening" bats far from the roads. This group, which includes *M. myotis, M. bechsteinii* and all long-eared bats (genus *Plecotus*), could be the most affected by high levels of background noise (Arletazz et al., 2001), and this may explain the road avoidance effect detected in our data. Recent studies suggest that traffic noise reduces foraging time and effort of *M. myotis*, presumably by masking rustling sounds made by moving arthropods and prey detection in noisy areas (Barber et al., 2009; Jones, 2008; Parris and Schneider, 2009; Schaub et al., 2008; Stone et al., 2009). For aerial feeding bats it is less clear that foraging will be adversely affected by traffic noise (Jones, 2008), but Szewczak and Arnett, (2006) report that foraging can be affected and discouraged by the emission of loud broadband noise. In the case of roads, traffic noise and traffic volume (visual disturbance) may act in synergy to exclude bats from habitats next to noisy and busy roads.

Riparian vegetation had the highest bat activity compared to the other habitats in the study area. Several authors consistently recorded the same pattern, higher relative bat abundances along with higher species richness on rivers and streams than on other surrounding habitats in Mediterranean landscapes (Rainho, 2007; Russo and Jones, 2003). Distances to these landscape features may be a limiting factor in the use of space by bats, particularly in our study area where water availability is scarce and unevenly distributed (Rainho and Palmeirim, in review). Riparian corridors are also used by bats as flight paths and /or foraging areas (Hein et al., 2008; Rainho, 2007; Rogers et al., 2006; Russo et al., 2005, Verboom and Huitema, 1997). Moreover, the higher bat activity recorded in streams next to roads suggests that streams with riparian gallery may be used as flyways to cross roads, though differences were not significant. Further research using different methodologies that are able to study in detail bat movements across the landscape, *i.e.* radiotelemetry, should be used to test this hypothesis. Another fact that should be evaluated is the connectivity of these linear network elements.

4.3 Spatial patterns of road kills

We have shown that the spatial pattern of road kills is influenced by different types of factors including habitat quality, road features, and bat species ecological traits. To our knowledge, this is the first attempt to model bat road kill occurrences.

The results of the variation decomposition analysis confirm the importance of landscape structure as the main factor influencing the likelihood of bat collisions with vehicles, followed by road features and bat ecology predictors, both with similar proportion of explained variation. Moreover, considering only the pure effects of each variable set, landscape characteristics still explained the major part of the road kills occurrences. The variables included in the final model were all indicative of ecological requirements and habitat preferences of bats. The positive relationship with dense *montados* and proximity to streams with developed riparian gallery, suggest that bats have a higher probability of being killed when foraging in their preferred habitats. In fact, *montado* areas and riparian vegetation are often highly suitable habitats to bat communities, because they provide shelter and habitat structure for insects (Allen et al., 2003; Ober and Hayes, 2008; Russo and Jones, 2003). *Montados* are often grazed by cattle and sheep providing abundant dung on which some insects feed, therefore increasing the availability of prey for some bat species (Rainho, 2007). Moreover, the structural complexity and diversity of *montados*, which can include open areas intermixed with forested areas with different tree densities, provide foraging areas for different bat guilds (Morris et al., 2010), including forest species like *B. barbastellus* and *M. bechsteinii* (Arlettaz, 1999; Schofield and Morris, 2000) and edge habitat specialists (e.g. *P. kuhlii* and *M. escalerai*) (Grodzinski et al., 2009; Siemers and Schnitzler, 2000). Thus, these areas are often high quality habitats for bats. Aquatic and riparian environments also have a positive influence on the occurrence of mortality because they provide water and are often associated with high abundance of prey. Additionally, riparian corridors provide flight paths for many bat species (Limpens and Kapteyn, 1991) which increases the risk of road kills when these paths are crossed by roads.

Traffic volume and speed of vehicles are generally regarded as important factors influencing road fatalities (Forman and Alexander, 1998; Jaeger et al., 2005; Ramp et al., 2006; Seiler, 2003; Trombulak and Frissell, 2000). It is widely accepted that large and medium sized mammals and birds avoid very high traffic volume roads, whereas higher fatality rates are more likely to occur on roads with moderate to low traffic adjacent to high quality habitat (Benítez-López et al., 2010; Clevenger et al., 2003; Grilo et al., 2009; van Langevelde et al., 2009). Our results do not conform to this view. In fact, road stretches with higher traffic volumes were those with higher road kill rates. This fact could be explained, because most individuals found road killed were pipistrelles. These species are considered generalist opportunists, often living in urban environments, with noise and other kinds of human disturbance and, as explained above, do not show road avoidance or attractiveness behaviour. Even for the habitat specialist *B. barbastellus,* roads are integrated in their foraging areas and, surprisingly, do not represent a barrier effect (Kerth and Melber, 2009). Therefore, for these bat species that do not avoid roads an increase in traffic intensity may reduce the chance of a successful road crossing (Jaarsma et al., 2006). Nevertheless, for other species, the scenario can be different. Kerth and Melber (2009) concluded that for *M. bechsteinii*, areas close to roads with high traffic volume and few underpasses were poor foraging areas and roads functioned as flight boundaries.

The results of our model show that the probability of bats being hit by a car increases in road segments with viaducts. These structures are often associated with elements of the landscape (e.g., riparian galleries, ponds) where usually bat foraging activity is enhanced. Furthermore, bats use regularly viaducts and underpasses for crossing the roads, while commuting along their flight paths in the evening, but also when switching between foraging areas during the night (Abbott, personal communication; Bach et al. 2004; Kerht and Melber, 2009; Limpens et al., 2005). These evidences suggest that on viaducts the probability of being road killed should be lower, in contrast with our results. Nevertheless, these structures often provide roosting places for common species (Keeley and Tuttle, 1999) increasing the probability of some individuals being road killed when leaving their roost for feeding or while active in the vicinity. Further studies are needed to clarify the role of bridges and viaducts in bat road fatalities.

The adoption of habitat use variables within predictive fatality modelling has been done only sporadically, despite many models suffering from poor explanatory power. However, Roger and Ramp (2009) reported the importance of incorporating these variables to improve the predictive ability of the models and reduce the number of other possible variables. We included activity data in bat road kills modelling and found, as expected, a strong positive relationship between the two variables. This association is particularly clear when hotspots of mortality and activity maps are overlaid, with higher mortality occurring in core activity areas which once again must reflect the probability laws (more abundance or higher intensity of use means higher probability of getting a road kill). This also explains why road kills have a higher probability of occurring near known roosts. All known roosts on the study area are mainly occupied by *P. pygmaeus*, between 50-200 individuals (Medinas et al. unpublished data) which on a few occasions share roosts with *P. kuhlii*. Both species are generalists when choosing roosting places (Dietz et al., 2009) and are often found in crevices of buildings and viaducts along the roads (Davidson-Watts and Jones, 2006; Flaquer et al., 2006).

Summarizing, the high quality habitats adjacent to roads, proximity to roosts and high traffic volume seems to be the main drivers of road casualties in bats. Most of the species on the study area are affected by roads, including threatened and poorly known species for which the impact of road kills on population trends may be particularly relevant. Implementing mitigation measures aiming to minimize fatalities of these species together with road fatalities monitoring programs should be, in our opinion, a priority. We showed that bats are more vulnerable during specific life-history periods, as lactation, mating, and migration to swarming and autumn roosts. Global bat activity seems not to be affected by the presence of roads although the passive listening species (*M. myotis*; *Plecotus spp.*) may avoid them. Globally our results support the opinion that road traffic could be a major source of bat mortality. Moreover, the impacts of mortality, barrier effect and habitat deterioration is species-specific. Furthermore, whatever is the most relevant in each context, the final result points to the same conclusion: roads negatively affect overall bat populations.

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Considerações finais:

É comummente aceite que as principais ameaças dos morcegos são a perda ou alteração de habitat, destruição ou perturbação dos abrigos (especialmente durante as épocas de hibernação e maternidade), o uso excessivo de pesticidas e a perseguição (Dietz et al., 2009; Mickleburgh et al., 2002). Porém, a conservação deste grupo faunístico pode ser afectada tanto ou mais por outros factores de ordem antropogénica, como é o caso da mortalidade causada por colisões com aerogeradores ou por atropelamento por veículos motorizados (Arnett et al., 2009; Cryan & Barclay, 2009; Lesiński, 2007, 2008; Lesiński et al., 2010; Sovacool, 2009; Willis et al., 2010). Lesiński et al. (2010) defende mesmo que as estradas podem ser a principal ameaça externa à viabilidade das populações de morcegos em certos locais.

Em território nacional ocorrem 27 espécies de morcegos, das quais 25 se distribuem no continente, encontrando-se todas elas abrangidas pela legislação nacional e internacional, nomeadamente a Directiva Habitats (Decreto-Lei nº140/99, de 24 de Abril), Convenção de Bona (Decreto nº103/80, de 11 de Outubro), EUROBATS – acordo para a conservação das populações de morcegos europeus (Decreto-Lei nº 31/95, de 18 de Agosto) e Convenção de Berna (Decreto-Lei nº316/89, de 22 de Setembro). Segundo Cabral et al. (2005) cerca de 38% das espécies que ocorrem em Portugal continental, possuem estatuto de ameaça, estando três espécies Criticamente em Perigo, uma em Perigo e cinco Vulneráveis, existindo ainda nove espécies classificados com “Informação Insuficiente”. Devido à protecção legal e ao elevado estatuto de ameaça de muitas espécies, é peremptório a monitorização de projectos que possam ter ou vir a ter impactos negativos sobre as comunidades (Bach & Rahmel, 2004). Esta realidade já se verifica para a maioria dos empreendimentos eólicos que têm vindo a ser construídos nos últimos anos em Portugal, porém no caso das estradas ainda existe uma falta de informação, associada a uma total inércia dos agentes de administração rodoviária nesta questão. Sendo assim, foi neste contexto que se enquadrou este trabalho, servindo como um promotor de linhas de investigação que visem diagnosticar correctamente o problema, tendo por base a identificação dos factores determinantes na mortalidade de morcegos num ambiente Mediterrâneo, bem como a descrição do padrão espacial e temporal da mortalidade e a quantificação do efeito da presença de estradas nas comunidades locais de morcegos.

No que se refere à mortalidade, os resultados do nosso estudo apresentam-se de acordo com os restantes trabalhos acima mencionados, nos quais as espécies localmente abundantes ou com níveis de actividade bastante grandes são as mais afectadas (e.g. Gaisler et al., 2009; Haensel and Rackow, 1996; Kerth and Melber, 2009; Kiefer et al., 1995; Lesiński et al., 2007, 2010; Limpens et al., 2005). Recolhemos 154 morcegos atropelados, pertencentes a 11 espécies, em que as mais frequentes foram *Pipistrellus kuhlii* e *P. pygmaeus*, representando cerca de 64% dos espécimes encontrados. Foram também recolhidas espécies com estatuto de ameaça ou pouco conhecidas, tal como, o *Barbastella barbastellus* ou *Rhinolophus ferrumequinum*. A maioria da mortalidade ocorreu entre o final do Verão e o princípio do Outono, contudo a mortalidade das espécies ameaçadas ocorreu principalmente durante o período da migração entre os abrigos de Verão e os abrigos de Inverno. Apesar do baixo número de indivíduos atropelados das espécies com estatuto de ameaça, a sua morte não natural pode ter um impacto significativo nas populações locais das espécies, devido aos pequenos tamanhos das populações e a sua raridade a nível nacional (Cabral *et al*., 2005). O número de machos atropelados é maior do que o das fêmeas, contudo este padrão inverte-se somente no período durante os nascimentos das crias e a sua amamentação. A mortalidade de juvenis só foi registada em Julho e foi uma pequena percentagem do total de atropelamentos, este resultado contraria outros estudos, nos quais os juvenis como resultado da sua inexperiência são mais vulneráveis aos atropelamentos que os adultos (Gaisler et al. 2009; Lesiński, 2007, 2008; Russell et al., 2008).

Segundo os nossos dados, os morcegos parecem não evitar a proximidade das estradas, porém como a nossa amostra é essencialmente constituída por indivíduos do género *Pipistrellus*, os nossos resultados podem estar enviesados, tendo em conta o seu carácter euritópico (Dietz et al., 2009; Russo and Jones, 2003). Todavia, no caso das espécies de morcegos que caçam utilizando o ruído produzido pelas presas, quando estas se deslocam, o padrão para ser diferente. Indivíduos com este tipo de comportamento de caça, só foram detectados a distâncias nunca inferiores a 1 km das estradas mais próximas. Este facto pode querer indicar que a presença das estradas pode diminuir a importância dessas áreas como zonas de alimentação. No contexto Mediterrâneo, as ribeiras agregam um conjunto de valências ecológicas muito importantes, dessa forma são locais onde existe grandes níveis de actividade e riqueza específica de morcegos (Rainho, 2007; Russo and Jones, 2003). Foi registado um incremento da actividade junto aos locais onde as ribeiras cruzam as estradas, comparativamente a locais longe das estradas. Os resultados podem indicar que os morcegos utilizam ribeiras com galerias ripícolas bem desenvolvidas para cruzar a estrada. Todavia, será essencial mais informação sobre os movimentos dos morcegos ao longo da paisagem e para isso será necessário equacionar novas metodologias, tal como a rádio-telemetria.

A relação entre o padrão da mortalidade de morcegos e as características da paisagem mostrou ser bastante evidente e foi o conjunto de variáveis mais significativo na explicação da variância, tendo sido registado um maior número de atropelamentos em locais onde a estrada atravessa habitats de grande qualidade, nomeadamente manchas de montado denso e ribeiras com galeria ripícola bem desenvolvida. Estas zonas providenciam locais de grande adequabilidade às comunidades de morcegos, porque contêm disponibilidade de abrigos e na maioria das vezes grandes abundâncias de insectos (Allen et al., 2003; Ober and Hayes, 2008; Russo and Jones, 2003). A seguir a este conjunto de variáveis, sucede-se as características da estrada e finalmente os requisitos ecológicos do grupo. Dessa forma, os resultados indicam também que o volume de tráfego, a presença de viadutos e a proximidade dos abrigos conhecidos contribui para o incremento da mortalidade de morcegos. Relativamente à actividade dos morcegos na área de estudo, encontrámos uma relação positiva forte entre as áreas com maior actividade e os pontos negros de mortalidade.

Os modelos de avaliação da probabilidade de atropelamentos explicam apenas uma escassa parte da variância das variáveis resposta. Dessa forma, alguns autores sugerem que a capacidade preditiva do modelo poderia ser maior, aquando da inclusão de alguns pormenores da paisagem (Manel et al., 1999; Osborne et al., 2001), distribuição dos recursos alimentares (Barrientos & Bolonio, 2009) e também de características específicas das espécies (Roger & Ramp, 2009). A informação obtida através dos modelos, associada com a identificação dos pontos negros de mortalidade, constitui a base para a gestão de rodovias mais integradas na paisagem e com menos impacto na vida selvagem.

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