Universidade de Évora
Mestrado em Biologia da Conservação

Avaliação da Ocorrência de Pontos Negros de Mortalidade por atropelamento em Strigiformes

Dissertação realizada por
Luis Alexandre Piteira Gomes

Orientador Prof. António Mira

Esta dissertação não inclui as críticas e sugestões feitas pelo júri.

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Índice

Resumo

O atropelamento é uma das principais causas de mortalidade de Strigiformes. Como predadores de topo, este poderá comprometer a persistência das suas populações. Para reduzir tais efeitos, medidas de mitigação deverão ser implementadas. A sua aplicação ao longo da totalidade das estradas não é financeiramente exequível, implicando a identificação dos locais onde a mortalidade é mais intensa (i.e. pontos negros). Para além disso, também é necessário reconhecer quais os factores que influenciam sua distribuição, o que permitirá delinear medidas mitigadoras. O desempenho de cinco métodos de identificação de pontos negros foi analisado utilizando informação de atropelamentos de Strigiformes, recolhida sistematicamente durante dois anos, ao longo de 311km de estrada no Sul de Portugal. A identificação dos factores determinísticos da mortalidade conseguiu-se através do desenvolvimento de modelos, usando a regressão logística binária e a Análise Factorial do Nicho Ecológico (ENFA). Os resultados sugerem que o método desenvolvido por Malo (2004) deve ser o escolhido para identificação dos pontos negros. O atropelamento destas espécies está associado a existência de boas condições de habitat para a ocorrência das espécies, e da existência de condições que promovem a ocorrência de episódios de caça sobre a via. A determinação destes factores permitiu a definição de um conjunto de medidas mitigadoras.

Abstract

Road fatalities are among the major causes of mortality for Strigiformes species, and may affect the population's perpetuity. The use of mitigation strategies must be considered to overcome this problem. However, because its application along the total length of all roads is not financially feasible, the locations where Strigiformes road-killing are more frequent (i.e. road fatality hotspots) must identified. Aside hotspots identification, factors that influence the occurrence of such fatalities should be recognized to allow mitigation measures delineation. We use road fatality data collected from 311km of south Portuguese roads during a two years period, to compare the performance of five different hotspot identification methods: Binary logistic regression; Ecological Niche Factor Analysis; Kernel density estimation; Nearest Neighbour Hierarchical Clustering and *Malo's method.* Binary logistic regression and ENFA modelling were also used for road-kill deterministic factors recognition. Our results suggest that *Malo's method* should be preferred to hotspot identification. The main factors driving owl road-killings are associated with the existence good habitat conditions for species occurrence, and the existence of specific conditions that promote hunting behaviour near roads. According to these factors several mitigation measures are recommended.

Introdução

O meio natural, como o conhecemos, há muito que sofre com os impactes "positivos"e "negativos" da actividade humana, sendo um bom exemplo deste facto a fragmentação que a paisagem natural tem vindo a ser alvo (Saunders et al., 1991; Forman & Deblinger., 2000), principalmente devido à construção de vias rodoviárias (Kuitunen et al., 1998; Vos & Chardon, 1998 ; Seiler, 2001). De uma forma geral os efeitos conhecidos destas estruturas sobre os ecossistemas podem ser agrupados em cinco grandes categorias: (1) Destruição de Habitat; (2) Poluição e Perturbação; (3) Corredor; (4) Mortalidade e (5) Barreira (Seiler, 2001).

As estradas são autênticas barreiras aos movimentos animais, sejam eles diários ou sazonais, promovendo o isolamento e impedindo o fluxo genético entre populações de diversas espécies (Rodriguez et al., 1996; Carr & Fahrig, 2001). Alguns autores atribuem a este isolamento, a extinção das populações a nível local (Saunders et al., 1991). Um dos impactes negativos mais importantes e mensuráveis que as vias rodoviárias provocam sobre as comunidades animais é a morte por atropelamento (Vignes, 1984; Garnica & Robles, 1986; Davies et al., 1987; Rodda, 1990; Prieto et al., 1993; Philcox et al., 1999; Carr & Fahrig, 2001; Malo et al., 2004).

Nas últimas décadas a morte por atropelamento parece ser o principal problema de conservação que afecta as aves de rapina nocturnas (Ordem: Strigiformes) (Hernandez, 1988; Newton et al., 1997; Frías, 1999; Fajardo et al., 2000; Meek et al., 2003; Ramsden, 2003). Dentro deste grupo, as mais afectadas pelo impacto directo das estradas são também as mais abundantes, designadamente o Mocho Galego (*Athene noctua*) e a Coruja das Torres (*Tyto alba*) (Gragera et al., 1992). Actualmente pensa-se que os indivíduos são frequentemente atropelados durante os voos de caça, a baixa altitude, ao tentar capturar micromamíferos que se movimentam nas bermas das estradas, tornando-se assim vítimas dos veículos (Nores & Moro, 1990; Hill & Hockin, 1992). As mortes entre os indivíduos desta ordem relevam-se relativamente à mortalidade de indivíduos de outras espécies, atendendo à sua posição mais sensível como predadores de topo (Palmeirim et al., 1994), que de uma forma geral apresentam densidades populacionais mais baixas que as espécies de níveis tróficos inferiores, sendo também mais vulneráveis a mudanças no habitat (Elias et al., 1998).

A mortalidade massiva destas espécies em locais em que a taxa de natalidade não é suficiente para compensar estas perdas, pode levar à extinção das populações locais (Andrews, 1990; Newton et al., 1991; Gragera et al., 1992; Fahrig et al., 1995; Hels & Buchwald, 2001). A mortalidade de um elevado número de juvenis também compromete a existência de novos casais reprodutores, podendo determinar a regressão de tais populações (Fajardo et al., 1992).

Dado o elevado número de Strigiformes atropeladas é necessário definir estratégias que permitam mitigar o efeito de tais estruturas. Para isso é fundamental conhecer quais os factores que os condicionam a ocorrência de atropelamentos e a forma como actuam (Clevenger et al., 2003). No entanto, a informação reunida acerca do atropelamento de aves de rapina nocturnas é escassa e inconclusiva (Hernandez, 1988; Gragera et al., 1992; Muntaner & Mayol, 1996; Fajardo, 2001). Para além de identificar quais as medidas potencialmente mitigadoras desta mortalidade é essencial conhecer também quais os locais onde a mortalidade é mais intensa (i.e. *pontos negros*), pois a aplicação de tais medidas seria financeiramente incomportável para a extensão total das estradas. A determinação de pontos negros de mortalidade para aplicação de tais medidas visa garantir uma maior eficácia na aplicação das mesmas.

Para atingir tais fins, face à complexidade dos factores envolvidos, o desenvolvimento de modelos preditivos da ocorrência de atropelamentos é uma boa ferramenta, pois permite: (1) identificar as variáveis que determinam a probabilidade de atropelamento e (2) identificar os pontos negros de mortalidade (Mac Nally, 2000). Alguns modelos preditivos foram desenvolvidos em estudos recentes (Finder et al., 1999; Nielsen et al., 2003; Malo et al., 2004; Saeki & Macdonald, 2004; Ramp et al., 2005), no entanto nenhum deles visava exclusivamente as aves de rapina nocturnas. A necessidade de desenvolver modelos exclusivos para espécies de Strigiformes justifica-se pelo facto de partilharem características específicas, tais como tácticas de caça, padrões de dispersão de juvenis associado a elementos da paisagem (Shawyer, 1987) e período de actividade quase exclusivamente nocturno (Mikkola, 1983), que alteram o ajuste dos modelos gerais quando aplicados a estas espécies.

Muitos métodos foram desenvolvidos para identificação de pontos negros (Everett, 1974). Modelos predictivos para identificação de pontos negros implicam a utilização da informação acerca da ocorrência de atropelamentos por secção de estrada

(Finder et al., 1999; Malo et al., 2004; Saeki & Macdonald, 2004; Ramp et al., 2005). Outros métodos implicam a modelação dos atropelamentos com base em informação pontual (Clevenger et al., 2003), ou a comparação das contagens de atropelamentos por secção de estrada com o número de atropelamentos esperado em caso de aleatoriedade determinado pela distribuição de Poisson (Malo et al., 2004). Algumas considerações foram feitas acerca da utilização destes e de outros métodos na determinação de pontos negros de mortalidade (Ramp et al., 2005), no entanto desconhecem-se estudos comparativos que permitam determinar qual método traduz melhor a realidade da ocorrência de atropelamentos.

Neste contexto, o presente estudo pretende: (1) compreender o padrão espacial de atropelamentos e identificar pontos negros em três espécies de Strigiformes num conjunto de estradas do sul de Portugal; (2) determinar qual método de identificação de pontos negros apresenta melhor performance nestas situações; (3) identificar quais os factores que determinam a probabilidade de ocorrência de atropelamentos de Strigiformes, permitindo inferir acerca das medidas de minimização adequadas para prevenir os atropelamentos destas espécies e (4) desenvolver modelos preditivos que possam vir a ser usados para determinar a localização de pontos negros em estradas para as quais não exista informação de atropelamentos. Para tal, monitorizaram-se quinzenalmente 311km de estrada, no Alentejo, na procura de carcaças de indivíduos das três espécies de Strigiformes mais abundantes em Portugal. As espécies alvo foram: Coruja das Torres (*T. alba*); Coruja do Mato (*Strix Aluco*) e Mocho Galego (*A. noctua*) (Figura 1).

Os resultados do presente trabalho foram sujeitos a publicação sob a forma de artigo científico, intitulado "**Owl road-kill hotspots location: Identification methods and Deterministic factors**", submetido à revista Biological Conservation.

Figura 1. Espécies alvo do presente estudo. Da esquerda para a direita: Coruja das Torres (*Tyto alba*); Coruja do Mato (*Strix Aluco*) e Mocho Galego (*Athene noctua*)

"Owl road-kill hotspots location: Identification methods and Deterministic factors"

Luís P. Gomes & António Mira

Unidade de Biologia da Conservação. Departamento de Biologia, Universidade de Évora. 7002-554 Évora (luispgomes@gmail.com)

Abstract

Road fatalities are among the major causes of mortality for Strigiforme species, and may affect the population's perpetuity. The use of mitigation strategies must be considered to overcome this problem. However, because its application along the total length of all roads is not financially feasible, the locations where Strigiforme road-killing are more frequent (i.e. road fatality hotspots) must identified. Aside hotspots identification, factors that influence the occurrence of such fatalities should be recognized to allow mitigation measures delineation. We use road fatality data collected from 311km of south Portuguese roads during a two years period, to compare the performance of five different hotspot identification methods: Binary logistic regression; Ecological Niche Factor Analysis; Kernel density estimation; Nearest Neighbour Hierarchical Clustering and *Malo's method.* Binary logistic regression and ENFA modelling were also used for road-kill deterministic factors recognition. Our results suggest that *Malo's method* should be preferred to hotspot identification. The main factors driving owl road-killings are associated with the existence good habitat conditions for species occurrence, and the existence of specific conditions that promote hunting behaviour near roads. According to these factors several mitigation measures were recommended.

Introduction

It is widely accepted that roads affect many aspects of ecosystems (Lodé, 2000; Seiler, 2001; Forman et al., 2003; Geneletti, 2003). Their impact on the environment includes both direct effects on the habitats destroyed during their construction, and changes in the ecosystem dynamics they run through (Forman, 1998).

Most of these effects alter the structure of wildlife populations adjacent to roads (Forman et al., 2002), by intensifying toxic environmental contamination, limiting wildlife movement, fragmenting wildlife populations, isolating animals from their mates, and leading to resource inaccessibility (Clevenger, 1996; Richardson et al., 1997; Forman & Alexander, 1998; Spellerberg, 1998; Lodé, 2000; Meunier et al., 2000; Develey & Stouffer, 2001; Seiler, 2001; Dyer et al., 2002; Forman et al., 2003).

The most visible effect of roads is the mortality caused by collisions between fauna and motor vehicles (Malo et al., 2004). The persistence of a population can be compromised, if additional non-natural mortality (i.e. road casualties) affects a significant proportion of the population, which is not compensated by higher birth rates (Andrews, 1990; Newton et al., 1991; Fahrig et al., 1995; Hels & Buchwald, 2001).

Road-kill seems to be the principal conservation issue affecting Strigiforme populations, besides other groups, over the last decades (Hernandez, 1988; Newton et al., 1997; Frías, 1999; Fajardo et al., 2000; Meek et al., 2003; Ramsden, 2003). According to several authors, it can drive the conservation status of some Strigiforme species to a situation of gravest concern (Fajardo & Pividal, 1994; Newton et al., 1997; Del Hoyo et al., 1999).

The information gathered about road casualties among nocturnal birds of prey is scarce and inconclusive, especially with respect to understanding which factors influence this mortality (Hernandez, 1988; Gragera et al., 1992; Muntaner & Mayol, 1996; Fajardo, 2001). The majority of studies suggest that these birds hunt, feed, breed and move along the road and road-sides (Fajardo et al., 1998; Meunier et al., 2000; Ramsden, 2003).

Given the extent of wildlife killed on the roads, there is a sense of urgency with respect to minimizing the number of fauna-vehicle collisions. For the purposes of reducing fauna road-kill, mitigating measures have been tested in various studies conducted world-wide. Investigators have examined the use of fences (Ludwig & Bremicker, 1983; Clevenger et al., 2001), mirrors to dissuade animals from crossing (Ujváry et al., 1998), road signs to inform drivers about the presence of animals on the road (Pojar et al., 1975), overpasses, underpasses, road-level crossings (Keller, 1999; Clevenger & Waltho, 2000) and whistles installed on vehicles (Romin & Dalton, 1992). But none of these measures has proven to be totally effective.

To take effective measures to mitigate wildlife road casualties, it is necessary to understand which factors determine their incidence, and to identify ways of influencing these factors (Clevenger et al., 2003). Because it is not financially feasible for most governments to create road-kill management strategies along the total length of all roads, it is necessary to choose locations where application of these measures will be most effective and efficient; in other words, to optimize the number of lives saved at as low a cost as possible. For such purposes, given the likely complexity of factors involved, it is best to generate a model to predict fauna-vehicle collisions, a model which: (1) identifies the factors that determine the probability of fauna-vehicle collisions, and (2) allow to predict the location of mortality hotspots (i.e. segments of the road with a disproportionately high number of fatalities) (Mac Nally, 2000). Some recent studies have provided predictive models for fatality occurrence (Finder et al., 1999; Nielsen et al., 2003; Malo et al., 2004; Saeki & Macdonald, 2004; Ramp et al., 2005), but none has focused exclusively on nocturnal birds of prey (i.e. Strigiformes).

Strigiforme species share certain characteristics that include: different hunting tactics than other predators; juvenile dispersal patterns associated with landscape structures (Shawyer, 1987); and almost exclusive nocturnal activity, thereby reducing energy efficiency (i.e. they cannot use warm air currents in flight, like diurnal birds of prey do) (Mikkola, 1983). These characteristics may alter the accuracy of prior general road-fatality model results, thereby warranting a specific model for understanding the factors that determine Strigiforme-vehicle collisions.

Besides model developing, there are literally dozens of different statistical techniques designed to identify 'hot spots' (Everett, 1974). But its performances in Strigiforme road-fatality hotspots identification are actually unknown.

The present study was developed to achieve four main goals: (1) to understand the spatial pattern of Strigiforme-vehicle collision locations, identifying hotspots along the surveyed roads; (2) to establish which is the best hotspot identification method for these road-kills (3) to identify the factors that influence the likelihood of Strigiforme road casualties along these roads; and then (4) to develop predictive models that can be used to identify hotspot locations for these road-kills on other roads. If the necessary care is taken, these predictive models might generate accurate, significant estimates of the impact of different roads on Strigiforme populations, and identify target areas for the application of mitigation measures (Malo et al., 2004; Ramp et al., 2005). With these aims in mind, segments of fourteen south Portuguese roads were surveyed for Strigiforme fatalities, once each fifteen days over a two-year period. The target species were the three most abundant nocturnal birds of prey in Portugual: the Barn owl (*Tyto alba*); the Tawny Owl (*Strix aluco*) and the Little Owl (*Athene noctua*).

Methods

Study area

All the surveyed roads are located in Alentejo, Southern Portugal (38°41' to 38°01' North and 8°41' to 7°40' East). These roads (hereafter termed the *surveyed road*) are all interconnected, linking two major cities, Évora and Beja, in addition to other small localities (Figure 1). They were inserted in an area where plain is the dominating topography, with altitudes ranging from 200m to 400m. The climate is Mediterranean and annual precipitation ranges from 500mm (SW) to 800mm (SO) (Rivas-Martínez, 1981).

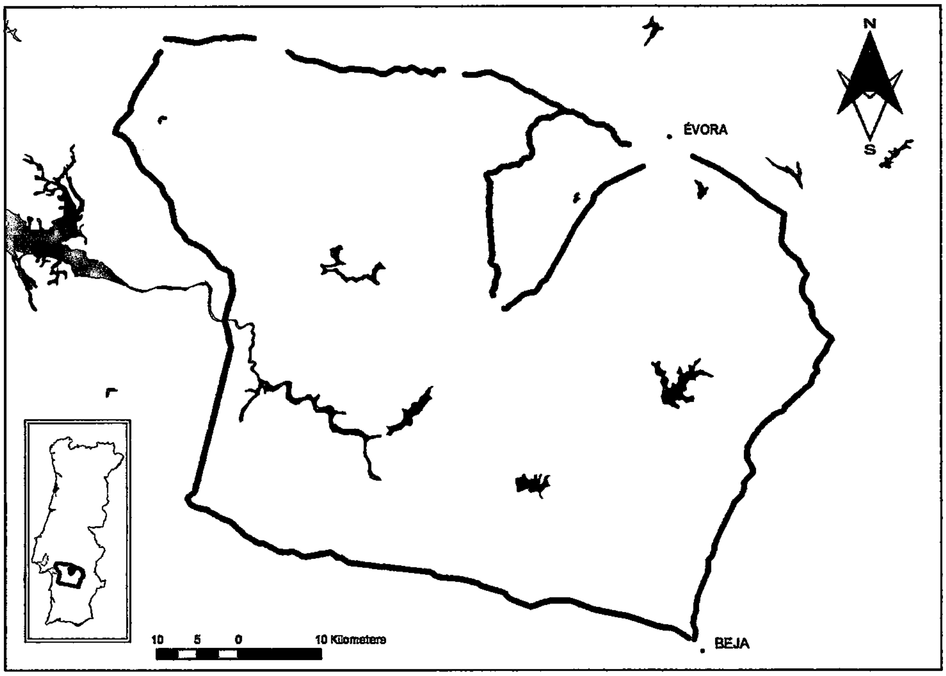

Fig 1. Map of the surveyed road and its relative location on Portuguese territory. Habitats with high density of trees are indicated by light grey, and water by dark grey.

Human-altered habitats dominate the landscape, including *montado* (i.e. a traditional multiuse system that has resulted in the degradation of the original Mediterranean forest dominated by holm and/or cork oaks) accounting for 40% of the area through which these roads pass; cereal crops (28%); vines/olive orchards (13%); pine and eucalyptus production forests (11%); and urban areas (8%) (IGP, 1990).

Generally, the surveyed roads had two lanes of traffic, one in each direction and an asphalt hard shoulder, with a global mean width of 7m. Different types of fences exist along 33% of the length of the surveyed road. Sometimes, along one or both road-sides, trees form small corridors along the road. The length of all surveyed segments and traffic volume, are exhibited in Table 1.

Table 1. Surveyed length and mean traffic volume of surveyed roads.

Surveyed segments roads	**Surveyed length**	**Mean traffic volume (vehicles/day)**
IP2	40 Km	5920
IC1	16 Km	---
EN2	16 Km	---
EN4	31 Km	8084
EN5	33 Km	9361
EN10	12 Km	8276
EN18	34 Km	8845
EN114	23 Km	13252
EN120	19 Km	---
EN121	22 Km	6508
EN257	4 Km	---
EN259	25 Km	5389
EN370	15 Km	---
EN380	21 Km	5944
Total	**311 Km**	—

Traffic volume data refers to summer months of 2001, and was supplied by Estradas de Portugal, E.P.E.. (—) means lack of data

Data collection

Road-kill survey

Strigiforme fatalities were counted once each fifteen days, over a two-year period, between July 2003 and July 2005. The survey was conducted by one observer traveling at 30km/h, driving along the road's hard shoulder. When a killed animal was found, the species was identified, and the location was recorded using a GPS device. To avoid double-counting, carcasses were removed from the road. The total length of road surveyed was 622 km, counting both sides of the road.

Eco-geographical Variables

Twenty-two variables were considered for inclusion in the models, all reported to be meaningful to Strigiforme fatality incidence by previous investigators (Mikkola, 1983; Hernandez, 1988; Petty, 1989; Marques, 1994; Massemin & Zorn, 1998; Meunier et al., 2000; Fajardo, 2001; Clevenger et al., 2003). Table 2 presents information about each variable, its structure, and other important features.

All the roads were subdivided into 500m segments, and each segment was characterized in terms of each variable. Some descriptors characterize the area through which the segment passes, defined by a buffer of 250m around the segment (Table 2). For some variables, like CTRPOS , CTRNEG and WBTYPE the most representative feature was registered. With respect to the variable DMINLO, the distance considered is relative to the center of the characterized segment. Some variables were transformed in order to soften the effects of extreme values (Table 2).

Land uses mapping was based on COS'90 (IGP, 1990), updated with field surveys. The GIS software used was Arcview GIS 3.2 ® (ESRI, 1999) and ArcGis 9 ® (ESRI, 2004).

Statistical analysis

Spatial analysis in search of fatality clustering

In order to identify global clustering in the spatial arrangement of Strigifome fatalities, Moran's I was calculated for each species' fatality dataset. The index compares the value of one variable, that represents the number of fatalities, at one determined location with the same value across all other locations, using for that comparison the distance between locations (Anselin, 1992). For this test, the number of fatalities in each 500m segment was associated with the center of the same segment. All counted fatalities were restricted to the road, so distance measurements were obtained using the distance along the road "network", despite the direct distance. For these calculations, Crimestat® III software was used (Levine, 2004a).

The degree of fatalities clustering was investigated using an adaptation of Ripley's K-function, the network K-function (Okabe & Yamada, 2001). Ripley's K-function describes the clustering of data over different spatial scales, by calculating the average number of points within a determinate distance, *d*, from each point in the dataset, then dividing it by the overall study area to give K(*d*). This process is repeated for increasing distances (O'Sullivan & Unwin, 2003). We used the Okabe & Yamada (2001) adaptation which modifies this statistic to work with distances along a network.

Table 2. Variables, their description and some important features.

Variable name	CODE	Description		Unit	Transformation	Source
Mean altitude	MEAALT	Mean ground altitude under road		Meters	-	Digital elevation model
Mean slope	MEASLP	Mean ground slope under road		Meters	LL/T.alba, A.noctua	Digital elevation model
Minimum distance to localities	DMINLO	Shortest distance to localities (>100 inhabitants)		Kilometers	-	-
Montado	MONTOT	Porportion of Montado (a)		Porportion	AA/A.noctua, S.aluco	Field Updated COS'90
Shrubland	SHRLND	Porportion of Shrubland (a)		Porportion	AA/T.alba	Field Updated COS'90
Cereal Crops	CERCRO	Porportion of Cereal Crops (a)		Porportion	AA/all	Field Updated COS'90
Pine plantations	PINEPL	Porportion of Pine plantations (a)		Porportion	AA/T.alba	Field Updated COS'90
Olive Orchads	OLIORC	Porportion of Olive Orchads (a)		Porportion	AA/A.noctua	Field Updated COS'90
Urban area	URBARE	Porportion of Urban area (a)		Porportion	-	Field Updated COS'90
Ripicule Vegetation	VEGRIP	Presence (1) / Absence (0) of ripicule vegetation		Porportion	-	Field Updated COS'90
Negative verges *vs.* contiguous area contrast	CTRNEG	Predominance of open habitats vs. closed habitats contrast (-1) / absence of negative contrast predominace (0)		-	-	Field
Positive verges *vs.* contiguous area contrast	CTRPOS	Predominance of closed habitats vs. open habitats contrast (1) / absence of positive contrast predominace (0)		-	-	Field
Width	WIDTHR	Road mean width		Meters	-	Field
Road topography	RDTOPO	Predominant road transversal topographic profile (b)	 (1) (2) (3) (rc) (4) (5) (6) (7) (i.e. absence of predominance)	-	-	Field
Relfectors	REFLCT	Presence (1) / Absence (0) of road reflectors		-	-	Field
Linear structures	LSTYPE	Presence of linear structures (a ; b)	(0) absence of these structures (rc) (1) water courses (2) storm drains (3) railways	-	-	Field
Fences	RDFENC	Presence (1) / Absence (0) of fences on road-sides		-	-	Field
Type of Water Bodies	WBTYPE	Presence of permament water bodies (a ; b)	(1) absence of permanent water bodies (rc) (2) permanent pond (3) reservoir	-	-	Field
Distance betwen trees	TDIS2S	Shortest distance between the trees of the two sides of the road	(1) 10-25 meters (2) 25-40 meters (3) 40-60 meters (4) +60 meters	-	-	Field
Trees density	TDENSI	Porportion of road contiguous area ocupied by trees (a)	(0) 0% (1) 0-10% (2) 10-30% (3) 30-50% (4) 50-70% (5) + 70%	-	-	Field
Diference of tree density	DDIFDE	Degree of diference between trees density of the two sides of road (a)	Small degree of diference (0) to high degree of diference (5)	-	-	Field
Wires and Post Density	DENWPO	Porportion of road lenght with electrical wires and posts		-	-	Field

Descriptors that characterize a 250m buffer around the road segments are identified by (a), and descriptors used as dummy variables are identified by (b). Dummy variable reference category is marked by (rc). Symbols used for transformation are (LL) for $\log(x+1)$, and (AA) for $Arcsen\sqrt{x}$.

In order to calculate the network K-function for fatalities among the three Strigiforme species, an ArcGis extension – Sanet 2.0 (Okabe et al., 2003) was used. The values of the network K-function for observed data then had to be compared with an approximation of expected values of network K-function for a random situation, which was generated by 1000 Monte Carlo simulations. For both the observed and expected situations, the incremental distance used for K-function value calculations was 500m.

The results were modified to show the incremental value of K for each additional distance, and the total was standardised to allow for comparisons between each species (Ramp et al., 2005). For a better understanding of this measure, the *L*-statistic was calculated, as the difference between the observed and expected K-function values. With the L-statistic, positive values signify clustering in that scale, and negative values signify dispersion (Ramp et al., 2005).

Modelling road fatalities

We developed two kinds of models: one using presence/absence data (Binary Logistic Regression) and other with presence-only data. In fact, when non-daily surveys are conducted, some of the carcasses can be destroyed by traffic or removed by some natural or un-natural cause between surveys, therefore some collision absences might be false (authors personal observations). This may alter the results and the accuracy of any presence-absence model. For this reason, besides generating a logistic regression model, a presence-only model was developed, using Ecological Niche Factor Analysis (ENFA).

BLR - Binary logistic regression

A model was developed for each species, using as the dependent variable the presence/absence of fatalities along 500m road segments. Absences were defined as segments where there were no recorded fatalities. The variables described for 500m segments were used as independent variables. The number of segments assigned the value of *absence* was higher than the number of segments assigned the value of *presence*. When this happens, the logistic regression within the function's domain is not symmetrical, but rather deviates towards the extreme that presents a higher number of segments (Barbosa et al., 2003). In this situation, in ecological studies, it is assumed that there is a difference between the logistic function and a species' response to the environmental conditions (Rojas et al., 2001). In order to avoid unbalanced models, a number of segments with the absence value, corresponding to the number of segments where presence was recorded, were randomly selected for further analysis. Therefore the information used for *T. alba*, *S. aluco* and *A. noctua* regression is relative to 294; 216 and 176 road segments, respectively.

A preliminary screening of the independent variables for collinearity was done prior to the analysis. Spearman rank correlation coefficients (r) were calculated. For pairs of variables with a correlation coefficient higher than 0,7 – meaning high collinearity (Tabachnik & Fidell, 1996; Clevenger et al., 2003) - only the more biologically-meaningful variable was retained for further analysis.

As a first-step for model building, as suggested by Hosmer & Lemeshow (2000), univariate logistic regression models were constructed and tested for each independent variable. The variables with a significant coefficient according Walt test at the 0,25 level were retained for multivariate analysis (Hosmer & Lemeshow, 2000).

Multivariate logistic regression was conducted, using all the selected variables, using the backward-stepwise selection procedure, with a 0,05 p-value threshold for entry and a 0,1 p-value threshold for removal (Pereira, 1996). The backward-stepwise variable selection method was chosen because (1) stepwise methods are the best option for exploratory work (Menard, 1995; Field, 2000); and (2) backward-stepwise selection is less likely than forward-stepwise selection to exclude independent variables involved in suppressor effects – therefore being at lower risk for generating type II error (Field, 2000). The resulting models, hereafter, will be called *environmental models*.

Because spatial autocorrelation was expected, the inclusion of an *autologistic term* (*autocovariate*; Augustin et al., 1998) as a new variable, allowed the development of an *autologistic model* (Augustin et al., 1998). This term gives a response at one location as a function of the responses at neighbourhood sites (Augustin et al., 1998), thereby taking into account the possible spatial autocorrelation of the dependent variable values. The calculated autologistic term is the same suggested by (Knapp et al., 2003), and for the present study is given by

$$\sum_{j \neq i} w_{ij} . y_j$$

where w_{ij} is the weighted distance (meters) between the 500m road segment i center and the center of the neighbour segment j, and y_j is equal to 1 if fatalities are present in segment j and 0 if they not. The weight distance was calculated by

$$\frac{d_{ij}^{-1/2}}{\sum_{j \neq i} d_{ij}^{-1/2}}$$

where d_{ij} is the distance (meters) between the 500m road segment i center and the center of the neighbour segment j. All distances were calculated over the road. The need to include the autologistic term in the model, is justified by the possible absence of explanation for spatial autocorrelation in the original set of variables (Odlund, 1988; Augustin et al., 1998).

The performance of resulting models (i.e. environmental and autologistic models) was evaluated using Pearson's Chi-square test and examining the area under the ROC curve (Receiver Operating Characteristics) – AUC (Sokal & Rohlf, 1995; Hosmer & Lemeshow, 2000; Manel et al., 2001). A *Jackknife* procedure was used to model validation, which consists in creating as many models as the number of cases, excluding one case at a time (Guisan & Zimmermann, 2000). The AUC also was used to evaluate the adjustment of Jackknife predictions to observed data. Also, the correct classification rate was obtained by calculating contingency tables.

The model (i.e. environmental or autologistic) used in further procedures was selected based on the value of the Akaike Information Criterion (AIC).

The coefficients derived from the selected models were applied to all 500m road segments, to produce the distribution of fatality occurrence probabilities. In order to identify the fatality hotspots predicted by the models, the 95th percentile of probability values were labelled (Ramp et al., 2005).

The analysis was carried out using the software package SPSS® 13.0 (SPSS, 2004).

ENFA – Ecological Niche Factor Analysis

In road fatality studies, as in ecological studies, accurate absence data are difficult to obtain; consequently, a modelling method that uses presence-only data must produce unbiased predictions of fatality hotspots. ENFA modelling is a presence-only method, identical to factor analysis, which compares a species' distribution in the ecological space (i.e. in the ecological variables) with the distribution of all sets of cells in the study area, producing suitability functions, and obtaining a number of uncorrelated factors that reflect the main environmental gradients within the study area (Hirzel et al., 2002a). This comparison leads to the determination of a multivariate niche occupied by the species, and it can be quantified by means of a *marginality* index and a *specialization* index (Hirzel et al., 2002a).

Through ENFA analysis, a value for marginality and another for tolerance were achieved. The first explains how the species mean differs from the global mean, and ranges from 0 to 1, '0' meaning that a species exists in the average conditions for all the study area and '1' meaning that the species tends to live in extreme conditions. The second term is the inverse of specialization, and explains how low the species' variance is compared to global variance. It likewise ranges from 0 to 1, '0' meaning that the species tends to live in a very narrow range of conditions and '1' that the species is not particularly restrictive in its living environment (Hirzel et al., 2002a). ENFA also produces a group of factors that explain species marginality and specialization. The first factor obtained with ENFA explains 100% of the marginality and some part of the specialization; the remaining level of specialization is explained by other factors. The amount of specialization explained by each one of the factors is represented by eigenvalues. The higher the absolute value of the coefficients attributed to each variable on the first factor, the further the species departs from the mean available habitat regarding the corresponding variable (Hirzel et al., 2002a). The positive and negative coefficient signs reflect whether the species prefers values that are higher than the study area mean or lower, respectively. For variable coefficients for the remain factors, only absolute values matter, so the higher these values are, the more restricted the range of the species with respect to the corresponding variable (Hirzel et al., 2002a).

Obviously, in this study the *niche* refers to the subset of segments in the ecogeographical space (relative to all the surveyed segments of road) where each one of the species fatalities has a reasonable probability of occurring. Interpreting ENFA results must take this concept into account. ENFA modelling was conducted using the Biomapper package 3.1 (Hirzel et al., 2002b). From the different algorithms that Biomapper had to use to build suitability maps for ENFA analysis, the geometric mean algorithm was chosen (Hirzel & Arlettaz, 2004). The used fatality data refers to the occurrence of each species road-fatalities for the 622 road segments of 500m. The independent variables used were the same that for BLR (Table 2). The variables that were not considered because of present constant distribution maps, overly-fragmented maps or nearly-bollean maps were: CTRNEG; CTRPOS; WIDTHR; DDIFDE; RDTOPO; LSTYPE and VEGRIP.

The MacArthur's broken stick method was used to determine the number of factors used to calculate suitability maps (Hirzel et al., 2002a). The accuracy of the model was assessed as the area under the ROC curve (AUC).

To permit carrying out the validation procedure, before the model construction, the original data set was split into calibration and validation sets, each one containing information about half of the road-segments. These segments were randomly selected. The calibration set was used to produce the model, which was later applied to validation set. The adjustment of its predictions to observed data was achieved by ROC curve (AUC).

The 95th percentile was used to determine the hotspot locations.

Alternative Hotspot identification methods

To identify fatality hotspot locations, besides modelling road-fatalities, three alternative methods were used: (1) kernel density estimation; (2) nearest neighbour hierarchical clustering (NNHC); and (3) comparing fatality occurrences within a Poisson distribution (Malo et al., 2004).

Kernel estimation, a density technique, identifies clusters by searching for dense concentrations of fatalities (Gitman & Levine, 1970). What this does is place a smooth and symmetrical kernel function over each point, estimating a density distribution by summing the individual kernel functions for all the locations that produce a smooth cumulative density function (Levine, 2004b). The width of the kernel function (i.e. the area of influence of each kernel function, or bandwidth) chosen for the present work was 500m for all species, making the estimation produced for all the species comparable. The choice of bandwidth size was made taking into account the reasonable scale at which mitigation measures might be taken (Ramp et al., 2005). The larger the bandwidth, the smoother the resulting cumulative density function (Gattrell et al., 1996). The kernel estimation for each species' fatalities was generated using Spatial Analyst toolbox for ArcGis®. The 95th percentile was used to determine the hotspot locations.

Nearest Neighbour Hierarchical Clustering (NNHC) is a method that identifies groups of points, based upon nearest-neighbour method criteria (Levine, 2004b). Consequently, this method defines a threshold distance, and compares that to the distances for all pairs of points (Levine, 2004b). To incorporate points in first-order clusters, the selected points have to be closer to one or more other points than the threshold distance. Afterwards, this method grouped these clusters with other clusters (second-order) by means of the same criteria, and this process was repeated until all points were grouped into one big cluster. The search for fatality hotspots, for all target species, was focused on point clustering (first-order clusters), discarding all the additional information provided by this method. The threshold distance chosen was the expected random linear nearest-neighbour distance for first-order nearest neighbours, and is calculated by

$$\text{Ld (random)} = 0{,}5\left[\frac{L}{N-1}\right]$$

where L is the total length of the road network and N is the total number of points (Hammond & McCullagh, 1978). In this context, the threshold distance was calculated for each species, using the total number of fatalities recorded for that species as N and the total length of the road as L. Note that distances between fatalities were calculated over the road.

The last method used to investigate the locations of fatality hotspots, hereafter referred as *Malo's method*, involved comparing the spatial pattern of fatality occurrences with that expected in a random situation, in which case the likelihood of collisions for each road segment exhibits a Poisson distribution (Malo et al., 2004). In this last situation, for segments of 500m, the probability of having x number of fatalities is given by

$$p(x) = \frac{\lambda^x}{\left(x!e^{\lambda}\right)}$$

where λ is the mean number of fatalities for 500m road segments. With the simple application of this equation, it was possible to determine the number of fatalities for 500m segments, above which the probability of occurrence in a random situation is approximately less than 2%, referred next as the *0,98 Threshold*. The suggests that high fatality rates should be distributed randomly along the road, and their aggregation in consecutive sections is very unlikely (Malo et al., 2004). Therefore, 500m road segments with a number of fatalities above the *0,98 Threshold*, especially over contiguous segments, were defined as hotspot. In the present study, the hotspots highlighted were defined by segments with more than two fatalities for *T. alba* (0,014 Poisson probability) and for *S. aluco* (0,003 Poisson probability), and by segments with more than one fatality for *A. noctua* (0,016 Poisson probability).

Note that Kernel and NNHC methods uses the locations where each road fatality occurred, while the *Malo's method* use fatality data gathered by 500m segments. Both the accuracy and applicability of all these methods used to identify fatality hotspots will be discussed further.

Performance of hotspot identification methods

The performance of the methods used to locate hotspots was provided by the following three measures - the relative number of kills that were prevented; the relative hotspot length; and the ratio among these measures (hereafter referred *KPvsHL ratio*), assuming that effective minimization strategies that prevented all the killings had been applied to the identified hotspots on the surveyed road, during the two-years.

Results

Road-fatalities surveys

Over the two years of observation, 593 Strigiforme fatalities were recorded. Among these, 52% were among *T. alba*, 27% were *S. aluco*, and 21% were *A. noctua*. The mortality index (i.e. fatalities/km/year) for each of the Strigiforme species along the surveyed road was 0,49 for *T. alba*, 0,25 for *S. aluco*, and 0,20 for *A. noctua*. All these contributed to an overall Strigiforme mortality rate of 0,94 fatalities/km/year.

Along the surveyed road, traffic volume was not correlated with Strigiforme mortality (Spearman's rho (r) =0,25; *P*=0,516). It also was not correlated with mortality rate in any one of the three species (*T. alba*: r =0,133; *P*=0,732; *S. aluco*: r =0,333; *P*=0,385; *A. noctua*: r =-0,067; *P*=0,865).

Strigiforme fatality clustering

As expected, significant spatial autocorrelation was identified in the distribution of fatalities for all the Strigiforme species (Table 3), meaning that the fatalities occurred in clusters, rather than being randomly distributed along the road.

Table 3. Moran's I, Z value and *P* value of road-fatalities for the three Strigiforme species

Species	Moran's I	Z	*P*
T.alba	0,087	21,770	0,0001
S.aluco	0,060	15,110	0,0001
A.noctua	0,076	18,850	0,0001

For *T. alba*, the *L*-statistic showed that clustering of fatalities occurred at scales up to 20km. Also for *S. aluco* and *A. noctua*, clustering at scales up to 20km was identified (Figure 2). In addition, *A. noctua* fatality clusters occurred regularly at scales between 20 and 80km (Figure 2).

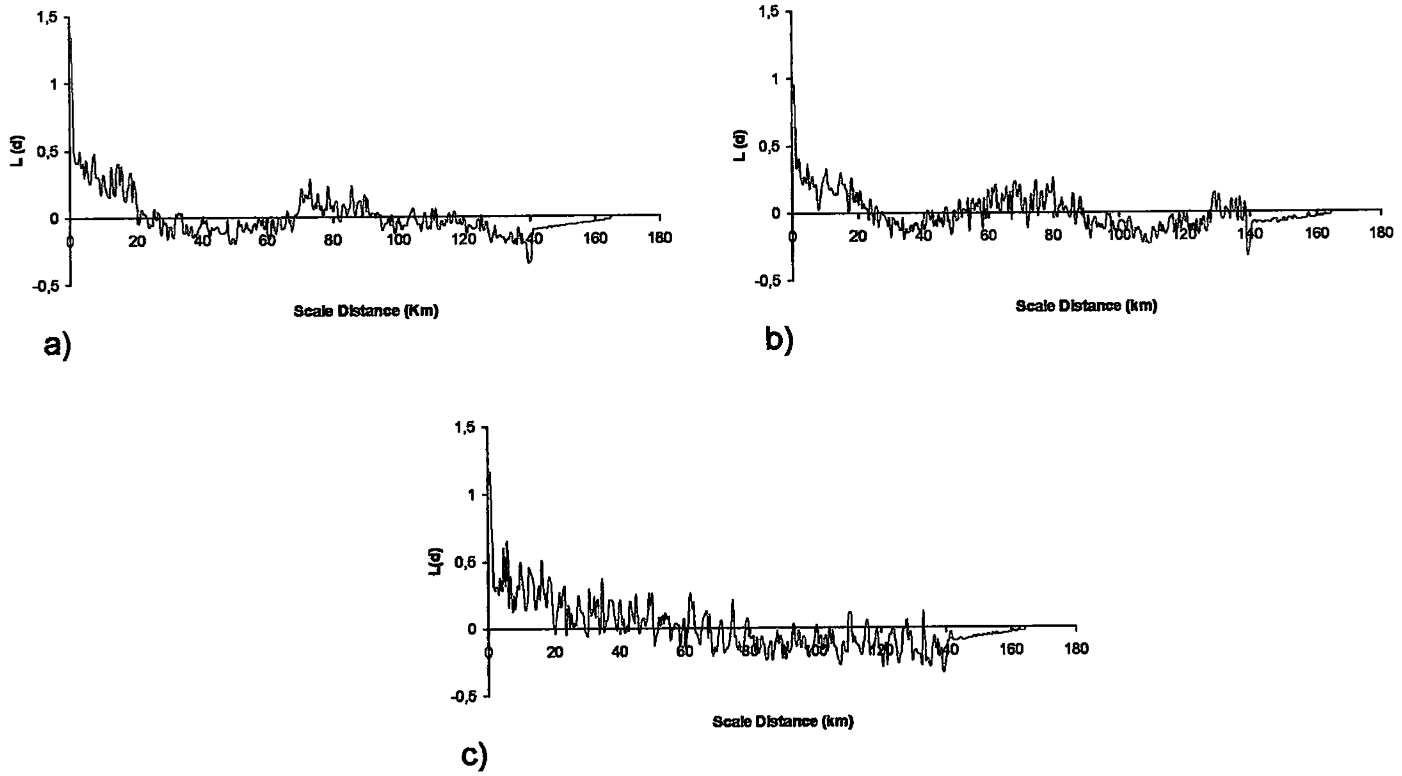

Fig 2. *L* statistic values for the network K-function for each species. a) *T. alba*; b) *S. aluco*; c) *A. noctua*.

BLR - Binary logistic regression

After Spearman rank correlation test and univariate logistic regression, the variables retained to perform multivariate analysis for each species fatalities were those presented in Table 4.

Table 4. Univariate logistic regression coefficients (ß), standard errors (S.E.) and significance level (*P* value), for descriptors retained for multivariate analysis in each species

		T.alba			*S.aluco*			*A.noctua*		
VARIABLES		**ß**	**S.E.**	***P***	**ß**	**S.E.**	***P***	**ß**	**S.E.**	***P***
MEAALT		-0,004	0,001	0,006	-0,005	0,002	0,001			
MEASLP		-1,279	0,742	0,085						
DMINLO								-0,273	0,064	< 0,001
MONTOT					0,903	0,251	< 0,001	-1,331	0,317	< 0,001
SHRLND		-0,883	0,346	0,011	0,018	0,006	0,003	-0,029	0,100	0,002
CERCRO		0,566	0,236	0,016	-1,321	0,328	< 0,001	0,867	0,307	0,005
PINEPL		-1,129	0,394	0,004	0,011	0,006	0,052	-0,015	0,009	0,104
OLIORC					-0,012	0,007	0,091	1,588	0,472	0,001
URBARE		-0,014	0,007	0,049						
VEGRIP		0,089	0,055	0,108						
WIDTHR		0,392	0,128	0,002	0,405	0,153	0,008	0,516	0,195	0,008
RDTOPO										0,178
	Type (1)							1,228	0,505	0,015
	Type (2)							-1,124	0,822	0,172
	Type (4)							0,417	0,785	0,595
	Type (5)							-0,276	0,930	0,767
	Type (6)							0,129	1,426	0,928
	Type (7)							0,535	0,930	0,566
REFLCT								0,706	0,180	0,028
LSTYPE				0,153						
	(1) water courses	0,558	0,877	0,524						
	(2) storm drains	1,173	0,901	0,193						
	(3) railway	0,693	1,041	0,505						
RDFENC		-0,555	0,266	0,037						
WBTYPE				< 0,001			0,003			0,003
	(2) permanent pond	2,350	0,625	< 0,001	3,040	1,039	0,003	1,149	0,678	0,090
	(3) reservoir	2,702	0,644	< 0,001	3,542	1,059	0,001	1,992	0,694	0,004
TDIS2S		-1,756	0,951	0,065	-0,287	0,104	0,006	0,125	0,110	0,240
TDENSI		-0,124	0,093	0,181	0,597	0,124	< 0,001	-0,610	0,156	< 0,001
DDIFDE								-0,428	0,301	0,154
DENWPO					-0,017	0,005	0,001	0,009	0,005	0,094
Autocovariate		20,835	2,799	< 0,001	29,828	4,833	< 0,001	20,117	3,831	< 0,001

All the models obtained by multivariate logistic regression (Table 5) showed significant improvements over the null model, and fit the data well (Table 6). The validation Jackknife procedure results suggest that all these models can be applied to other roads (Table 6). In all situations, the autologistic model performed better than the environmental model in terms of goodness of fit and model validation (Table 6). Using Akaike Information Criterion, the models selected to predict the location of fatality hotspots for these species were the autologistic models.

T. alba

The autologistic model for *T. alba* included three variables besides the autologistic term (Table 5). The proportions of pine tree habitat and of urban area were negatively associated with the occurrence of *T. alba* fatalities. Additionally, the presence of permanent water bodies (i.e. permanent ponds and reservoirs) near the road was significantly associated with fatalities.

S. aluco

Three variables were included in this autologistic model besides the autologistic term (Table 5). The incidence of *S. aluco* fatalities appeared to be positively associated with the following features proximal to the road: 1) the proportion of montado; 2) the density of trees; and 3) the presence of permanent water bodies.

A. noctua

The autologistic model included three descriptors besides the autologistic term (Table 5). Distance to localities was negatively associated with *A. noctua* fatality incidence, such that the shorter the distance, the higher the mortality rate, while the proportion of cereal crops and the presence of reflectors at road sides were positively associated.

Table 5. Environmental and autologistic regression models for each species.

T.alba

Environmental model						**Autologistic model**					
Variables		**ß**	**S.E.**	**Wald**	***P***	**Variables**		**ß**	**S.E.**	**Wald**	***P***
SHRLND		-1,237	0,475	6,782	0,009	**PINEPL**		-1,304	0,437	8,906	0,003
PINEPL		-1,801	0,489	13,568	<0,001	**URBARE**		-0,021	0,008	7,342	0,007
URBARE		-0,024	0,008	9,798	0,002						0,059
WIDTHR		0,405	0,169	5,787	0,016	**WBTYPE**	**2**	1,304	0,682	3,657	0,056
RDFENC		-1,524	0,338	20,382	<0,001		**3**	1,603	0,686	5,457	0,019
					<0,001	*Autocovariate*		18,628	3,106	35,963	<0,001
WBTYPE	**2**	2,704	0,714	14,350	<0,001	Constant					
	3	3,137	0,738	18,060	<0,001						
TDIS2S		-0,196	0,109	3,219	0,007						
Constant		-3,761	1,021	13,563	<0,001						

S.aluco

Environmental model						**Autologistic model**					
Variables		**ß**	**S.E.**	**Wald**	***P***	**Variables**		**ß**	**S.E.**	**Wald**	***P***
MONTOT		0,850	0,293	8,403	0,004	**MONTOT**		0,699	0,303	5,309	0,021
					0,001						0,017
WBTYPE	**2**	3,438	1,059	10,529	0,001	**WBTYPE**	**2**	2,592	1,093	5,621	0,018
	3	3,940	1,089	13,081	<0,001		**3**	3,096	1,123	7,606	0,006
TDENSI		0,515	0,134	14,735	<0,001	**TDENSI**		0,297	0,157	3,583	0,058
Constant		-4,989	1,113	20,096	<0,001	*Autocovariate*		16,575	5,954	7,749	0,005
						Constant		-6,670	1,280	27,148	<0,001

A.noctua

Environmental model					**Autologistic model**				
Variables	**ß**	**S.E.**	**Wald**	***P***	**Variables**	**ß**	**S.E.**	**Wald**	***P***
DMINLO	-0,300	0,077	15,167	<0,001	**DMINLO**	-0,291	0,077	14,179	<0,001
CERCRO	1,265	0,353	12,815	<0,001	**CERCRO**	0,642	0,376	2,925	0,087
OLIORC	1,230	0,517	5,662	0,017	**REFLCT**	0,779	0,379	4,233	0,040
REFLCT	0,776	0,373	4,320	0,038	*Autocovariate*	13,104	4,363	9,019	0,003
Constant	-0,519	0,386	1,814	0,178	Constant	-2,055	0,739	7,735	0,005

ß - regression coefficient, S.E. - standard error of the regression coefficients, Wald - Wald statistics, *P* - significance level.

Table 6. Chi-square, discrimination ability estimates, validation Jackknife procedure results and Akaike Information Criterion (AIC) for environmental and autologistic models for each species

		Chi-square test		Correct classification rate	ROC		Jackknife procedure ROC		AIC
	Model	χ^2	*P*		AUC	*P*	AUC	*P*	
T.alba	**Environmental model**	85,841	<0,01	0,710	0,793	<0,01	0,757	<0,01	335,730
	Autologistic model	93,468	<0,01	0,710	0,810	<0,01	0,787	<0,01	322,102
S.aluco	**Environmental model**	60,318	<0,01	0,690	0,784	<0,01	0,754	<0,01	247,121
	Autologistic model	69,396	<0,01	0,727	0,816	<0,01	0,778	<0,01	240,043
A.noctua	**Environmental model**	48,898	<0,01	0,727	0,777	<0,01	0,750	<0,01	205,090
	Autologistic model	52,012	<0,01	0,727	0,784	<0,01	0,756	<0,01	201,976

Ecological Niche Factor Analysis - ENFA

T. alba

The obtained model (Table 7) explained 78% of all the information. Fatalities appeared to occur under conditions that were almost like the mean conditions along the surveyed road (global marginality = 0,368), albeit under a relative wide range of conditions (tolerance = 0,736). Examination of the marginality factor suggested that the variables describing high road-kill segments that are most different from mean road conditions are mean altitude, the presence of fences, and the proportion of shrub-land and pine tree habitats (Table 7). The signal of score values demonstrated that road-kills are associated with the absence of fences, and with smaller values of the other three variables (Table 7). Nine percent of the specialization was explained by the marginality factor, the remaining essentially being related to the presence of permanent bodies of water and fences, the proportion of pine tree habitats, and urban areas (Table 7). The model's fit was good (AUC = 0,751, p<0,001). The validation procedure demonstrated good performance of the model (AUC = 0,710, p<0,01). Five factors were used to configure the hotspot location map (Figure 3).

S. aluco

For this species, fatalities seemed to occur under relatively specific conditions (tolerance = 0,594), and exhibited a moderate difference from mean conditions (global marginality = 0,586). The model explained 81% of the data. Fatalities tended to occur for higher values of tree density and proportion of montado habitat, and for lower values for mean altitude and proportion of cereal crops, versus global conditions (Table 7). After the first factor, the remaining specialization was explained by the other two factors, which were associated with the presence of permanent bodies of water and the mean slope (Table 7). The goodness of fit was good (AUC = 0,761, $P<0,001$). The model also exhibited good performance when validation was tested (AUC = 0,721, $p<0,01$). The hotspot map was configured using three factors (Figure 4).

A. noctua

A. noctua fatalities tended to occur under relatively distinct conditions from global mean conditions (global marginality = 0,778) and in a moderate range of the tested descriptors (tolerance = 0,656). Fatalities seemed to occur preferentially in locations near localities, with lower values for proportion of montado habitat and tree density, and higher values for proportion of olive orchard habitat (Table 7). The marginality factor only explained 7% of the specialization, the remaining mostly associated with the presence of permanent bodies of water and the proportion of shrub-land habitats. The amount of information explained by this model was 78%, showing good fit (AUC = 0,747, $p<0,001$). The validation procedure revealed that the model performs well (AUC = 0,732, $p<0,001$). Four factors were used to configure the fatalities hotspot map (Figure 5).

Table 7. Results of Ecological Niche Factor Analysis. Showing the four most important variables in each factor, sorted by decreasing absolute values of the coefficients.

T.alba

Marginality	Specialisation			
Factor 1 (9%)	Factor 2 (17%)	Factor 3 (11%)	Factor 4 (10%)	Factor 5 (9%)
MEAALT (-0,51)	WBTYPE 2 (0,66)	PINEPL (-0,57)	RDFENC (-0,55)	URBARE (0,46)
RDFENC (-0,35)	WBTYPE 3 (0,64)	SHRLND (0,46)	MEAALT (0,38)	CERCRO (0,44)
SHRLND (-0,28)	SHRLND (0,19)	TDENSI (-0,30)	WBTYPE 3 (0,37)	TDENSI (0,35)
PINEPL (-0,27)	DMINLO (0,19)	WBTYPE 3 (0,27)	MEASLP (0,37)	WBTYPE 2 (0,31)

S.aluco

Marginality	Specialisation	
Factor1 (26%)	Factor2 (22%)	Factor3 (13%)
TDENSI (0,53)	WBTYPE 2 (0,72)	MEASLP (0,73)
CERCRO (-0,39)	WBTYPE 3 (0,61)	WBTYPE 3 (0,30)
MONTOT (0,33)	MONTOT (-0,13)	WBTYPE 2 (0,26)
MEAALT (-0,30)	TDENSI (-0,12)	RDFENC (-0,25)

A.noctua

Marginality	Specialisation		
Factor 1 (7%)	Factor 2 (26%)	Factor3 (12%)	Factor4 (10%)
OLIORC (0,56)	WBTYPE 3 (-0,58)	WBTYPE 3 (-0,67)	SHRLND (-0,67)
DMINLO (-0,36)	PINEPL (0,50)	WBTYPE 2 (-0,64)	DMINLO (0,43)
MONTOT (-0,35)	WBTYPE 2 (-0,35)	REFLCT (0,16)	TDENSI (-0,29)
TDENSI (-0,31)	MONTOT (-0,32)	PINEPL (-0,11)	MONTOT (0,29)

The percentage values after each factor refers to the amount of specialization explained by that factor.

Hotspot identification methods

For *T. alba*, fatality kernel density estimation highlighted three especially dense hotspots (Figure 3), in addition to a number of small section clusters. The remaining methods were able to identify these locations as fatality hotspots; however BLR and ENFA failed to mark at least one of the locations where fatalities occurred at higher frequency. These two methods highlighted mostly continuous hotspots, whereas the other methods revealed a more fragmented hotspot distribution (Figure 3). The over-abundance of fatalities along two sections of road, (Figure 3) each of approximately 20km length, are reflected in the *L*-statistic.

For *S. aluco,* the kernel method identified nine particularly dense hotspots, all located in the proximity of high tree density habitats (Figure 4). Some of the other methods failed to identify these locations as hotspots (Figure 4). The proximity of five of these dense hotspots is reflected in the *L*-statistic, which illustrated clustering at scales up to 20km.

The majority of hotspots identified by the kernel method for *A. noctua* was located in road-sections adjacent to low tree density habitats (Figure 5). The wide distribution of road sections with fatality over-abundance, near Beja, may explain the clustering with scales up to 20km and regularly at greater scales revealed by the *L*-statistic (Figure 5). One dense hotspot occurred approximately 20km from Beja (Figure 5). All utilized methods exhibited slight differences when the locations of highlighted hotspots were analysed.

Note that, for all three species, ENFA signalled several sections of road without any observed over-abundance of fatalities. (Figure 5).

Considering the KPvsHL ratio (Table 8) the most effective method for Strigiforme road-kill hotspot identification was *Malo's method*, which means that allows preventing a higher number of road fatalities at lower costs than the other methods, if minimization measures are applied to identified hotspots. On the other hand, the BLR and ENFA methods provided the worst results. Note also that the kernel density estimation procedure performed about as well as *Malo's method*.

Table 8. Performance measures results for hotspot identification methods, assuming that effective road-kill minimization measures were applied to the identified hotspots on surveyed road, by a two years period.

Hotspot Identification Methods	*T.alba*			*S.aluco*			*A.noctua*		
	KP	HL	Ratio	KP	HL	Ratio	KP	HL	Ratio
BLR	23,30%	6,59%	3,54	18,99%	5,31%	3,58	20,97%	5,14%	4,08
ENFA	18,77%	5,31%	3,53	17,72%	5,31%	3,34	12,10%	5,95%	2,03
Kernel	66,02%	7,75%	8,52	61,39%	6,07%	10,11	66,94%	5,85%	11,44
Malo's method	52,75%	5,95%	8,87	20,88%	1,93%	10,82	48,39%	4,02%	12,04
NNHC	47,90%	5,95%	8,05	34,18%	4,98%	6,86	49,20%	7,07%	6,96

KP – Road kills prevented; HL – Hotspot length; Ratio - KPvsHL ratio

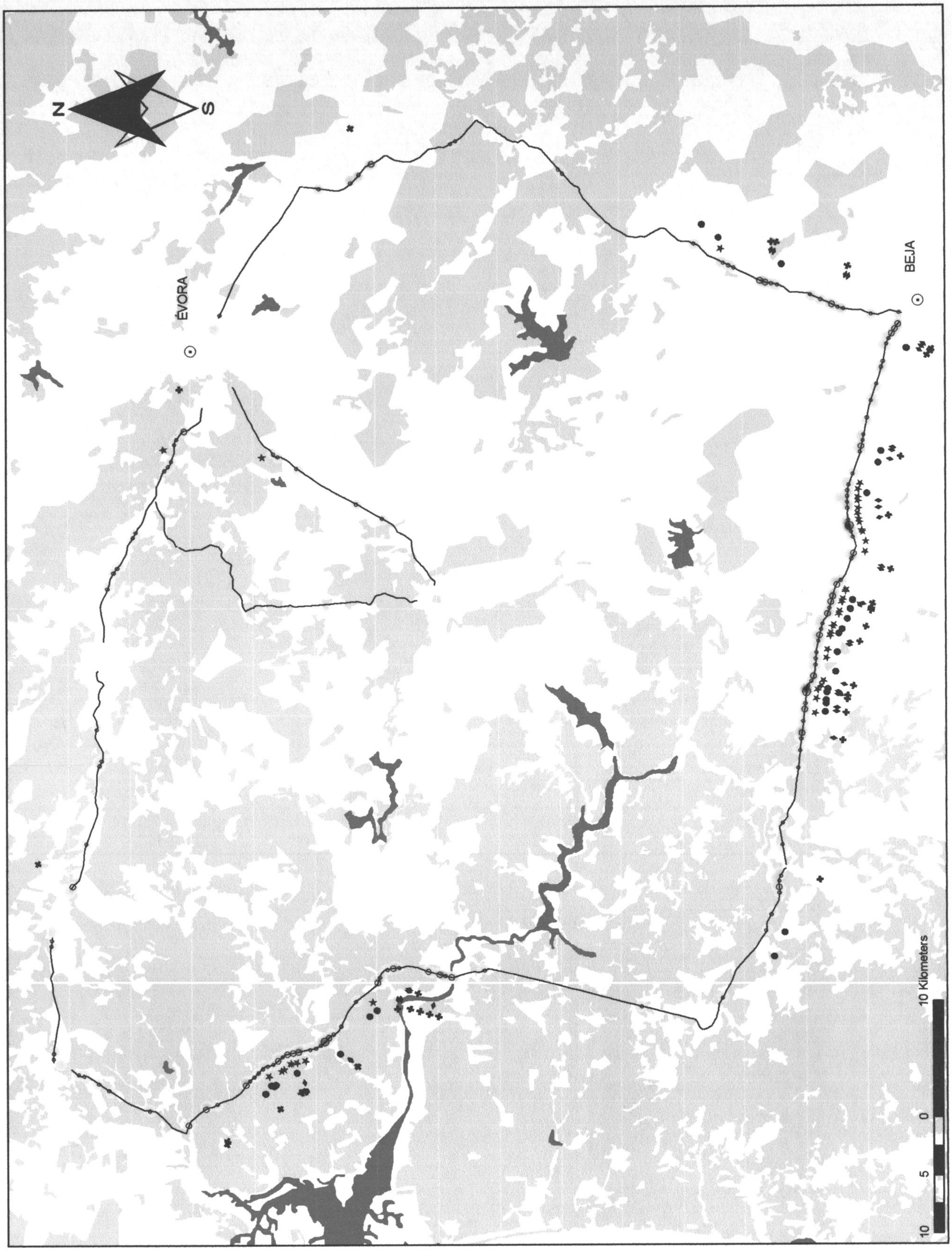

Fig 3. Map obtained by the five hotspot identification methods for *T. alba* road fatalities. Kernel density estimation results are represented by black colour gradient under the road line. Darker shading reflects higher density. Hotspots identified by the other methods were marked by ★ (BLR), ● (ENFA), ♦ (NNHC) and ✚ *Malo's method* placed aside the road. One symbol refers to a 500m road section. The size of circumferences over road line represents the observed mortality in each 500m segment.

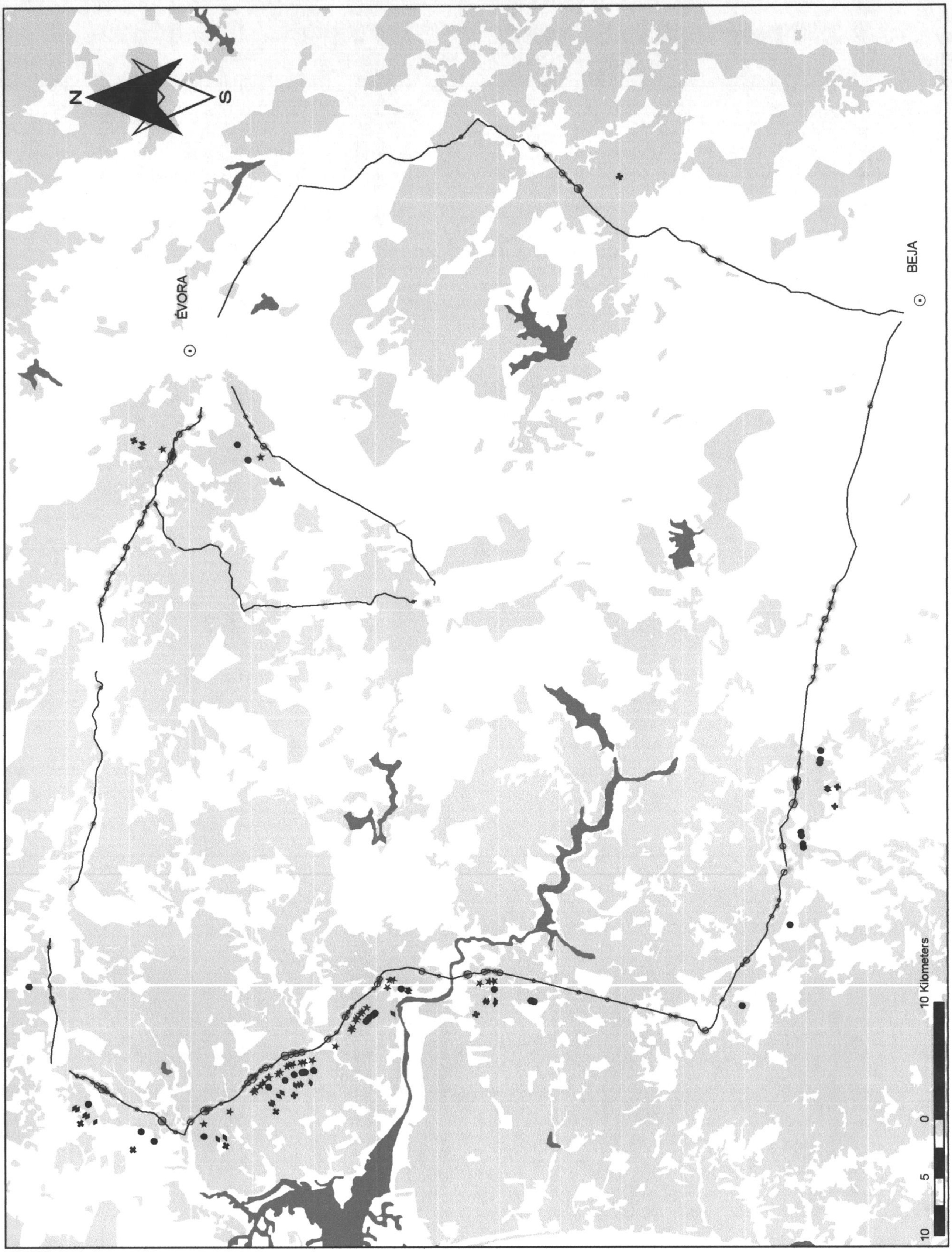

Fig 4. Map obtained by the five hotspot identification methods for *S. aluco* road fatalities. Kernel density estimation results are represented by black colour gradient under the road line. Darker shading reflects higher density. Hotspots identified by the other methods were marked by ★ (BLR), ● (ENFA), ♦ (NNHC) and ✚ *Malo's method* placed aside the road. One symbol refers to a 500m road section. The size of circumferences over road line represents the observed mortality in each 500m segment.

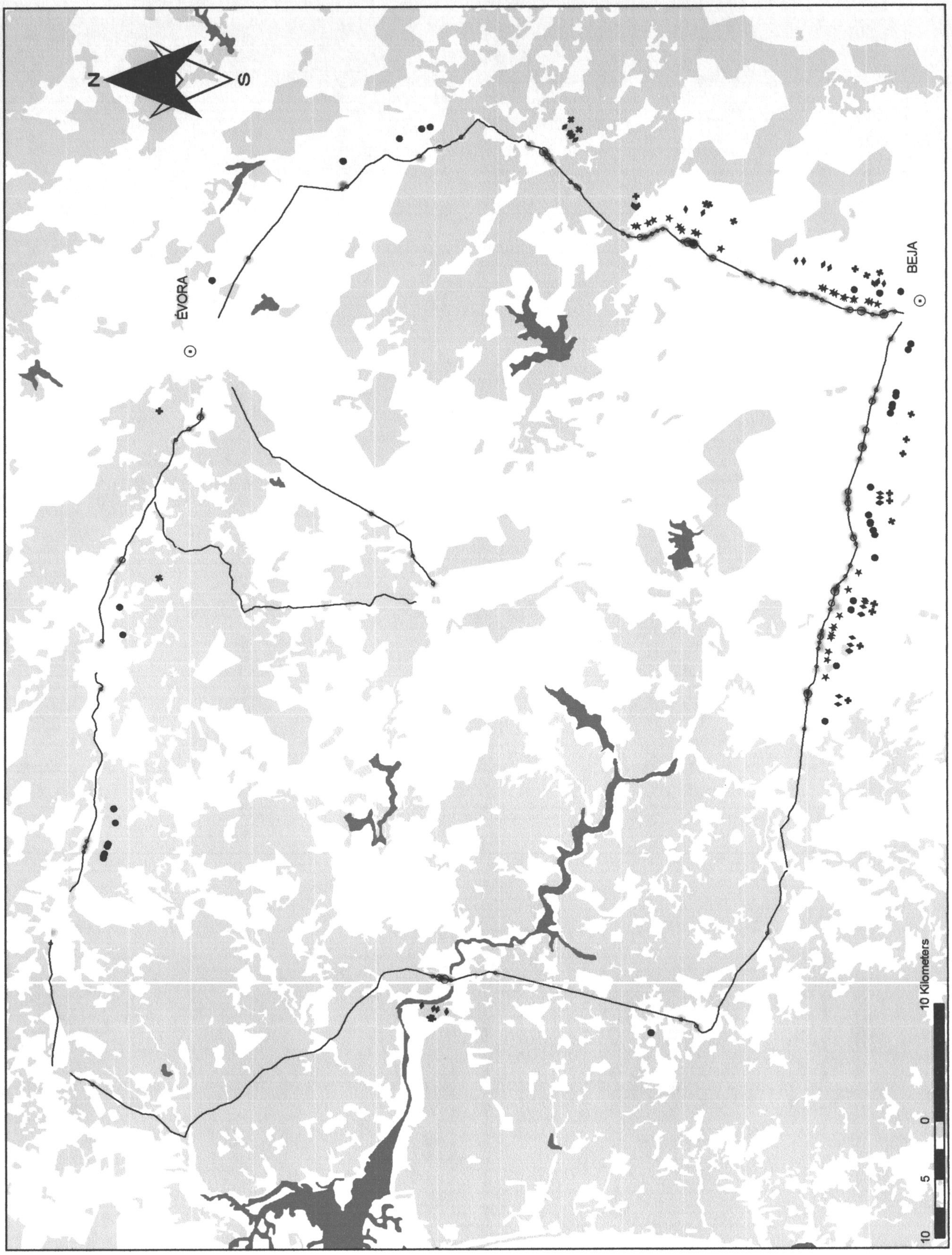

Fig 5. Map obtained by the five hotspot identification methods for *A. noctua* road fatalities. Kernel density estimation results are represented by black colour gradient under the road line. Darker shading reflects higher density. Hotspots identified by the other methods were marked by ★ (BLR), ● (ENFA), ♦ (NNHC) and ✚ *Malo's method* placed aside the road. One symbol refers to a 500m road section. The size of circumferences over road line represents the observed mortality in each 500m segment.

Discussion

Road collisions are considered to be the major cause of mortality for the Strigiforme species (Baudvin et al., 1991; de Bruijn, 1994; Taylor, 1994). Consistent with these earlier studies, the high mortality rates observed in the current study demonstrate a particularly large impact of road traffic on nocturnal birds of prey populations. However, the lack of population studies on each target species, and hence the lack of data on the size of these populations, did not allow for us to assess the true impact of the observed deaths on these populations. Besides the direct impact of vehicle collisions, road traffic may promote long-term fragmentation of populations by decreasing the occupancy of usual nesting sites (Ramsden, 2003), thereby affecting genetic exchanges (Mikkola, 1983; Lodé, 2000). All these effects on Strigiformes might affect the perpetuation of its populations.

The high rates of road mortality observed among the Strigiforme species seem to be associated with the species' almost exclusive nocturnal activity, and with behavioural adaptations to such conditions. Strigiformes, as nocturnal species, are not able to use warm air currents in flight, reducing their energy efficiency (Mikkola, 1983). Consequently, they use a variety of structures (i.e. trees, fences, electrical wires and posts) that are distributed along road-sides, to serve as perches, thereby focusing their activity in areas, like roads, that harbour such structures. Additionally, these areas generally provide a relative abundance of food (Bourquin, 1983; Fajardo et al., 1992). All these factors likely increase the likelihood of vehicle versus animal collisions, at least relative to other raptor species. Moreover, at night, it is more likely that car lights cause temporary blindness in individuals that attempt to cross the road, further inducing collisions (Hernandez, 1988). Other studies have suggested that the relative abundance of inexperienced juveniles crossing roads might be the reason for a greater number of fatalities occurring during times of dispersion (Massemin et al., 1998).

The most traffic-affected species was the *T. alba*, with more than twice the mortality observed for the other two species. This difference may be due to the ubiquity of the species, especially in areas surrounding the surveyed road (Fajardo *et al.*, 1992). However, it also may be due to some specific behavioural, anatomic and/or physiologic features present in this species, which increases its vulnerability to traffic collisions (Muntaner & Mayol, 1996; Clevenger et al., 2003; Erritzoe et al., 2003). This species does not retain as much body fat ($\approx 5\%$ of body mass), which affects the amount of energy stores that accumulate and the species' tolerance to low temperatures (Martínez & López, 1995; Massemin & Handrich, 1997; Hoyas & López, 2002). These limitations coalesce with high activity periods, when males provide food for females during courtship-feeding (Baudvin et al., 1991; Taylor, 1994), with high energy spent by females during the fledging and post-fledging stages (Taylor, 1994), and with starvation during severe winters, all of which may produce poor body conditions (Newton et al., 1991). This results in a decrease in the birds' accuracy and efficiency of behavioural responses to outside stimuli, increasing the probability of road-kill episodes. Additionally, it generally is accepted that *T. alba* usually fly 2-5m above the ground while hunting (Baudvin, 1986), a height which increases its probability of colliding with vehicles travelling on the road.

Factors that determine road casualties

In a general way, the models developed by means of BLR and ENFA exhibit satisfactory accuracy in estimating the probability of fatality occurrence and identifying predictors of this outcome. Across the three species, the selected BLR models fit the data better than the ENFA models did, suggesting that fatality absences are well accounted for. The experimental use of ENFA for stochastic phenomenon modelling over linear structures, for the first time, as far as we know, gave rise to a variety of issues that had to be circumvented. As a map-based modelling method, the use of descriptor maps with information constrained to a line should generate a higher degree of map fragmentation. Biomapper® software does not accept constant, nearly-Boolean or overly-fragmented variable maps (Hirzel, 2004). Variable maps that behave in this way must be excluded, potentially eliminating descriptors that explain a good percentage of the data. Among the seven variables excluded for this reason, one (road width) was included in the group of variables that comprise the models developed using BLR. Besides this, an interpretation problem may occur with ENFA models, when a small degree of specialisation is explained by the first factor (i.e. marginality factor); what occurs is that it becomes impossible to associate the remaining specialization to higher/lower values of the factor variables.

T. alba

T. alba is a eclectic species that is associated with several different habitats; however, it exhibits a preference for open land habitats, mainly with cereal crops (Mikkola, 1983; Lourenço et al., 2002), a characteristic that may explain why fatalities seem to be negatively associated with the proportion of pine tree habitat.

Despite commonly nesting near urban areas (Mikkola, 1983), a negative association was found between urban setting and number of fatalities. The reason for this is not entirely clear. However, it is possible that higher degrees of human activity taking place in these areas makes the habitats inadequate for hunting or for other activities that involve low altitude flight.

Typical prey of this species consists of small mammals (i.e. voles, shrews) that tend to occupy grassland areas undisturbed by agriculture (e.g. road verges), as well as nearby storm drains, water courses and permanent bodies of water surrounded by grassy vegetation (Bourquin, 1983; Mikkola, 1983; Fajardo et al., 1992; Van Der Reest, 1992). The presence of these structures at the road-side makes the area near the road a great hunting area, suggesting that individuals are attracted there by the availability of food (Meunier et al., 2000; Ramsden, 2003). This may increase the frequency of Strigiforme movements along and across the road, thereby increasing the probability of road-kills. Additionally, the scarcity of water, particularly in summer months, can also explain why fatalities concentrate near water bodies.

According to Mikkola (1983), *T. alba* commonly uses a flight-hunting technique to capture its prey. The presence of any structure that could prevent low-altitude continuous flight, near the road, may reduce the frequency of road-kill events. This may explain why the presence of fences appears to reduce fatalities.

S. aluco

This species, in contrast to *T. alba*, seems to depend upon dense woods of holm and cork trees and well-structured montado habitats (Mikkola, 1983; Elias et al., 1998; Lourenço et al., 2002; Lourenço et al., 2004), consistent with the positive association found between the occurrence of fatalities and the presence of montado habitat and high tree density. This also explains the association, found by ENFA, between fatalities and smaller proportions of cereal crops. *S. aluco* tend to use the old oak and cork trees for their nests and to optimize their view of the ground, facilitating both their search for and capture of prey (Mikkola, 1983; Cramp, 1992; Taylor, 1994; Hagemeijer & Blair, 1997; Ramsden, 2003). The distribution of identified hotspots almost exclusively near high tree density habitats is consistent with this hypothesis.

As the main food items for this species are similar those of *T. alba*, bodies of water near roads offer the same food source, potentially explaining the increased incidence of fatalities when such a body of water is nearby. Also for *S. aluco* the scarcity of water can explain the fatality distribution relative to water body locations.

A. noctua

Both model methods identify, for *A. noctua*, *distance to localities* as a significant inverse predictor of fatalities; the greater the distance, the lower the probability. This is in agreement with what we actually know about this bird, a species that seems to be closely associated with human-altered habitats, where the most important requirements are food availability (i.e. small mammals and insects) and the presence of hollow trees to place nests (Exo, 1992; Árias, 1994; Tomé et al., 2004). This also can explain the association between road fatalities and olive orchards. Olive orchards, besides being rich in hollow trees (one single olive tree can provide up to six breeding sites) also harbour grass patches around the base of their trunks, where prey is abundant (Fajardo *et al.*, 1998).

Besides occupying human-altered habitats, these birds tend to avoid woods with high vegetation density (Mikkola, 1983; Cramp, 1992; Tucker et al., 1994; Hagemeijer & Blair, 1997; Fajardo et al., 1998; Lourenço et al., 2004). Hernandez (1988) identified the lack of trees along the road as a factor responsible for increasing road casualties in this species. They trend to prefer more open areas, like cereal crops, (Cramp, 1992; Elias et al., 1998) which might increase the likelihood of being road-killed near such areas. This hypothesis is reflected in the distribution of identified hotspots, almost exclusively near low tree density habitats.

Temporary blindness of *A. noctua* individuals by car headlights is known to be responsible for a significant number of causalities (Hernandez, 1988). Road-side reflectors would be expected to increase this phenomenon and the resultant deaths, as the BLR model predicts.

Identification of fatality hotspots

Many methods have been utilized to identify the locations of fatality hotspots. Traditionally, the road is divided into segments of varying length, and predictive models are developed using the information (i.e. fatality numbers and possible explanatory variables) obtained from each one (Finder et al., 1999; Malo et al., 2004; Saeki & Macdonald, 2004; Ramp et al., 2005). Others approaches being proposed include modelling fatality data based upon point locations (Clevenger *et al.*, 2003), and performing *Malo's method* to assess fatality counts by segment (Malo *et al.*, 2004). However, most of these methods convert presence-only data to binary data (i.e. presence vs. absence), obtaining absences from segments with no recorded fatalities (Ramp *et al.*, 2005). Misclassification of absence data can result, due to the constant traffic that could displace or remove carcasses between surveys. To circumvent this problem, kernel density estimation has been suggested as an appropriate approach when only data on fatality presence exist (Ramp *et al.*, 2005).

The ability to localize hotspots varied considerably between the different methods tested, but not between the different species. Despite providing accurate estimates of fatality likelihood, the BLR and ENFA models exhibited the weakest performance when used to identify fatality hotspot locations. This demonstrates the amount of information that is lost in these two models, during the conversion from quantitative to presence/absence data (i.e. binary data) or into presence-only data, losses which are significant in the localization of hotspots. As discussed in a previous section, the use of ENFA for stochastic phenomenon modelling over linear structures may raise some issues that compromise model performance.

As expected, the kernel density estimation method performed well, but it was not the most sensitive method, despite the use of actual fatality locations, instead of 500m segments. The use of the 95th percentile for hotspot identification could result in discarding some fatalities, fatalities that might have contributed to higher values in the smooth density function for those hotspots. Conversely, *Malo's method* includes all fatalities that contribute to hotspot identification. Comparing all the various methods, *Malo's method* exhibited the greatest ability to detect the hotspot locations. When applied to the surveyed road, it accounted for the smallest effort, if minimization measures are applied (i.e. short hotspot length), to prevent the largest number of kills. However, despite its capacity to detect fatality hotspots, *Malo's method* does not allow for easy adjustments in the intensity of target hotspots. This feature is strongly related to the length of used segments and the amount of fatalities recorded by kilometre. Because the kernel method performed equally well as *Malo's method*, if regulation based upon the amount of effort and resources available is necessary, the former method seems more appropriate.

NNHC was better at identifying fatality hotspots than either BLR or ENFA, but worst than the other methods. The main weakness of the NNHC method is that it can identify two fatalities as a cluster, if those two fatalities occur closer together than the threshold distance, which may not be very useful.

Despite the previous performance considerations, BLR models can provide a good approximation of road fatality hotspots, when no fatality data are available. In such situations, prior to autologistic model application, the environmental model should be used to estimate the presence/absence of road-fatalities, so as to allow for the calculation of autologistic term values (Seoane et al., 2003).

Mitigation measures

It is widely accepted that Strigiformes are attracted to roads and their surroundings, because of environmental conditions and their required energy expenditures in pursuit of food (Bourquin, 1983; Hernandez, 1988; Taylor, 1989; Baudvin et al., 1991; Fajardo et al., 1992; de Bruijn, 1994; Taylor, 1994). Besides the relatively good habitat provided adjacent to most roads, hunting issues seem to be the principal cause for road casualties in these species. Consequently, mitigation measures must focus on these issues.

Altering the road proximity to certain habitat features, in accordance with owl hunting behaviours, could decrease road casualties. It seems obvious that reducing the area of undisturbed grassland will decrease the number of small mammals near roads, lowering the number of owls killed by traffic (Fajardo *et al.*, 1992). This also should reduce the tendency of Strigiformes to use these areas for hunting. However, care must be taken when a target road crosses the distribution area of some species of conservation concern that depends upon grassland habitats, as was the case with our surveyed road crossing the distribution area of Cabrera's vole (*Microtus cabrerae*; conservation status = vulnerable; (Cabral *et al.*, 2006)). Many populations of this species appear to depend upon road-side grassy areas to maintain their population and the connectivity between them during dryer summers (Pita *et al.*, 2006; Santos *et al.*, 2006). Also, certain measures, like planting short bushes, should decrease the visibility and accessibility of small mammals (Baudvin *et al.*, 1991).

Another way to reduce the interest of owls in road-side areas would be to minimize the proportion of good hunting grounds a short distance from the road, for example by draining small permanent ponds that can support a grassland habitat for most of the year, decreasing the attraction of Strigiforme species to these locations. Respecting the importance of these ponds for other *taxa* - especially during dryer seasons - new, replacement ponds must be created further from the road.

If possible, in *S. aluco* hotspots located in woody areas, trees could be removed or transplanted from the road side, reducing the number of perches close to the road. Conversely, in *T. alba* hotspots, located in open land habitats, fences and trees should be placed near to the road, and shrubs as well.

Lastly, because *A. noctua* seem to resting on the road where there isn't an adequate number of perches (Hodson, 1962), the installation of reflectors that only reflect the light upwards may reduce the temporary blindness these individuals suffer with oncoming headlights. Our study did not support Hernandez' (1988) suggestion that *A. noctua* suffer fewer road mortalities when high perches (>2m) are available.

Conclusions

To our knowledge, this is the first time that a study has been conducted systematically surveying such a long stretch of road, over as long a period as two years, in order to study factors predicting road-kill among Strigiforme species. We believe that the quality of our data permit us to make inferences regarding Strigiforme behaviours with confidence. Note also that is the first time that such information has been obtained in Portugal.

In any attempt to mitigate the adverse influence of roads on Strigiforme populations, the first critical step is to accurately identify the location of fatality hotspots. Our results indicate that *Malo's method* and kernel density estimations are the best methods for this purpose. This is true when fatality location data exist; but, when they do not, predictive models can provide a good approximation of hotspot locations. During model construction, priority should be given to regression methods that use quantitative data, rather than binary data.

After identification of fatality hotspots, one must strive to understand the factors that influence fatality clustering, therefore identifying potential minimization strategies to apply to these target areas. In these situations, the development of predictive models using BLR allows for the identification of deterministic variables that influence road-mortality.

The variables identified as determinants of Strigiforme road fatalities generally were associated with good habitat conditions in areas crossed by the roads, and with the existence of specific conditions that facilitate hunting behaviour near roads.

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O atropelamento é actualmente considerado uma importante causa da mortalidade em Strigiformes (Baudvin et al., 1991; de Bruijn, 1994; Taylor, 1994), situação que se reflecte no índice de mortalidade calculado para as espécies alvo deste estudo. A mortalidade global foi de 0,94 atropelamentos/km/ano, para a qual contribuiu o elevado número de atropelamentos registado para *T. alba* de 0,49 atropelamentos/km/ano atingindo aproximadamente o dobro da mortalidade de cada uma das restantes espécies. Tanto as características anatómicas, comportamentais e fisiológicas de *T. alba*, como a sua ubiquidade em áreas próximas da estrada podem estar na origem desta elevada taxa de mortalidade. Pelo facto de *S. aluco* e *A. noctua* serem espécies menos ecléticas relativamente ás condições óptimas do habitat, que no presente caso se restringem a uma porção da área circundante à totalidade da estrada, obteve-se um índice de mortalidade inferior para estas espécies relativamente à anterior.

A mortalidade destas espécies parece estar associada à existência de boas condições de habitat nas áreas atravessadas pela estrada, e da ocorrência de condições que promovem um elevado número de episódios de caça junto da mesma. Enquanto que a ocorrência de atropelamentos de *S. aluco* parece estar associada a habitats com predominância de árvores como o montado, em *A. noctua* tende a preterir este tipo de habitats ocorrendo principalmente na proximidade de habitats de origem antrópica que ocorrem perto das localidades. Os atropelamentos de *T. alba* tendem a ocorrer em habitats abertos como pseudo espetes cerealíferas, estando negativamente associados à proporção de pinhal (*Pinus sp.*) junto da estrada. Todas as Strigiformes estudadas têm como presas espécies que ocorrem em zonas de elevada densidade de gramíneas, como micromamíferos e insectos (Bourquin, 1983; Mikkola, 1983; Fajardo et al., 1992; Van Der Reest, 1992). A presença destas estruturas nas bermas da estrada e a respectiva abundância em presas torna-a um óptimo local de caça para estes predadores (Meunier et al., 2000; Ramsden, 2003). A existência de massas de água permanentes nas imediações da estrada, e da vegetação associada ás suas margens contribui para um aumento da frequência de episódios de caça e consequentemente da mortalidade de rapinas nocturnas associada á colisão com veículos, nomeadamente em *T. alba e S.*

aluco. Os modelos desenvolvidos também sugeriram que a presença de cercas junto da estrada tende a diminuir a mortalidade em *T. alba*, talvez porque em habitas abertos onde é possível a caça em voo (i.e. "flight hunting technique") estas estruturas dificultem a aproximação ao solo e a captura da presa. Por ultimo a mortalidade de *A. noctua* parece estar associada á presença de reflectores junto da estrada, que maximizam a frequência com que sucedem os episódios de encadeamento/atropelamento desses indivíduos.

A minimização da mortalidade por atropelamento, nestas espécies, deverá passar pela alteração das características da área circundante à via que potenciam a sua utilização como zona de caça. Reduzindo a abundância de gramíneas nas bermas parece obvio que se reduz substancialmente o interesse que estas zonas suscitam nas Strigiformes, no entanto há que ter em atenção o facto de que em algumas situações a persistência de outras espécies poderá depender da existência dessa vegetação. Outra forma de reduzir o interesse nas zonas próximas à estrada pode passar pela drenagem de alguns charcos temporários, que impossibilitarão a fixação de populações de micro mamíferos nas suas margens, resultando numa diminuição dos atropelamentos destas rapinas. No entanto a importância destes habitas para outros *taxa* poderá implicar a criação de massas de água alternativas a uma maior distância da estrada. Por último sugere-se a remoção/translocação das árvores da proximidade da estrada nos pontos negros identificados para *S. aluco,* e a colocação de cercas, arvores e arbustos junto da estrada nos pontos negros identificados para *T. alba.*

A distribuição dos atropelamentos não ocorre aleatoriamente ao longo da estrada, tendo sido identificados diversos pontos negros de mortalidade para as diferentes espécies. Dos modelos de identificação de pontos negros testados, apesar da dificuldade de comparação entre eles, inerente as diferenças nas suas abordagens, a Regressão Logística e a modelação mediante a Análise Factorial do Nicho Ecológico (ENFA) demonstraram em todos os casos uma performance fraca. O uso da Regressão Logística apenas se justifica quando, na ausência de informação relativa a atropelamentos, é necessário obter uma aproximação da localização dos pontos negros para as referidas espécies. Dos restantes métodos, o descrito por Malo (2004) foi o que obteve uma melhor performance..

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