UNIVERSIDADE DE ÉVORA

ESCOLA DE CIÊNCIA E TECNOLOGIAS

DEPARTAMENTO DE BIOLOGIA

Road and landscape fragmentation effects on tawny owls: density, population trend, and intra- and inter-year territory occupancy

Shirley Therese van der Horst

Orientação: Rui Lourenço

Mestrado em Biologia da Conservação

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Efeito das estradas e da fragmentação da paisagem na coruja-do-mato: densidade, tendência populacional e ocupação do território intra- e inter-anual

Resumo A urbanização é um dos maiores factores que impulsiona mudanças na paisagem e na biodiversidade e os seus efeitos ainda não são entendidos na totalidade. Apesar da coruja-do-mato (*Strix aluco*) não estar ameaçada a nível europeu ou global, é uma das aves que é frequentemente encontrada atropelada no sul de Portugal. Determinámos que a densidade de coruja-do-mato era mais baixa perto de estradas principais, com pouca diferença entre secundárias e de terra, mas a tendência populacional era negativa em estradas secundárias e principais. As variações inter e intra-anuais da presença de coruja-do-mato eram ambas mais elevadas em territórios perto de estradas secundárias, sugerindo uma elevada instabilidade. Os nossos resultados fazem-nos crer que os esforços de conservação devem-se focar tanto em estradas secundárias como principais, e nos seus efeitos primários e secundários.

Palavras-chave fragmentação da paisagem, dinâmica populacional, efeitos das estradas, interpolação espacial, *Strix aluco*, coruja-do-mato

Road and landscape fragmentation effects on tawny owls: density, population trend and intra- and inter-year territory occupancy

Abstract Urbanization is one of the major movements that impulses landscape and biodiversity change, and its effects have yet to be fully understood. Although the tawny owl (*Strix aluco*) is not threatened at European or global scale, it is one of the birds that is often found as roadkill in southern Portugal. We determined density values to be lower near main roads, and to have little difference between secondary and dirt roads, yet trend to be negative for main and secondary roads. Inter and intra-year variations in territory occupancy for the tawny owl in our study area were both highest in territories near secondary roads, suggesting high instability. Our results lead us to believe that conservation efforts should be focused on both main and secondary roads, and on both their primary and secondary effects.

Keywords landscape fragmentation, population dynamics, road effects, spatial interpolation, *Strix aluco*, tawny owl

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1. Introduction

Urbanization is one of the major movements that impulses landscape change and, consequently, a large amount of the effects on biodiversity. Structures like both residential and non-residential buildings, and the road networks that connect them are the main impact-creating objects that urbanization provides (Morelli *et al.,* 2015). Effects can be both positive and negative, but a large amount of studies show that the negative effects are prevalent (Reijnen and Foppen, 2006). It is also important to note that in a study from Rheindt (2003), a differential level of sensitivity to road effects was suggested, depending on the species of bird under study. A more recent study by Ware *et al.* (2015), confirmed divergent responses at both species and individual levels, and associated this with various factors, including: energy loss to find another resting place for those who decide to leave, and increased food availability for those who decide to remain, marking it down to a 'choice', after weighing of pros and cons.

Linear structures such as roads have permanent impacts on both the habitat and the species (Rheindt, 2003; Borda-de-Água *et al*., 2010), and their quantity and use is expected to rise as population and urbanization grow (Planillo *et al.,* 2015). Roads create both direct and indirect effects on populations. Direct effects include both wounding, and mortality, of a diverse and abundant number of animals (Coffin, 2007; Karlson *et al*., 2014). Collision with vehicles is a major source of bird mortality, of which a large part consists of individuals of the Strigiformes group (owls). Indirect effects may include: (1) various types of pollution, such as chemical, light and noise (which may decrease breeding success; Reijnen and Foppen, 2006); (2) fragmentation (Lesbarrères and Fahrig, 2012); (3) facilitated human access and invasion attempts by exotic species (Coffin, 2007; Planillo *et al.,* 2015); (4) edge effects; (5) barrier effects (Borda-de-Água *et al*., 2010; Grilo *et al*., 2014); (6) effects on a genetic level (e.g. Ascensão and Mira, 2005).

Visual signal reception, on which nocturnal birds rely in part, may be hindered due to the artificial lighting that roads create from dawn until dusk (Lourenço *et al*., 2013). Also, there may be a large will to avoid these stimuli by default, resulting in less individuals near the roads. Indirect effects such as these, however, depend on the road itself and the surrounding landscape, as roads surrounded by high tree or bush density will have mitigated indirect effects, whereas if the surrounding habitat is very open, the light, noise and chemical pollution will travel further (Reijnen and Foppen, 2006).

Fragmentation is often defined by a large section of habitat that is broken into patches, divided by habitat that is different from the original (Fahrig, 2003). The construction of roads leads to fragmentation, dividing large areas into smaller patches, many times leading to the segregation and consequent disappearance of species from the area (Redpath,1995), and this may be worsened by barrier effects (Karlson *et al*., 2014). It is likewise important to note that the effects of roads are not restricted to the immediate areas around the structures, and may extend several kilometres, depending on behaviour and biological needs of the species, and characteristics of the road and surrounding land (Planillo *et al.,* 2015; van der Ree *et al*., 2015). Even so, it is important to recall that roadside habitats are used as refuge for many small mammals and insects, and as feeding sites for necrophages (Coffin, 2007). Some animals may also use less busy roads as corridors – commonly these species are generalists and many times they are exotic or invasive species (Coffin, 2007; Planillo *et al.,* 2015).

Edge effects like higher temperatures during the day and larger wind speeds, are felt more keenly due to the clearing of vegetation that road structures imply, whilst barrier effects impede normal animal dispersion, foraging and migration (Borda-de-Água *et al*., 2010; Grilo *et al*., 2014) - they are felt most keenly by land-bound animals, but many flying animals are affected by them too (Karlson *et al.*, 2014). These and fragmentation result in a genetic effect, disrupting the flow, isolating meta-populations (e.g. Ascensão and Mira, 2005).

Many studies conducted on these infrastructures demonstrate the impact they have on bird populations which reside near or somehow interact with these roads (Rheindt, 2003). These impacts are generally more noticeable around roads with a greater flux of traffic, and many studies appoint noise pollution as the possible principal cause for a negative reaction from birds (Summers *et al*., 2011; McClure *et al*., 2013). Due to the diminished capacity for receiving and interpreting conspecific calls, and reduced perception of potential predators, noise pollution seems a plausible hypothesis, nevertheless there are many other factors that could potentially justify the generalized aversion of birds to roadside habitats (Summers *et al*., 2011). Grade and Sieving (2016) found that noise from a main road disallowed proper reaction to anti-predator information in birds, although they were unsure whether road noise masked vocal information, or monopolized the cognitive function of the birds.

Nocturnal birds such as owls are often victims of roads impacts, and may avoid main roads, as some studies suggest (Grilo *et al.,* 2014; Silva *et al.,* 2012). A decrease in predatory birds may be associated with an increase in prey species, due to a phenomenon called predation release (Fahrig and Rytwinski, 2009; Rytwinski and Fahrig, 2013), which may affect other species indirectly at a local scale. As owls are generally top predators, fluctuations in their population numbers may influence various trophic levels, thus their conservation is key to maintain balance.

Studies performed on the effects of roads are often focused on mortality rates and the location of mortality ‘hotspots’. Some authors support that roadkill quantity is not culpable for the lower densities found near main roads, since many of the individuals hit are juveniles, which often possess a naturally high mortality rate. However, birds that live for longer and use roadsides to forage (like some owls may), can be an exception to this affirmation (Reijnen and Foppen, 2006). The volume of roadkill on roads suggests a strong relation with the quantity of traffic, the velocity at which the cars travel, and the width of the road (Santos *et al*., 2013; van der Ree *et al*., 2015). Collision avoidance with oncoming vehicles depends on the correct identification of the vehicle, the evaluation of threat and the use of evasive countermeasures. Threat evaluation and resulting reactions can be affected by vehicle approach speed, as the individual may not have enough time to evade before the vehicle reaches them (DeVault *et al*., 2015; Lima *et al*., 2014). As secondary roads generally have a lower top speed limit than main roads like highways and large national roads, and have a lower traffic volume; it is plausible that main and secondary roads may possess differing effects on animals (Reijnen and Foppen, 2006).

There is a less marked association between mortality and driver visibility or quality of surrounding habitat, the latter being subjective to the species of nocturnal bird (Santos *et al*., 2013). However, Kociolek *et al.* (2015) affirm that it is important to avoid constructing or updating roads near important land-features, like preferential nest or roosting sites such as old trees with hollows, that tawny owls (Strix aluco) enjoy. Tawny owls are typically sit-and-wait predators, using perches (electrical poles, trees, fences) to survey for prey (Sergio *et al.,* 2007). Also, as Strigiformes are mainly nocturnal, when there is an absence of warm air currents that aid flight, their flight spends more energy, and to mitigate this energy loss, owls often use perches. Both this and, mainly, their sit-and-wait hunting tactic, may augment risk of vehicle-animal collision if the structures are near roads (Gomes *et al*., 2009). If roads are constructed through habitats containing these attractive elements, mitigation measures should be concentrated around areas of high mortality or ‘hotspots’, as it is logistically impossible to apply them throughout the whole length of road networks (Gomes *et al*., 2009; Grilo *et al*., 2014). To mitigate effects of road construction, like the aforementioned wind speed, trees are often planted along the roadside (Sousa *et al.,* 2013), and roads surrounded by high tree density are more likely to see tawny owls as roadkill (Gagné *et al*., 2015). Also, birds have varying degrees of susceptibility to vehicle-collision throughout the year, being higher in the breeding period when adults find and dispute territories and young disperse (Kociolek *et al*., 2015).

Despite the quantity of studies focusing on mortality, studies of the effects the roads have on abundance of a species are scarce (Fahrig and Rytwinski, 2009). However, Brotons and Herrando (2001) studied the effect of main roads on metapopulations of forest birds, and found that probability of occurrence diminished the closer the site was to a main road. Grade and Sieving (2016) found that due to the reduced perception or interpretation of anti-predator information near main roads, the density of prey birds may fall, due to increased predation success. Also, Silva *et al.* (2012) found that tawny and little owl (*Athene noctua*) abundance was lower near roads with high traffic density. Although owls are often found as roadkill, it is assumed that it is the indirect effects that are felt more keenly and have more effect on population dynamics, rather than the direct mortality of the individuals (Summers *et al*., 2011; Silva *et al*., 2012).

Habitats near main roads will be of inferior quality for owls, and therefore should be occupied by individuals of lower quality, which are often represented by immature individuals or floaters that wish to avoid territorial disputes (Silva *et al.,* 2012). Territories further from main roads, generally, represent high-quality habitats. Thus, if low-quality individuals occupy inferior-quality territories, high-quality individuals should hold the superior-quality territories. These low-quality territories may be considered 'sinks', where population replenishment is too slow to balance out the number of fatalities (due to, for example, a busy road). However, these territories may still be maintained, if there is a 'source' habitat that sustains it, as individuals disperse from the high-density (source) area (Pulliam, 1988). These 'sinks' may be ecological traps, as high-quality individuals may settle here occasionally, if surrounding areas all have high densities of individuals. Ecological traps are defined as territories where breeding or survival chances are sub-optimal, yet individuals prefer it notwithstanding (Donovan and Thompson, 2001). This preference may be 'forced' if source habitats with optimal resources are all occupied and the individual is incapable of ousting a territorial couple.

Figure 1 Tawny owl *(Strix aluco)* Illustration by Shirley Therese van der Horst

The target species of this study is the tawny owl, its distribution extending throughout much of mainland Portugal, with exception of the inland north region and lower Alentejo where there are larger agricultural patches (Lourenço *et al*. 2015a). Tawny owls are highly territorial and call throughout the year. While there are peak periods when the individuals announce their territory possession spontaneously, they will generally defend it when provoked, even amongst the quieter months (Redpath, 1994). Territories located in habitats with high density of suitable forest reveal more frequent spontaneous calls, possibly due to a higher density of 'neighbour' owls (Redpath, 1994).

Flight behaviour of the bird, its size, and the size of its home range can affect the probability of collision. Tawny owls characteristically have a low flight pattern (Kociolek *et al*., 2015), are of medium build and have large home ranges, of 12 to 24 ha (Redpath 1995). This means they are generally quite susceptible to vehicle-collision. Besides having large home ranges, tawny owls have large mean dispersal distances (14.5 km; Grilo *et al.,* 2014), meaning they are more likely to settle in an unknown territory, potentially one of lower quality like one near a main road. They are also more likely to suffer mortality during their dispersal trip (they encounter more roads than if their dispersal was shorter). This is especially common in sub-adults, possibly due to their inexperience when dealing with vehicles (Grilo *et al.,* 2014).

Habitat fragmentation may greatly affect the tawny owl, due to its dependence on forested areas for its vital activities. Even so, it seems to support some degree of fragmentation, if the surrounding habitat is not completely deprived of trees or of other structures that may serve as a perch (Redpath, 1995). That said, they do require a minimum small wooded patch of habitat to breed, and often other areas where they may be detected (for example more urban areas) are used solely for foraging (Ranazzi *et al.*, 2000)

Being, in general, top predators, it is unlikely that the diminished capacity of detecting possible predators (due to the aforementioned noise and light pollution) is a limiting factor for the tawny owl, and yet evidence exists that these birds may be under risk of predation by apex predators like, for example, the eagle owl (*Bubo bubo*), in a phenomenon known as superpredation (Lourenço *et al*., 2011; Lourenço *et al.*, 2013; Lourenço *et al.,* 2015b; Sergio *et al.,* 2007). This incident seems mainly influenced by food stress. Possibly, in a habitat where fragmentation has degraded structure, and therefore potentially lowered the levels of prey available, superpredation may be augmented (Lourenço *et al*., 2011; Lourenço *et al.*, 2013; Lourenço *et al.,* 2015b). However, even though some prey numbers may fall due to the roads, roadside habitats seem to provide suitable habitat for an elevated number of small mammals (Sabino-Marques and Mira, 2010; Galantinho *et al*., 2017), as these areas usually have denser vegetation, owing to the absence of influence from grazing animals and agriculture. This attracts owls, which may prove fatal if they choose to hunt here (Massemin and Zorn, 1998; Pereira *et al*., 2011). In some cases, beetles may also be an attractive food choice on roads for tawny owls (Finnis, 1960).

Tawny owls are the perfect model species for this study. The usage of playback vocalizations in this study almost guarantees to incite a response. Even if the owls do not spontaneously vocalize, as mentioned before, they will often answer a challenging call even in the quieter months (Redpath, 1994). Besides this, they are a generalist species, eating amphibians, reptiles, small mammals, birds, invertebrates and even fish (König and Weick, 2008), and a top predator. Thus, any change in their population may affect population dynamics for many other species that coexist with them, and they may act as an umbrella species for conservation efforts.

This study brings a new look on the situation, as it has not yet been studied what the effects of roads are on the population dynamic over various years. We wish to determine if (1) the locations near to roads present a more negative population trend in relation to those locations far from roads, and (2) if the locations near roads have more noticeable instability in their intra and inter-year territory occupancy by the tawny owl. Furthermore, we explore if main and secondary roads have similar effects on tawny owls. The temporal scale in this study is near long-term, as it extends over the period of a decade (using pre-collected information in the same area), which will allow a decent analysis of population trend. Also, during the listening points performed during this study, a large expanse of terrain was covered, totalling 523 points.

To realise the above mentioned ideas, five objectives were set for this study: **(1)** determine the effect of roads and consequent fragmentation of the landscape on the populational density of the tawny owl, using information collected during the period of 2015/2016; **(2)** determine the effect of roads and consequent landscape fragmentation on the populational trends of the tawny owl in the study area in the period of 2005 to 2016; **(3)** determine the effect of roads and consequent landscape fragmentation on the inter-year stability of tawny owl territories (2005, 2007, 2009, 2011 and 2015/2016); **(4)** determine the effect of roads and consequent landscape fragmentation on the intra-year stability of tawny owl territories (2015/2016); **(5)** compare individual aggressiveness and evaluate the quality of tawny owl territories near and far from main roads using the measure of success of captures in these aforementioned locations.

2. Methodology

2.1. Study Area

The census points were located within the areas of the MOVE, LifeLines (Linear Infrastructure Networks with Ecological Solutions, LIFE14 NAT/PT/001081), and REFER (Ecological Study of the Évora-Elvas/Caia Corridor) projects; and within the 10x10 km quadrats of the 2nd European Breeding Bird Atlas (EBBA 2). The study of demographic stability over the years will be applied solely to the points completed within the area of the MOVE project, as it is in this area that data from anterior years (2005,

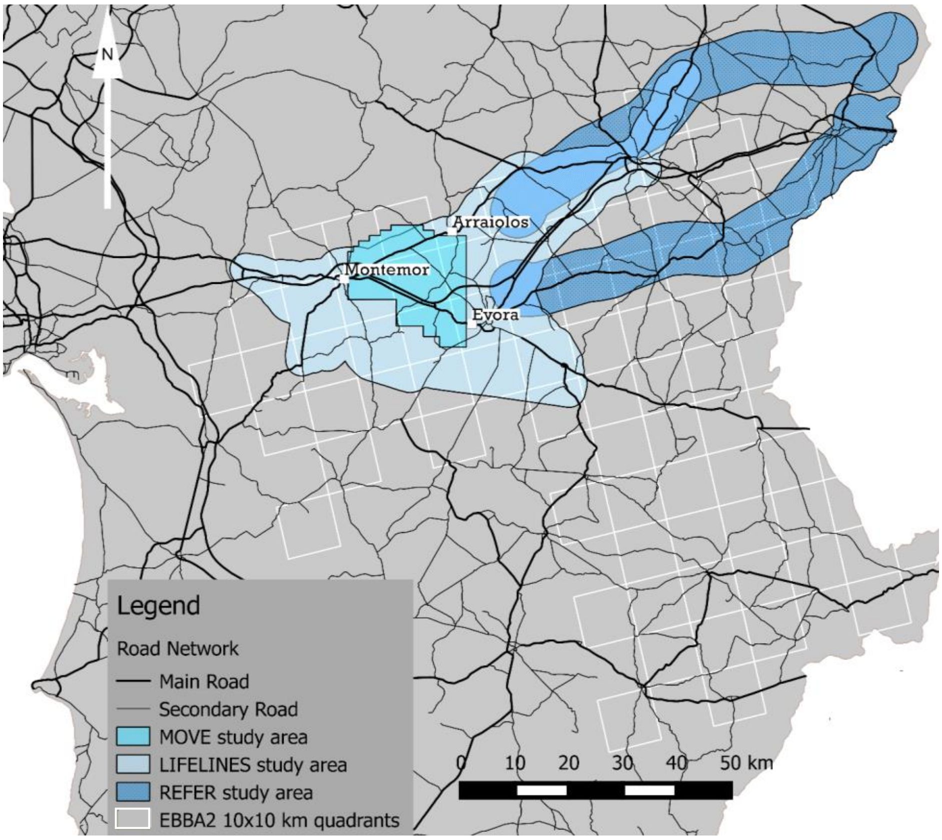

Figure 2 Study areas where listening points were performed

2007, 2009 and 2011) has been collected, as presented in the articles by Silva *et al*. (2012) and Santos *et al.* (2013). The farthest-reaching study area (**Figure 2**) is comprised between Vendas Novas (to the west) and Caia (to the east). The northern limit is variable from east to west, Monforte being the most extreme locality. The southern limit extends between São Cristóvão (southwest) and Barrancos (southeast). The more specific area of the MOVE project (**Figure 3**) is confined by Montemor-o-Novo, Santiago de Escoural, Évora and Arraiolos.

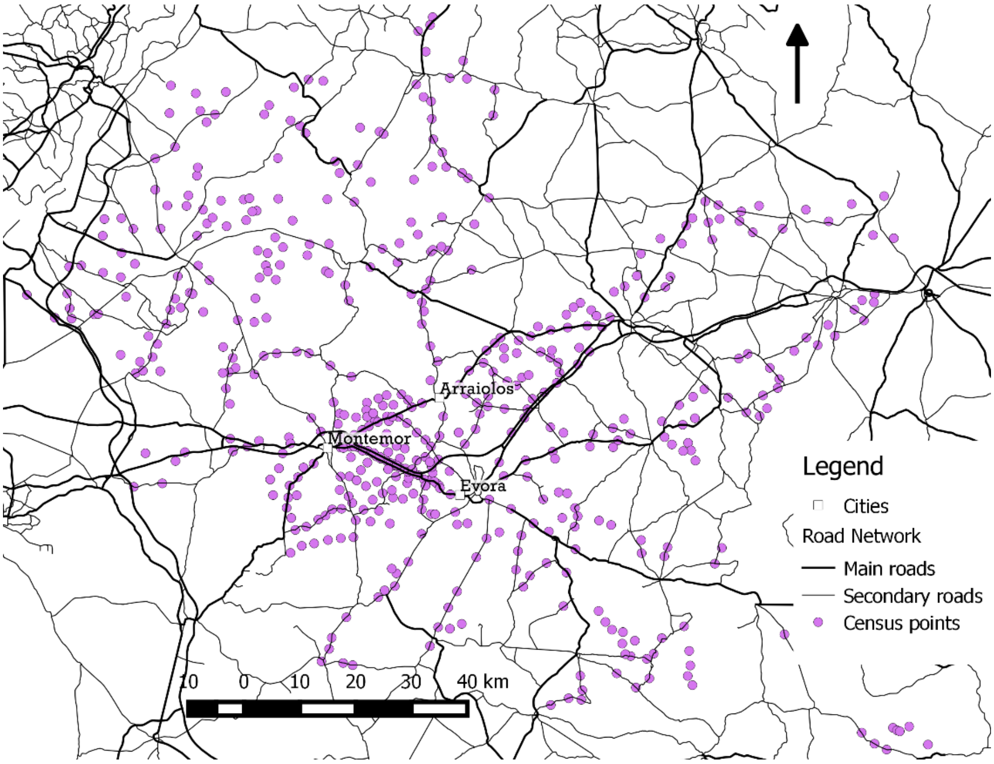

Figure 51 Census points in study area

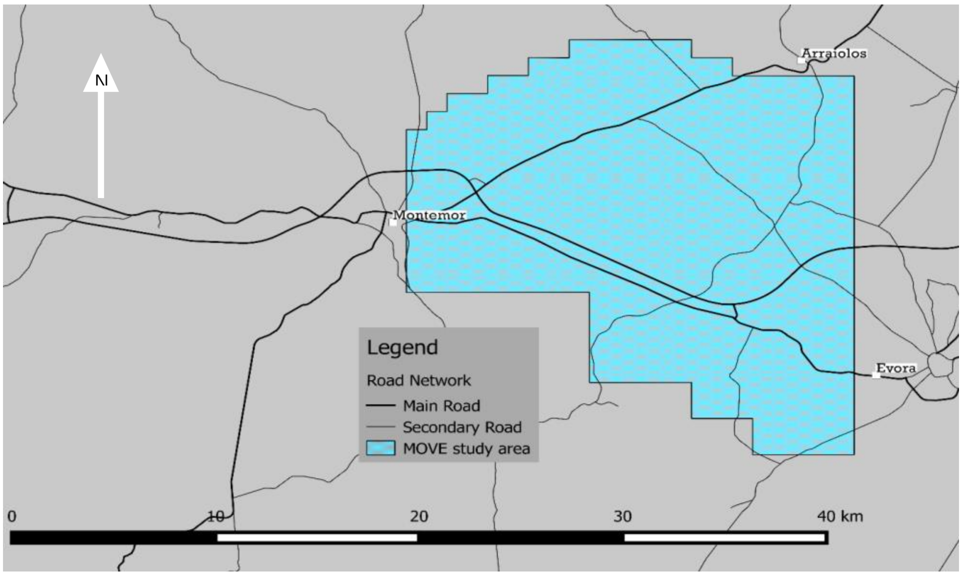

Figure 4 MOVE project study area and nearby cities

The MOVE project study area boasts a Mediterranean climate, with warm and dry summers, and mild winters, with between 500 and 800 mm of mean annual precipitation. The terrain is characterized by an undulating relief, where *montado* (an agro-silvo-pastoral system typical to the Iberian Peninsula) of cork (*Quercus suber)* and holm oak (*Quercus rotundifolia*) are dominant (close to 50% of the area), mixed in with a large portion of agricultural lands. Less common are olive tree plantations, orchards, vineyards, plantations of *Eucalyptus* spp. and maritime pine (*Pinus pinaster*), areas of intensive agriculture and urban expanses (Silva *et al*., 2012; Santos *et al*., 2013). The study area is traversed by various types of roads: (1) main roads, like the AE6 highway (25 km), national roads with high traffic density (57 km); (2) secondary roads, with moderate to low traffic (61 km); (3) dirt roads, unpaved roads which give access to agricultural areas and estates (Silva *et al.,* 2012; Santos *et al*., 2013). Road type was defined according to criteria used by Silva *et al.* (2012): main roads consisted of highways and highly-used national roads with between 400 and 1700 vehicles passing every 8 hours; secondary roads consisted of municipal roads and less-used national roads, with generally less than 170 cars passing within 8 hours; dirt roads consisted of agricultural accesses with very low traffic, and low maximum vehicle speed, making a collision unlikely.

2.2. Census Methodology

The methodology applied in 2015/16 was adapted from Silva *et al.* (2012) and Santos *et al*. (2013), which was used to collect the information from the MOVE project points in the years 2005, 2007, 2009 and 2011.

The points were chosen as to allow an even-as-possible coverage and so that, whenever possible, 1500 metres was left between them (occasionally the minimum being 800 m). For the points already established for the MOVE project in former years, a close-as-possible replication of the points was always attempted. Even so, it was not always possible, due to recent structures of fencing being constructed and closure of formerly public access terrains. In total, 523 listening points were executed in the period between November of 2015 and February of 2016, plus 64 repeat points (within the MOVE study area) were done in May of 2016. Listening points began 15 minutes after sunset and typically took 3 to 4 hours, depending on the number of points to be completed each night. The listening points were performed on nights with favourable meteorological conditions, in avoidance to days of heavy rainfall or strong winds.

For each listening point, characteristics included, the name of the point, the geographic coordinates, the name of the observer or observers, the date, the time of initiation, the habitat type (*montado*, olive tree plantation, eucalyptus plantation, agricultural, riparian, …), the road type (main, secondary or dirt), the wind (null, moderate or strong), the presence or absence of fog, and the moon phase (1 to 5, new moon being 1 and full moon being 5).

After arrival at the point, a spontaneous listening period (10 minutes) was initiated, wherein any nocturnal bird calls were identified and registered. Upon ending this period, a second period was initiated with a playback of the vocalization of two species, consisting of the following procedure: playback of the 1st vocalization (1 min 30 s), listening to determine if there is an answering call (3 min), playback of the 2nd vocalization (1 min 30 s), listening to determine if there is an answering call (4 mins). All the playback tracks consisted of 30 seconds of vocalization, followed by 30 seconds of silence and then another 30 seconds of vocalization, for a total of 1 minute and 30 seconds. Terminating these two phases, the point is considered completed, totalling 20 minutes. The tawny owl playback track was always played, and the second species was chosen depending on the surrounding habitat. It is also important to stress that the 1st playback track was always of the smaller of the two species, followed by the larger. This is due to possible aggression or predation of smaller species by larger, resulting in potential restriction of a smaller species' vocal activity, if the larger bird's vocalization is played first (Lourenço *et al.,* 2013).

Whenever spontaneous or response calls were heard, direction, approximate distance, sex of the individual (when possible), period during which the call was made (spontaneous listening; 1st playback and 3-minute listening period; 2nd playback and 4-minute listening period), any obvious movement made by the individual (approximation or distancing to the observer, over-head passes, …) and the individual territories and pairs were registered.

Differing from the MOVE points done in past years (2005, 2007, 2009 and 2011) which were performed in the period between March and May of each year, the points in 2015/16 were completed between November 2015 and February 2016, due to the fact that the species in focus (tawny owl) begins its reproductive period earlier (in the Fall) and maintains vocal activity throughout the year, except in August, when they are moulting and generally remain silent (Mikkola, 1983; Cramp, 1985; Lourenço *et al*., 2013).

The repetition of 64 points in the MOVE study area during May 2016 was performed with the intended purpose of allowing comparison with former years and to analyse the occupation of tawny owl territories during the same reproductive period. This last case is achieved with comparison of the results gleaned during visits in November and December (pre-reproduction) and May (end of the reproductive period).

2.3. Inverse Distance Weighting Interpolation (IDW)

To provide a graphical image of the relationships between tawny owl abundance and roads, a raster map was created using Inverse Distance Weighting interpolation of tawny owl abundance data, in QGIS Desktop 2.13.3 (Quantum GIS Development Team, 2015). Inverse Distance Weighting interpolation uses surrounding values to predict a value for unmeasured cells, giving more importance or weight to the values closest to the prediction location. Cell size was set at 1000x1000 m. An overlay of layers was added to aid interpretation. A layer was added where suitable forest type habitat for tawny owl occupation were classified as darker regions in the map, and lighter regions as any other type of habitat that is not optimal for tawny owl occupation, such as, for example and predominately, agricultural lands. Suitable habitat was defined from various woodland type uses of the soil, as categorized in the 'Carta de Uso e Ocupação do Solo de Portugal Continental'. As such, agroforestry systems, woodland of leafy trees, woodland of conifers, mixed woodland and open woodland were considered suitable habitat. Another overlay layer represents main roads with thick lines and secondary roads with thinner lines. A third and final overlay adds the names of three locals within the study area, to further aid orientation.

2.4. Statistical Analysis

The populational parameters to be analysed are as follows: (1) population density – number of territories at each listening point; (2) population trend (2005-2016); (3) inter-year variation of territory occupancy; (4) intra-year variation of territory occupancy. The statistical analyses were performed with the usage of a listening point as a unit. The location of the listening point was not used as a random effect in the analysis of trend and inter-year variation, because several points had no repetitions (or were moved between years), and consequently models using "point ID" as random factor (GLMM or LMM) did not converge or displayed severe warnings.

2.4.1. Data exploration and methodological bias

All variables were initially checked for normality and heterogeneity through histograms and boxplots. The variables were analysed through generalized linear models for their effect on the number of tawny owl territories. To check for possible methodological bias, the factors that can influence the detection of tawny owls were scanned prior to the analysis of the effects of roads (see **Annex 1**). According to the results, only the variable "month" was included in the further analysis (see **2.4.2**).

2.4.2. Effects on tawny owl density (2015/16)

A multi-model inference procedure was used to analyse the possible effect of roads on tawny owl density. A series of generalized linear models (Poisson distribution) were created and posteriorly, run through a model averaging process (MuMIn). The number of tawny territories in each point count (count data) was used as response variable, while the three explanatory variables were: proportion of woodland within a 1 km radius (henceforth referred to simply as 'proportion of woodland' – woodland being the suitable woodland habitat for tawny owl occupation, previously mentioned and defined in section **2.3**); road type (road nearest to the point), and month. All model combinations were performed, and models were compared by their AICc (Burnham and Anderson, 2002). The null model was included in the comparisons as a measure of model fitness. The set of best competing models was considered to be those models with ΔAICc < 2.0 (Burnham and Anderson, 2002). Models were validated using diagnostic plots.

The variable distance to the nearest main road (m) showed a non-linear distribution, therefore we evaluated its effect using a generalized additive model (GAM), with the aim to detect a potential distance threshold in the negative effect of major roads.

All statistical analyses were performed in the software R, version 3.3.0 'Supposedly Educational' (R Core Team, 2016), with the packages MuMIn (Barton, 2016), mgcv (Wood, 2011) and gplots (Warnes *et al*., 2016).

2.4.3. Population trend

Population trend was analysed using a generalized linear model (GLM) using the collective data of 2005, 2007, 2009, 2011 and 2016. As mentioned before, points were replicated through the years as faithfully as possible. The model included tawny owl abundance (number of breeding pairs) as response variable and year as explanatory variable. Statistical procedures were the same as described in the sub-section above.

A co-plot was created to compare the tawny owl population trend associated with each road type (major, secondary and dirt roads).

2.4.4. Inter-year variation of territory occupancy

To detect the effect of roads on inter-year variation of territory occupancy the standard deviation, coefficient of variation and mean number of tawny owl territories were used as response variables in linear models. The road type was used as explanatory variable. Significance was set at $p < 0.05$.

2.4.5. Intra-year variation of territory occupancy

To detect the effect of roads on intra-year variation of territory occupancy, the difference in the number of territories in each sampling plot from beginning to end of the breeding season was used as response variable. A series of competing linear models (LM; Normal distribution) were created with the explanatory variables distance to nearest main road (m), proportion of woodland and road type. Models were compared by their AICc (Burnham and Anderson, 2002). The null model was included in the comparisons as a measure of model fitness. The set of best competing models was considered to be those models with $\Delta AICc < 2.0$ (Burnham and Anderson, 2002). Models were validated using diagnostic plots.

2.5. Capture attempts

The behaviour of individuals during capture attempts were compared between attempts near main roads and attempts off-road (farther than 1km from main and secondary roads). This comparison may give indication on the quality of territories and individuals occupying areas near and far from main roads. We used mist-nets for capturing individuals, luring them with the playback of male and female tawny owls duetting. Four behavioural response types were considered: (1) attack and capture; (2) approach but no attack; (3) response but no approach; (4) no response. Data resulting from capture attempts was analysed through a Chi-squared test and bar-graphs, due to the small sample size being inadequate for further statistical analysis.

3. Results

In the resulting interpolation, green patches or high tawny owl density, were often found associated to larger patches of suitable habitat and secondary roads, whereas low densities were associated to non-suitable lands or main roads (**Figure 5**). However, in some zones, this is not evident, yet it is important to remember that interpolation creates values where there is no data, based on surrounding data, so the results may have a lower accuracy. In areas where density is low in seemingly more suitable situations, or high where one would expect lower density, another variable may be in effect, which is not considered here. However, in general, for its visual value, we consider the interpolation is still relevant, although the following statistical results more accurately depict the reality.

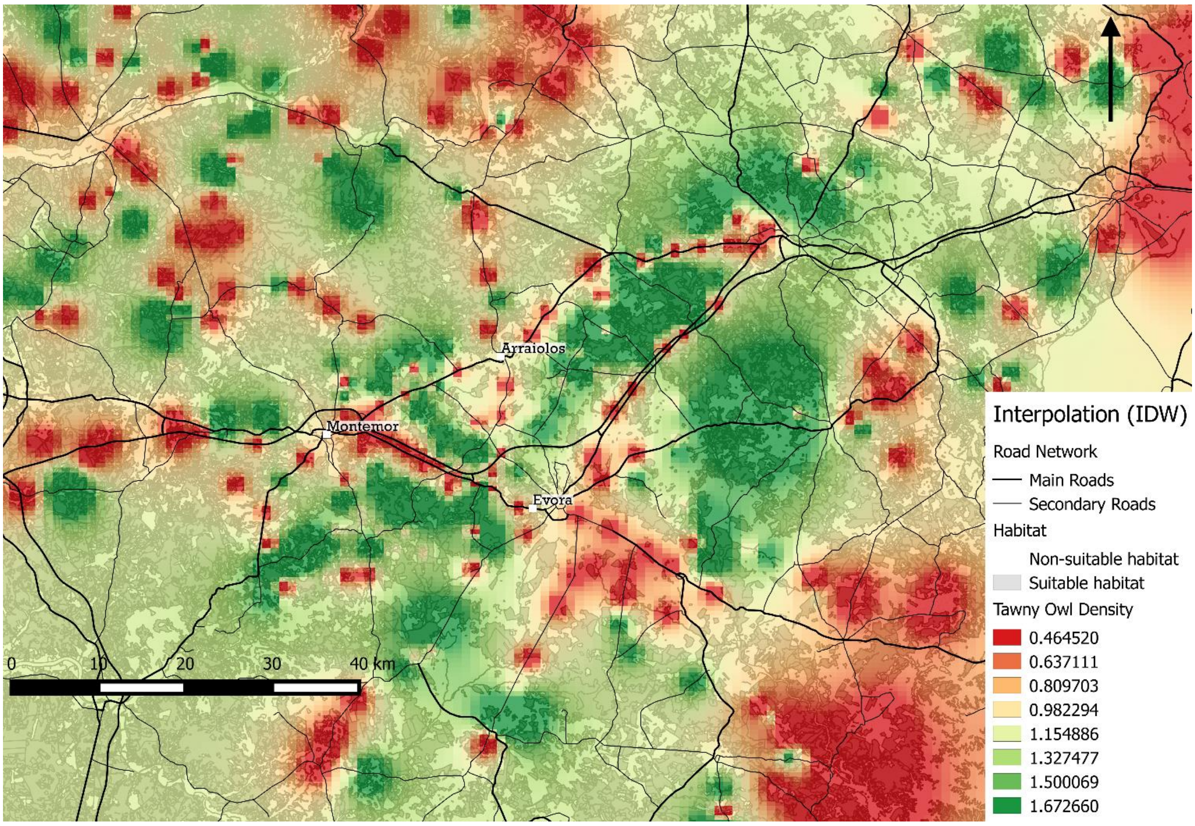

Figure 5 Inverse Distance Weighting Interpolation (IDW) of the density of tawny owls in the study area, with overlay of the road network (main and secondary roads) and habitat type (non-suitable and suitable). Green patches indicate high density of tawny owl, whereas red patches indicate low density of tawny owls. Thick lines represent main roads, and thin ones, secondary roads. Dark regions are suitable tawny owl habitat, light regions are non-suitable.

3.1. Effects on tawny owl density (2015/16)

The set of best models explaining tawny owl density included the variables road type, proportion of woodland and month. The best model contained the three variables and had a probability of 58% of being the best model among the candidate models (w = 0.58), while the second model, consisting of the variables proportion of woodland and month, held the probability of 42% of being the best model (**Table 1**).

The variables proportion of woodland and road type both held a higher relative importance of 1, when compared to the variable month, with a relative importance of 0.58 (**Table 2**).

Table 1 Component models of the multi-model inference analysis of variable effect on tawny owl density (2015/2016)

Component models	*df*	*logLik*	*AICc*	*ΔAICc*	*w (Akaike weight)*
Road type + Proportion of woodland + Month	7	-507.92	1030.14	0.00	0.58
Proportion of woodland + Road type	4	-511.33	1030.76	0.62	0.42
Road type + Month	6	-518.87	1049.97	19.83	0.00
Road type	3	-524.44	1054.94	24.80	0.00
Proportion of woodland + Month	5	-524.57	1059.29	29.15	0.00
Proportion of woodland	2	-527.94	1059.92	29.77	0.00
Null model	1	-544.61	1091.23	61.09	0.00

Table 2 Conditional model average results and relative variable importance, resulting from analysis of variable effect on tawny owl density (2015/2016)

Variable	*Estimate*	*SE*	*Adjusted SE*	*z*	*P*	*Relative variable importance*
(Intercept)	-0.6509	0.2082	0.2088	3.117	0.0018	
Nov:Dec	-0.1244	0.1441	0.1446	0.860	0.3896	0.58
Nov:Jan	0.0725	0.1303	0.1307	0.555	0.5792	
Nov:Feb	-0.2917	0.1576	0.1582	1.844	0.0651	
Dirt:Main road	-1.1829	0.2912	0.2922	4.049	≤ 0.001	1.00
Dirt:Secondary road	0.1595	0.1060	0.1064	1.500	0.1337	
Proportion of woodland	0.8432	0.1804	0.1810	4.658	≤ 0.001	1.00

More tawny owl territories were detected during listening points where the proportion of woodland was larger (**Figure 6**).

Tawny owl density was lowest near main roads, but there was no significant difference between dirt and secondary roads (**Figure 7**).

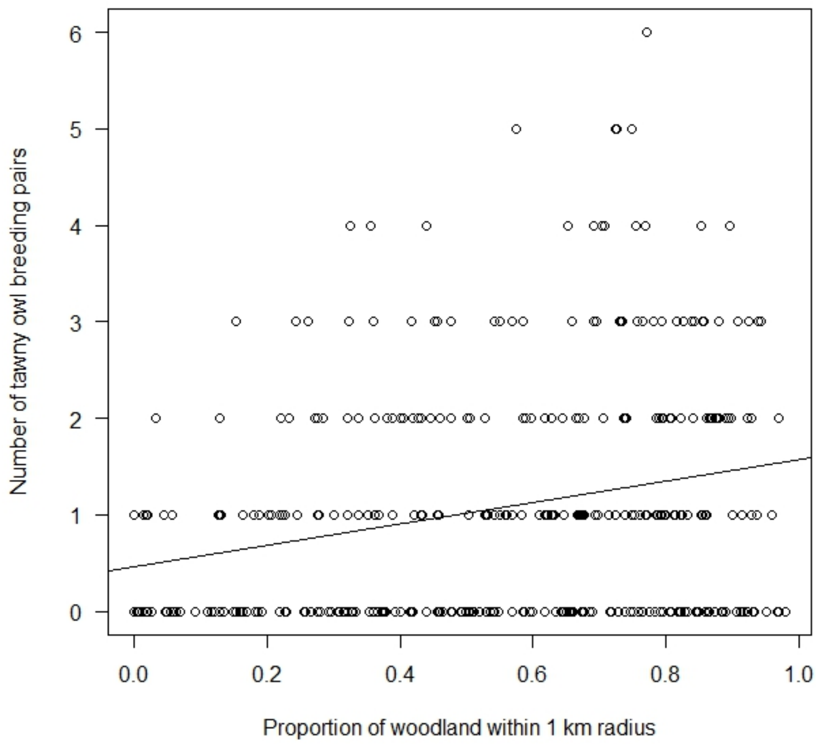

Figure 6 Plot of relation between tawny owl breeding pairs and the proportion of woodland, with GLM regression line

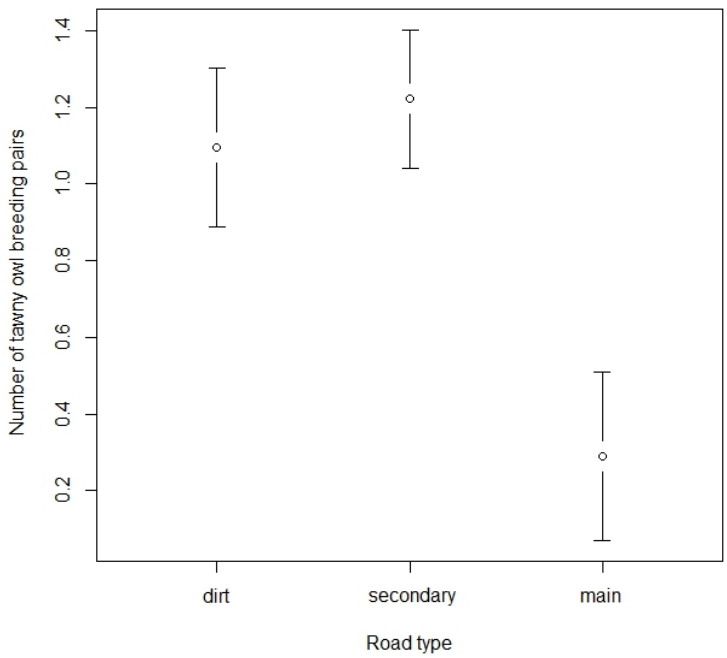

Figure 7 Plot of means of relation between tawny owl breeding pairs and road type, with intervals of confidence at 95%

AICc difference (ΔAICc) between the null model and the GAM model (**Table 3**) was calculated to be greater than 2.0 (16.75367), validating the explanatory power of the variable "distance to the nearest main road (m)".

Table 3 AICc output for both the null model (M0), and the model containing the variable distance to nearest main road (m) (M1)

Model	***AICc***
Null model	1222.92
Distance to nearest main road (m)	1206.17

The abundance of tawny owls increased with distance to the nearest main road until approximately 5000m. From here, these variables sowed an inverse relationship (with tawny owl numbers decreasing the further away the point was from a main road) (**Figure 8**).

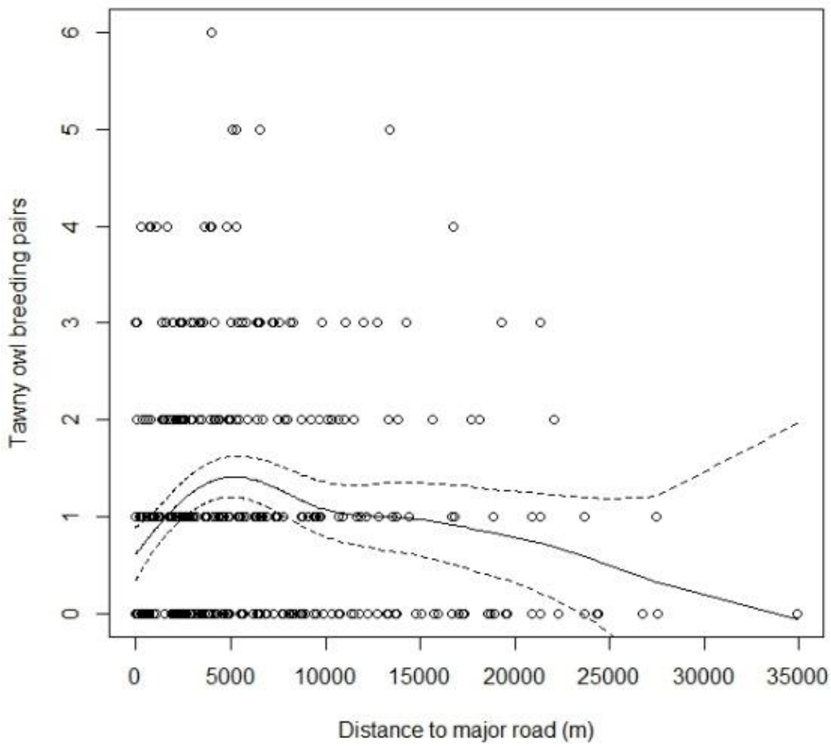

Figure 8 Plot of GAM between tawny owl breeding pairs and distance to the nearest main road (m), with intervals of confidence at 95%

3.2. Population trend

It was possible to note a decrease in the number of tawny owl breeding pairs throughout the years 2005 to 2016, suggesting a possible decrease in the regional population of the tawny owl (**Table 4**) (**Figure 9**).

Table 4 Output of generalized linear model analysing population trend through the years (2005/07/09/11/15/16)

Variable	*Estimate*	*SE*	*z*	*P*
(Intercept)	51.70	27.01	1.914	0.056
year	-0.026	0.013	-1.905	0.057

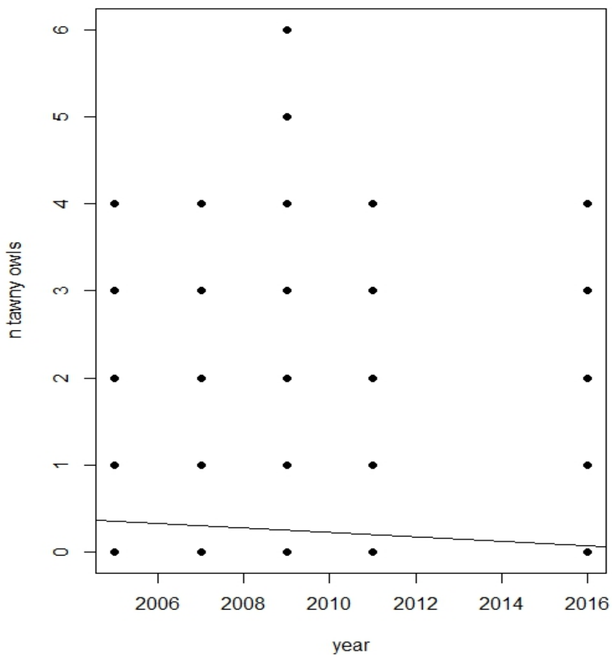

Figure 9 Plot of tawny owl breeding pairs for each year when data was collected (2005, 2007, 2009, 2011 and 2016), with trendline

Tawny owl decrease was more noticeable at points near main roads and secondary roads, with a similar decline on secondary and main roads, although main roads held the lowest numbers of tawny owls throughout the years. Points considered dirt road saw a slight increase in tawny owl numbers over the ten-year period (**Figure 10**).

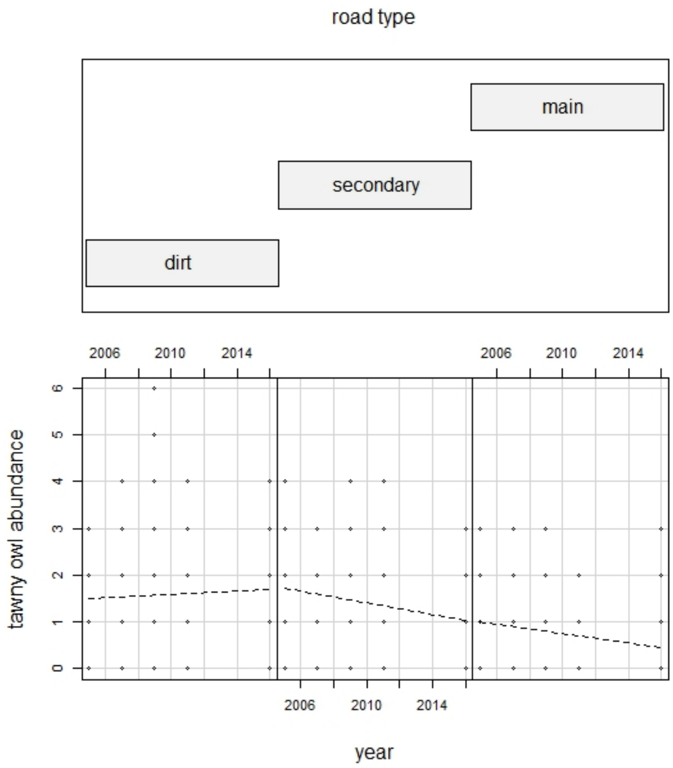

Figure 10 Co-plot of tawny owl breeding pairs for each year when data was collected (2005, 2007, 2009, 2011 and 2016) and road type, with regression lines

3.3. Inter-year variation of territory occupancy

The coefficient of variation for inter-year territory occupation was highest for main road type (**Figure 11** and **Table 5**), whereas the standard deviation was largest for secondary roads (**Figure 12** and **Table 6**). This means that along the years there has been a larger variation in the number of territories near secondary roads. However, when considering the coefficient of variation, which gives a relative amount of variation, main roads showed greater variation along the years than secondary roads.

Table 5 Linear model output of coefficient of variation (tawny owl breeding pairs) in function of road type

Variable	*Estimate*	*SE*	*z*	*P*
(Intercept)	0.7885	0.1025	7.691	≤ 0.001
Dirt : Secondary road	0.2265	0.1824	1.242	0.2197
Dirt : Main road	0.6047	0.1578	3.833	≤ 0.001

Table 6 Linear model output of standard deviation (tawny owl breeding pairs) in function of road type

Variable	*Estimate*	*SE*	*z*	*P*
(Intercept)	0.9500	0.0814	11.664	≤ 0.001
Dirt : Secondary road	0.1933	0.1468	1.317	0.193
Dirt : Main road	-0.1595	0.1249	-1.277	0.207

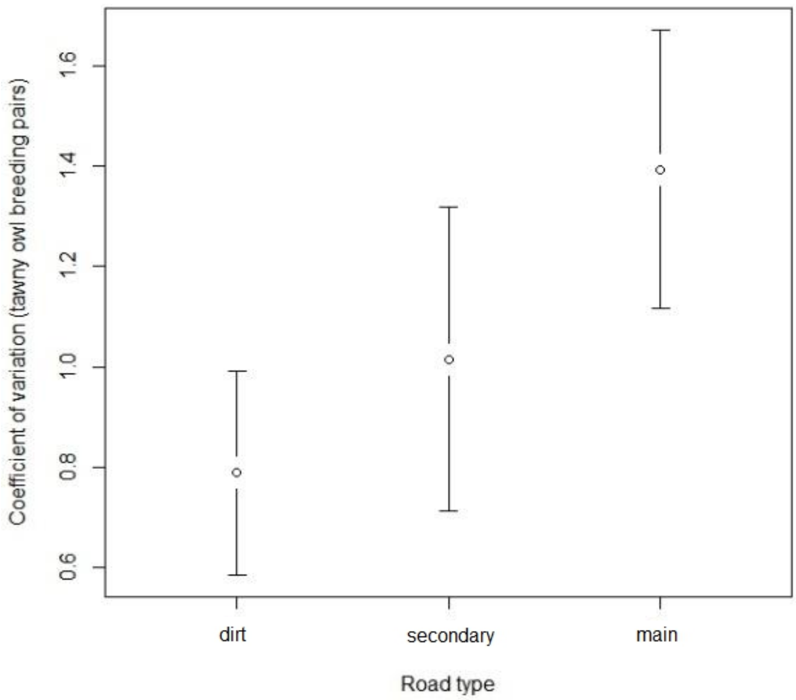

Figure 11 Plot of coefficient of variation (tawny owl breeding pairs) for inter-year (2005, 2007, 2009, 2011 and 2016) data and with road type, with intervals of confidence at 95%

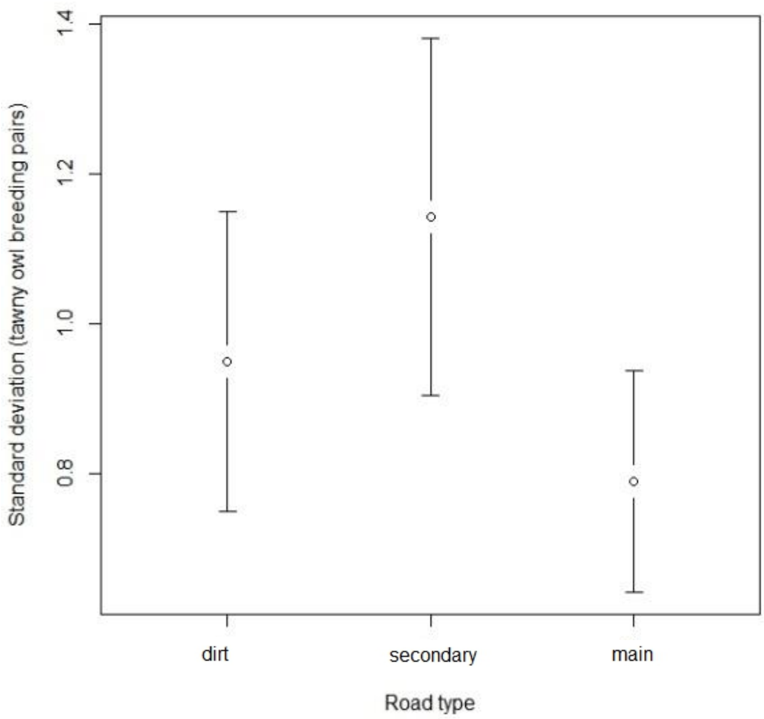

Figure 12 Plot of standard deviation (tawny owl breeding pairs) for inter-year (2005, 2007, 2009, 2011 and 2016) data and with road type, with intervals of confidence at 95%

Similarly to the observed for the 2015/16 data, mean inter-year abundance was greatest for dirt road points and lowest for main roads (**Figure 13 and Table 7**).

Table 7 Linear model output of mean (tawny owl breeding pairs) in function of road type

Variable	*Estimate*	*SE*	*z*	*P*
(Intercept)	1.5370	0.1363	11.275	≤ 0.001
Dirt : Secondary road	-0.2370	0.2458	-0.964	0.339
Dirt : Main road	-0.8120	0.2090	-3.886	≤ 0.001

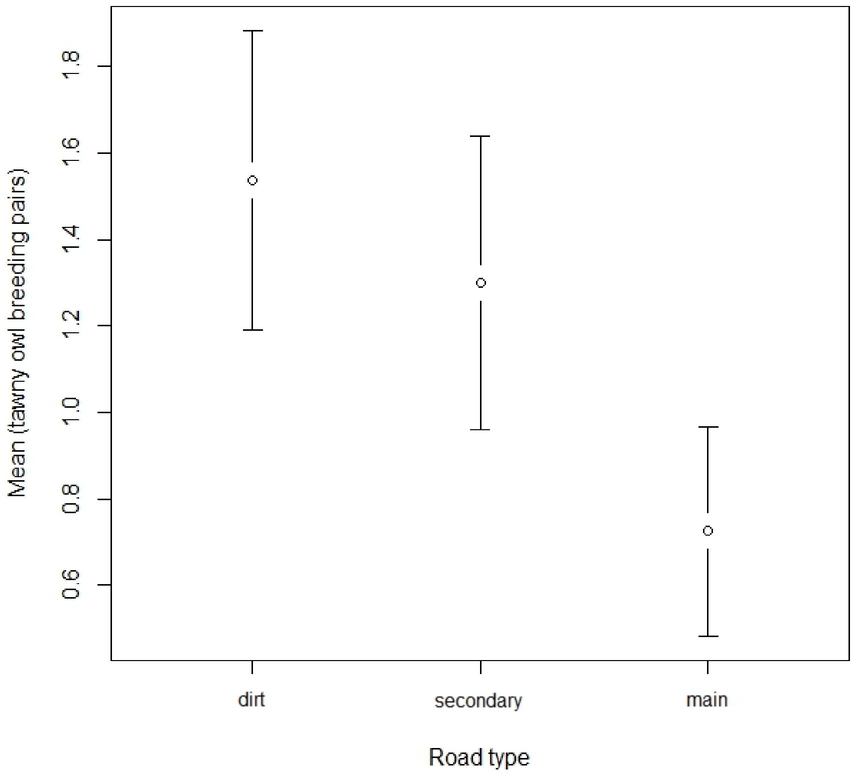

Figure 13 Plot of mean (tawny owl breeding pairs) for inter-year (2005, 2007, 2009, 2011 and 2016) data and with road type, with intervals of confidence at 95%

3.4. Intra-year variation of territory occupancy

Of the component models, the best model, consisting of the variables road type and distance to nearest main road (m), had the most probability of being accurate (w=0.43) (**Table 8**). The second-best model only included road type. Relative variable importance was greatest for the variable road type (0.69), followed by the proportion of woodland (0.58) (**Table 9**).

Table 8 Component models of the multi-model inference analysis on the intra-year database. The models are built with linear models of all possible combinations of the three variables: road type (main secondary or dirt); proportion of woodland; distance in metres to nearest main road

Component models	*df*	*logLik*	*AICc*	*ΔAICc*	*w (Akaike weight)*
Road + Proportion of woodland	5	-96.64	204.42	0.00	0.43
Road	4	-98.35	205.44	1.02	0.26
Distance to nearest main road	3	-100.04	206.51	2.09	0.15
Distance to nearest main road + Proportion of woodland	4	-99.30	207.33	2.91	0.10
Proportion of woodland	3	-101.25	208.94	4.52	0.05
Null model	2	-103.67	211.55	7.13	0.01

Table 9 Conditional average results and relative variable importance resulting from the output of multi-model inference analysis on the intra-year database

Variable	*Estimate*	*SE*	*Adjusted SE*	*z*	*P*	*Relative variable importance*
(Intercept)	0.4173	0.6457	0.6539	0.638	0.5233	
Dirt : Main road	0.1522	0.4522	0.4612	0.330	0.7413	0.69
Dirt : Secondary road	-1.071	0.4252	0.4346	2.464	0.0137	
Proportion of woodland	-1.008	0.6005	0.6132	1.644	0.1002	0.58
Distance to nearest main road	-0.0190	0.0082	0.0084	2.275	0.0229	0.25

The variation within the same year is close to null the closer the territory is to a main road, becoming a negative tendency as the distance to a main road grows (**Figure 14**). Intra-year variation seems to be positive with less proportion of forest and negative with a larger proportion (**Figure 15**).

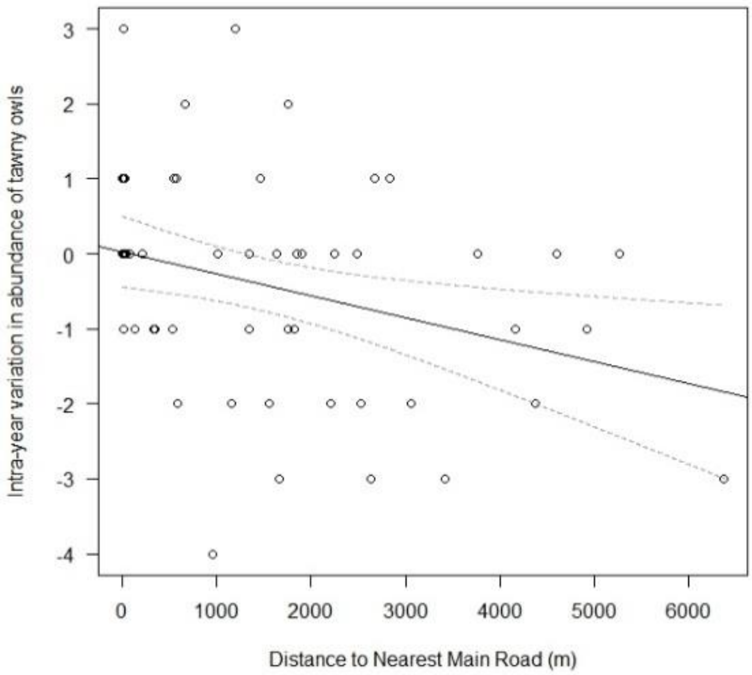

Figure 14 Plot of intra-year variation in tawny owl abundance in function of distance to nearest main road (m), with intervals of confidence at 95%

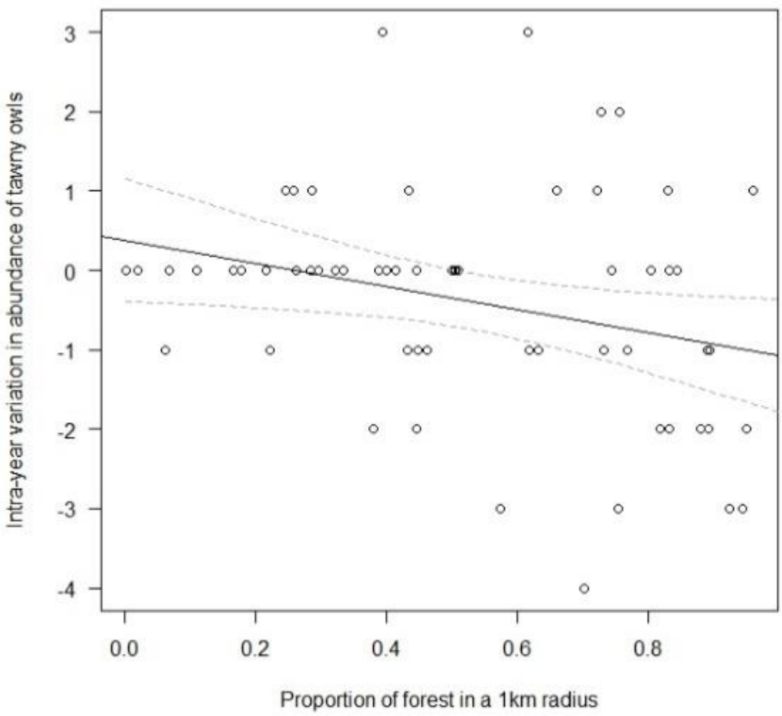

Figure 15 Plot of intra-year variation in tawny owl abundance in function of proportion of forest in a 1 km radius, with intervals of confidence at 95%

Intra-year variation is next to null for main and dirt roads, whereas secondary roads experience a negative variation, i.e. they lose territories between the beginning and the end of the breeding season (**Figure 16**).

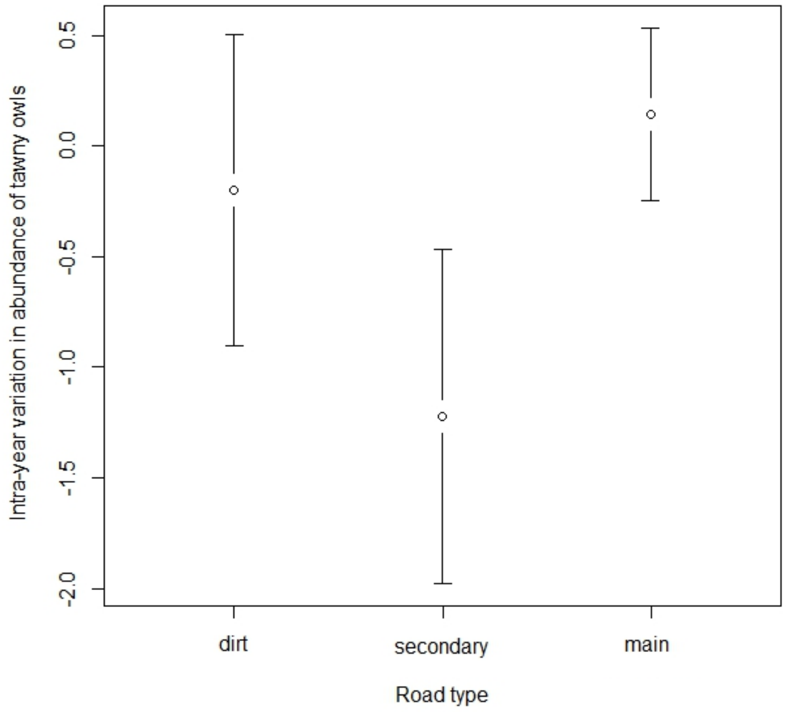

Figure 16 Plot of means of intra-year variation in tawny owl abundance in function of road type, with intervals of confidence at 95%

3.5. Capture attempts

Main and dirt road capture attempts were found to have significant differences between them in terms of response by tawny owl individuals (**Table 10**).

Table 10 Results of the Chi-squared test on capture attempts data

X^2	*df*	*p*
14.248	3	0.0026

Overall, attempted captures near major roads mostly yielded an absence of response (70%), attempted captures near dirt roads mostly had a successful capture result (71.4%) (**Figure 17**).

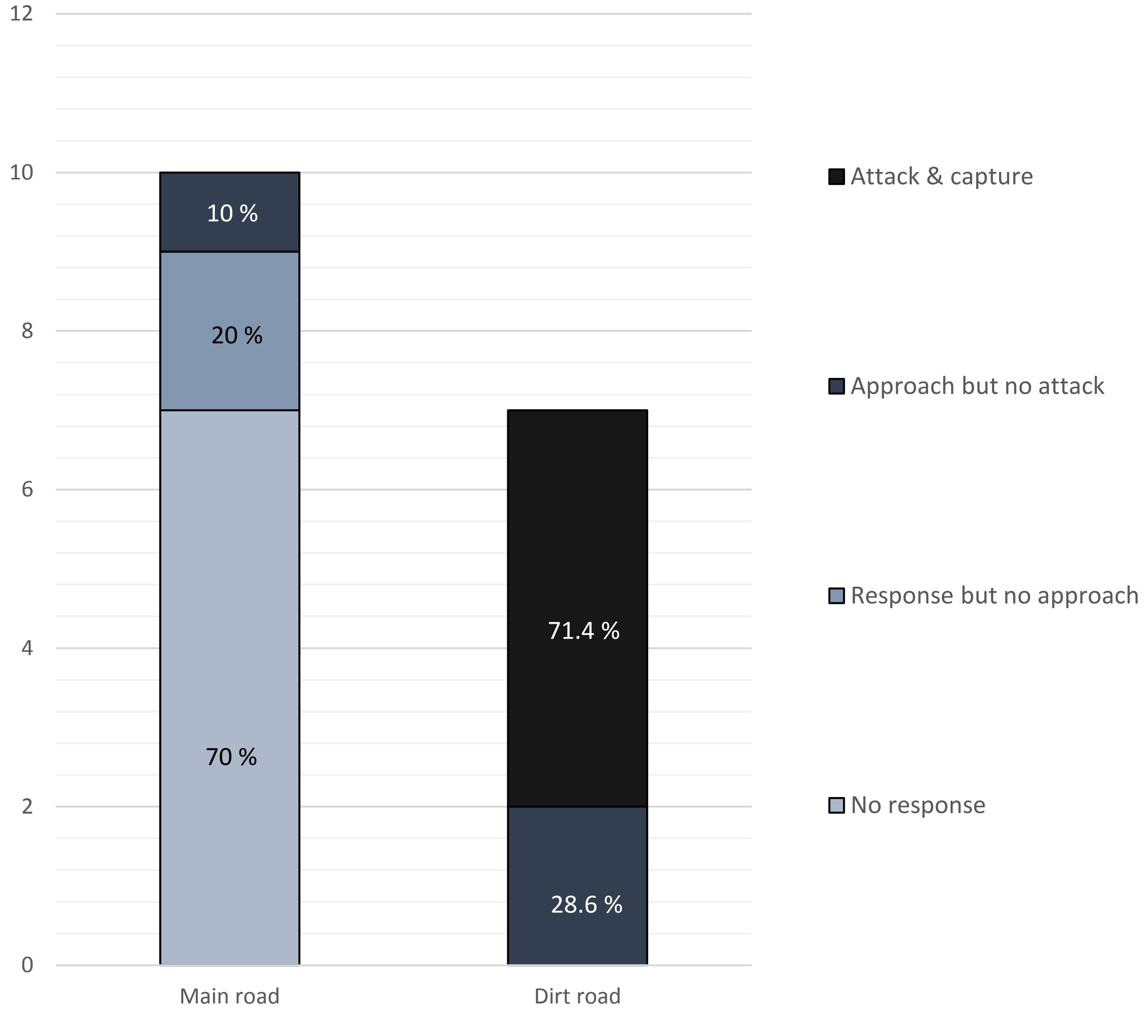

Figure 17 Bar-graph of the number of each response type to attempted captures near major roads and dirt roads. Y axis represents the number of times each situation occurred at each road type. Within the bar sections, the percentage that the number of each reaction type represents for road type is present

4. Discussion

Besides the well-known effect of increased mortality on owls near roads, the evidence gathered in this study demonstrates that roads have a clear disturbance effect on tawny owl populations, by reducing their density and occupancy rate. It is, however, essential to stress the different effects caused by main and secondary roads.

Tawny owl density was much lower for listening points that were located near main roads, most likely associated with avoidance due to various types of disturbances, that create territories of low quality adjacent to these roads. There will be a low number of attempted establishments in territories like these, and an even lower number that are maintained throughout the entirety of the breeding season. However, the difference between secondary and dirt roads was minimal, potentially due to the lower traffic volume on secondary roads being enough to make any effects tolerable for tawny owls, especially if competition is lower or there is a decent food availability that makes up for it. Dirt roads, of course, generally represent the best option, as there is minimal human disturbance in the form of transport, often a good supply of food, and high quality of habitat (if habitat is adequate for their needs, i.e. mature holm or cork oak forests). Even if sites near dirt roads are all high quality, the various habitat types (varying degrees in proportion of woodland suitable for tawny owls) proved to have an effect, due to the dependency of tawny owls on wooded areas, who rely on them for their vital activities. Density was found to grow as distance to the nearest main road did. As clarified, territories further away from main roads are of a higher quality, yet at approximately 5 km, the density dropped slowly, which seems contradictory to this affirmation. This is potentially due to two facts: either less points were performed at these longer distances, or the points at this distance have, by chance, a smaller proportion of woodland and are more agricultural, making it more improbable to find nesting tawny owl pairs. It is also important to note that there will be varying levels of detectability throughout the year. Tawny owls, although having a near year-round vocal activity, will have periods where they are more (breeding period) and less (incubation or moulting periods) willing to announce their presence (Mikkola, 1983; Cramp, 1985; Lourenço *et al*., 2013). Also, dispersing juveniles may answer calls when the imitation vocalizations are reproduced, and later leave in search of a better territory, leading to the false belief that there is an established territory in the zone.

In a recent study by Lourenço *et al.* (2015a) using NOCTUA (long-term monitoring program of Strigiformes and Caprimulgiformes in Portugal; GTAN-SPEA, 2016) information, the five-year trend was slightly positive for the tawny owl, yet the ten-year trend calculated in this study revealed itself to be negative for our study area. There may be regions where trend is positive enough to 'mask' zones of negative trend at a national scale. Also, when analysed with road type in mind, trend was slightly positive for dirt roads, but negative for both secondary and main roads (even though main roads always held the lowest values), revealing that although tawny owl density did not seem adversely affected by secondary roads, the overall trend through the decade (2005-2016) was negative for both secondary and main roads. With this, we can affirm that it is not only main roads that adversely affect tawny owl population and act as a population sink. We tend to assume main roads are where most collisions with vehicles happen, as besides a larger traffic volume and speed, trucks are a lot more common. Beyond having a larger chance of collision with an animal due to their greater proportions, trucks also have larger pressure changes around them, leaving turbulence behind them as they pass (although cars too, do this, at a smaller scale) that can pull the bird towards the vehicle or ground as they fly near it, stunning or killing it without direct contact (Ramsden, 2003). Even so, the lower volume of traffic on secondary roads may lull the birds into a false sense of security, and they may pass more often over the road while hunting, for example. Although mean speed of vehicles is lower on secondary roads, cars may still travel at speeds fast enough that, combined with the stun factor of headlights, may be sufficient to cause a fair amount of collisions, thus presenting secondary roads as a 'hidden trap'.

A negative population trend may also result from changes in the vehicles themselves and in road policies. With vehicles becoming more sophisticated each year, it is also important to consider that noise volume produced is reduced, or, as in the case of hybrid or electricity-powered cars, is almost eliminated, which, although decreasing secondary effects like noise pollution, may increase probability of collision, if the animal is unable to detect the moving object in time. Besides this, fire-prevention policies have been remodelled after 2005, a year when there was an inordinate quantity of wildfires across the country (around 50% more than average; Instituto da Conservação da Natureza e das Florestas, 2017). Since then, roadside vegetation has been maintained more strictly, following the law: Decreto-Lei n.º 124/2006 (al. a) do n.º 1 do artigo n.º 16. Being so, one of the few attractive qualities of roadsides, i.e. having so much prey available (particularly small mammals), will have diminished with these practices. As one of the main compensatory features of busy roads was the availability of a larger number of small mammals like rodents (Sabino-Marques and Mira, 2010; Galantinho *et al*., 2017), who often settled at roadsides, where vegetation grew unchecked and untouched by agricultural practices. The new practices will have likely dislodged these prey animals, and food availability will have fallen, making establishment of a mating pair here even more unlikely over the years.

With inter-year variation in the number of tawny owl territories, we found the coefficient of variation to be lowest for main roads. As explained previously, very few males attempted to settle in these low-quality territories, where the cons mostly outweigh the pros, at the start of the breeding season. These males either abandon these territories due to their unfavourable attributes or collide with vehicles during the time they spend there. The results suggest that areas near roads represent inferior quality territories, and accordingly they will be occupied mainly by less territorial or 'lower quality' males (Penteriani, 2003). To clarify, lower quality males are defined as those males who either are more subordinate or younger, or attempt to establish territory too late into the breeding season (most-often in the case of migrating birds). These males are essentially barred from high-quality territories due to their incapability to oust higher-quality males (Sergio and Newton, 2003).

On the other hand, the largest variation in the number of territories during both inter and intra-year studies was found for secondary roads, suggesting a high instability, both within the year (2015/16) and during the years (2005 to 2016). Territories near secondary roads will be of a higher quality to the ones near main roads, and therefore will have potential for more pairs to attempt establishment at the beginning of the breeding season. In comparison, main roads will always have a low number or no breeding pairs present, as demonstrated by mean territory values, being that secondary roads had slightly less than dirt roads, but main roads had very low mean territory numbers. As such, secondary roads have more potential to have a wider range of territories along the years and within the same year, whereas main roads generally will not surpass a maximum of one territory within or between years, due to a low number of attempted and successful establishments. On the contrary, territories near dirt roads are of a high-quality and will most likely be disputed, every year and within the year, by highly territorial 'superior-quality' individuals.

To review concisely: (1) main roads disrupt habitat quality for tawny owls – limiting occupancy, either by disturbance or mortality, and this was shown by the poor performance of this road type, for all the analysed variables; (2) secondary roads do not severely disrupt habitat quality, allowing occupation and relatively good densities, yet they may act as ecological traps, revealing lower probability of occupation along the breeding season and a negative population trend. If off-road is the prime quality habitat for tawny owls, individuals may establish near secondary roads while waiting for a vacant space far from roads (in high-quality areas). However, although lower, disturbance, and especially mortality risk near secondary roads may still be relevant, thereby negatively affecting tawny owl populations by acting as ecological traps (animals are attracted to a dangerous area by some factors such as conspecific-free space or a larger food availability).

Considering proportion of woodland to be an appropriate measure of tawny owl density, as they are forest-dependent species (Redpath, 1995), it is possible to affirm that higher fragmentation of the landscape (and lower proportion of woodland) will result in lower quantities of optimal habitat for tawny owls. This fragmentation will be intensified not only by large patches of agricultural land, which represents a less suitable environment for tawny owls due to low tree density (Redpath, 1995), but also by main roads, as their surrounding habitat is sub-optimal. We found intra-year variation to have a minimal difference when considering less or more proportion of woodland, yet tawny owl density was found to be adversely affected by smaller amounts of woodland, as mentioned previously. However, values seem more dispersed where there is too little or too much proportion of woodland, suggesting that although tawny owls do need some wooded areas to survive, they also need open patches with lower proportion of woodland where they can hunt, for example. This infers that a balance between wooded and open areas may be preferred.

Tawny owl individuals in higher-quality territories should be more willing to defend their territories from conspecific intruders, therefore, they should display more aggressive responses to capturing attempts using playbacks of a conspecific breeding pair. Capture attempts were unsuccessful near main roads, and mostly yielded a total absence of response. Low quality territories, like those presented by habitat near main roads, will be defended by lower quality males; who are less territorial and therefore less likely to engage intruders aggressively; choosing instead to return call from afar or have no reaction, as the territory, to them, is not worth a fight. Also, initial detection may have been resultant of a temporary establishment without a female correspondence, which led to the male abandoning the territory post-1st-detection, in search of something more favourable. Attempted captures near dirt roads mostly had a successful capture result, and when they did not, there was a visible or audible approach of the individual or individuals towards the playback call. High quality territories, like those near dirt roads, will be protected by high quality pairs who are more territorial and active when defending territory. An individual on its own may not be so inclined to defend a territory. If a pair has claimed a territory, both will usually defend it, and each party usually has a more aggressive response to intruders of the same sex, which is why a playback with both female and male vocalization were used during capture attempts.

Those variables that were not considered after the initial tests for methodological bias (**Annex 1**) had no influence on the detection of tawny owls, as playback calls were used to attempt to glean as many detections as possible (as mentioned before, tawny owls are very territorial and will usually respond to intrusions), and avoid false absences. If the methodology had considered only spontaneous calls, it is possible that the variables that were not considered during the rest of the study would have affected detection rates. For example, Penteriani *et al.* (2010) found that moonlight affected spontaneous call frequency in eagle owls, which was higher on moonlit nights. Also, Lourenço *et al*. (2013) found vocal activity to be inhibited by eagle owl presence. However, when tested in this study, neither moon phase and visibility, nor playback of eagle owl vocalization, had any apparent effect on the results.

5. Conclusions

In light of what was discussed during this study, despite the tawny owl being of Least Concern in Europe and globally (BirdLife International, 2015) and the aforementioned NOCTUA study (Lourenço *et al.,* 2015a) suggesting that its trend was tentatively positive in Portugal, there should be cause to consider applying conservation measures to mitigate both the primary and secondary effects of roads, as we may assume it will benefit not only the tawny owl, but all nocturnal raptors who suffer from these linear structures. Also, population trend is currently unknown at European and global scale (BirdLife International, 2015). The negative trend found for both secondary and main roads in the study area suggest that it is not only main roads that affect nocturnal raptors in a detrimental way, and therefore, when conservation efforts are planned, secondary roads should not be excluded. Efforts should, also, focus not only the primary goal of stopping collision with vehicles, but also on reducing noise, light and chemical pollution that result from the usage of roads, and therefore should stretch beyond the immediate area around roads.

Fencing, or structures that make flying animals rise above roads, may be a plausible solution for mitigating mortality rates. Walls would equally do the job and may lessen some noise pollution, although noise may also be lessened by advances in road construction and car mechanics. Light pollution may be contained if lamps are programmed to shut off after a certain hour, when traffic is lessened in the early hours of the morning.

Thinking in terms of conservation messages that one may take away, tawny owls may be considered good indicators to localize mortality hotspots that may be shared with other species, as these owls are of a decent size and therefore easily spotted as roadkill. Also, with this study we confirmed that secondary roads do too have effects on populations, and may present themselves as 'hidden traps', which is understandably problematic, as mitigation efforts are usually applied solely to main roads. To further understand how the interaction between the populations and the roads works, and how to better potential solutions, future studies focusing on the following possibilities would be recommended: (1) explore at which level fragmentation turns suitable habitat into non-suitable habitat (as mentioned, tawny owls support some degree of fragmentation – but how much?); (2) further study into the indirect effects of roads on the populations; (3) explore how far these indirect effects extend beyond the immediate area surrounding roads, depending on nearby habitat type.

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Annex 1. Methodological Bias in tawny owl (*Strix aluco*) census using call playback

The aim of this analysis was to evaluate which factors influence the detection of tawny owls in census using call playback, and therefore understand if these factors may create methodological bias. The factors found as affecting owl census will be considered in the subsequent analysis of the effects of roads on tawny owl density.

The census was conducted as described in methods (section **2.4.**).

During the statistical analysis, first, all variables were tested for to check normality in distribution, homogeneity of variances, and to detect outliers. Then all factors were tested for a significant effect on abundance of tawny owls, using generalized linear models. Significance was set at $p < 0.05$. Models were validated using diagnostic plots. All analyses were performed in the software R 3.3.0.

Observer effect

There were four observers in total (Ana, Rui, Fer and Shirley). The level of experience of all observers was high, still some differences could be expected (**Table A1**).

Table A1. Output summary of generalized linear model analysing observer effect on number of tawny owls detected.

Variable	Estimate	SE	z	P
(Intercept)	-0.0120	0.0776	-0.154	0.88
Fer	0.1731	0.1094	1.582	0.11
Rui	0.2592	0.1039	2.496	0.013
Shirley	0.0345	0.1681	0.205	0.84

The number of territories detected by one of the observers, Rui, was found to be statistically significant, carrying out, on average, point counts with a higher tawny owl abundance (**Figure A1**). However, we attribute this to the fact that this observer completed more listening points on dirt roads, where the probability of detecting a tawny owl was greater. It was determined that in fields tests, no notable difference in observer detection abilities were perceived, and, as such, the variable was not considered in the analysis of the effect of roads on owl density.

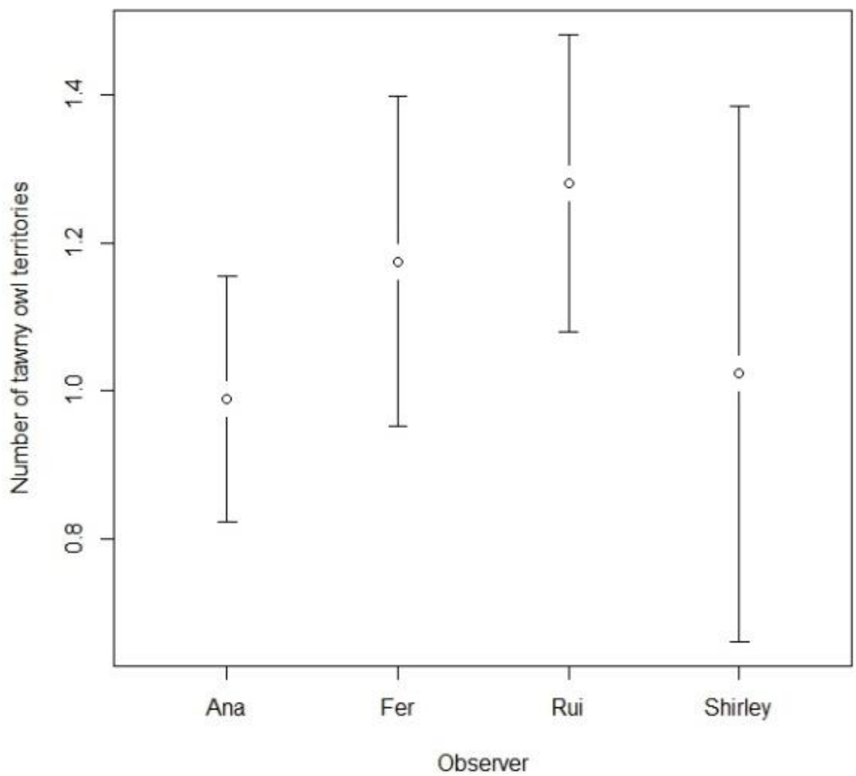

Figure A1. Plot of means of observer effect on number of tawny owl territories detected.

Eagle owl playback effect

Here, the point counts were compared when using only tawny owl playback and when using tawny owl followed by eagle owl playback (**Table A2**).

Table A2. Output summary of generalized linear model analysing eagle owl (*Bubo bubo*) playback effect on number of tawny owls detected.

Variable	Estimate	SE	z	P
(Intercept)	0.2151	0.0803	2.678	0.007
Eagle owl vs tawny owl playback	-0.0167	0.0989	-0.168	0.87

No significant differences were found between points where eagle owl playback was used and where it was not, therefore the variable was not considered relevant.

Hour of the night effect

We used the explanatory variable "minutes after sunset" (continuous variable) to evaluate the effect of the hour of the night when the census was performed (**Table A3**).

Table A3. Output summary of generalized linear model analysing the effect of the hour of the night on number of tawny owls detected.

Variable	Estimate	SE	z	P
(Intercept)	0.0993	0.0779	1.275	0.202
Minutes after sunset	0.0002	0.0005	0.414	0.679

No significant effect was found to be associated with the hour of the night and therefore the variable was not considered applicable.

Seasonal effect

To analyse the seasonal effect on the vocal activity of tawny owls we considered the month in which the point count was performed (November to February) (**Table A4**).

Table A4. Output summary of generalized linear model analysing the seasonal effect on number of tawny owls detected.

Variable	Estimate	SE	z	P
(Intercept)	0.1930	0.0729	2.647	0.008
Nov:Dec	-0.1538	0.1141	-1.348	0.18
Nov:Jan	0.1234	0.1031	1.197	0.23
Nov:Feb	-0.3992	0.1318	-3.029	0.002

This variable proved significant, showing that February was the month in which the points counts had lower tawny owl abundance (**Figure A2**). This difference may be related to phenological differences, influenced by the phases of reproduction, so the variable was considered in the analysis of the effects of roads.

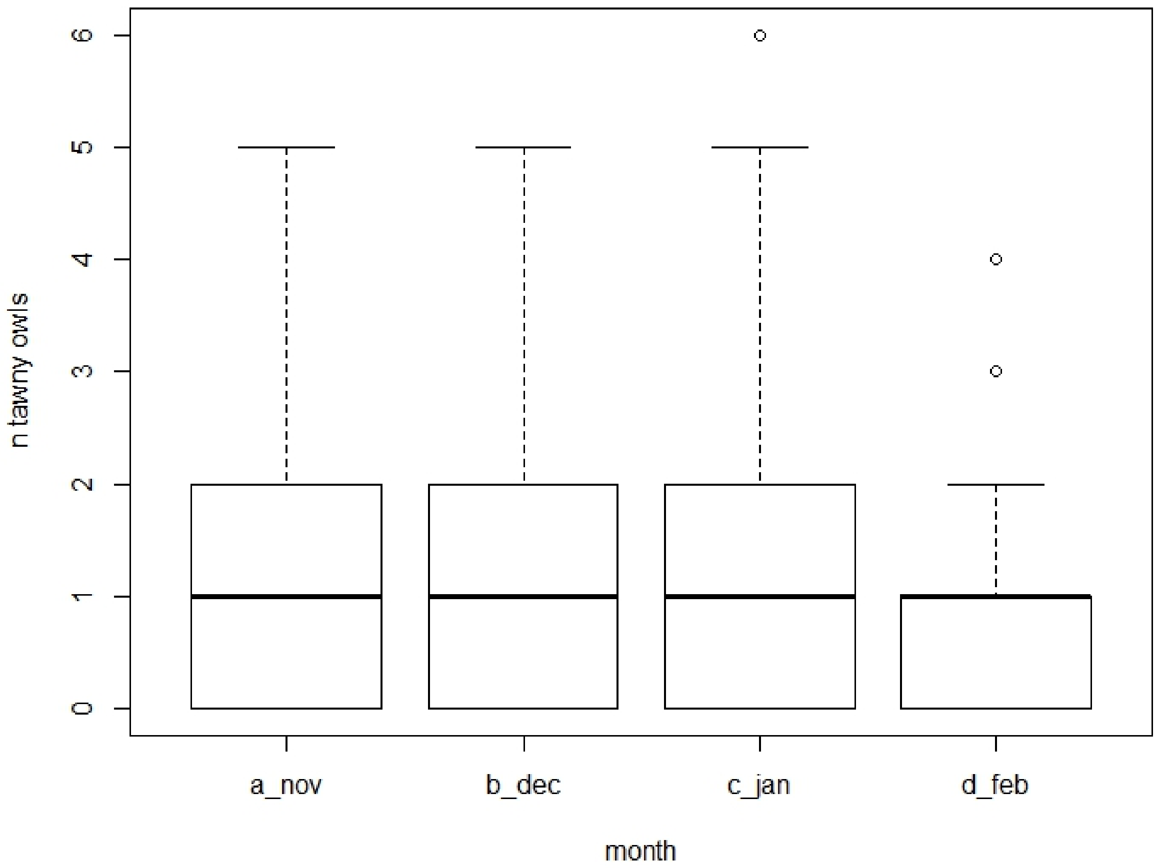

Figure A2. Variation of the abundance of tawny owls in point counts according to the month in which the census was done. Boxplot showing median, upper and lower quartiles and range.

Wind effect

Wind effect was measured by three classes (1 -low wind speed, 2 - moderate wind speed, 3 - high wind speed) (**Table A5**). When in doubt, wind classes were agreed between observers.

Table A5. Output summary of generalized linear model analysing effect of the wind (1 – low speed, 2 – moderate speed, 3 – high speed) on number of tawny owls detected.

Variable	Estimate	SE	z	P
(Intercept)	0.1446	0.04237	3.413	≤ 0.001
1:2	-0.2660	0.1845	-1.441	0.15
1:3	-0.8378	1.001	-0.837	0.40

No significant effect was found to be associated with wind and therefore the variable was not considered.

Cloud cover effect

Cloud cover effect was measured using three classes (1 – up to 33% cloud cover, 2 – between 34% and 66% cloud cover, 3 – between 67% and 100% cloud cover) (**Table A6**). When in doubt, cloud cover classes were agreed upon between observers.

Table A6. Output summary of generalized linear model analysing effect of cloud cover (1 – up to 33% cloud cover, 2 – between 34% and 66% cloud cover, 3 – between 67% and 100% cloud cover) on number of tawny owls detected.

Variable	Estimate	SE	z	P
(Intercept)	0.1294	0.0457	2.828	0.005
1:2	0.1295	0.1401	0.924	0.36
1:3	-0.1477	0.1436	-1.029	0.30

No significant effect was found to be associated with cloud cover and therefore the variable was not considered.

Moon phase effect

The variable moon phase considers both the phase of the moon (1 – up to 20% visible, 2 – between 21% and 40% visible, 3 – between 41% and 60% visible, 4 – between 61% and 80% visible, 5 – between 81% and 100% visible) and whether the moon was visible at the time the point was being done (0 – not visible, 1 - visible) (**Table A7**). Moon visibility percentages were checked with a reliable lunar calendar. The variable was also arcsine-transformed, due to its circular nature.

Table A7. Output summary of generalized linear model analysing effect of moon phase (1 – up to 20% visible, 2 – between 21% and 40% visible, 3 – between 41% and 60% visible, 4 – between 61% and 80% visible, 5 – between 81% and 100% visible) + moon visibility (0 – not visible, 1 – visible) on number of tawny owls detected.

Variable	Estimate	SE	z	P
(Intercept)	0.1105	0.0525	2.105	0.04
Moon phase	0.0374	0.0746	0.501	0.62

No significant effect on tawny owl detection was found to be associated with moon phase and therefore the variable was not considered.