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"Unless you try to do something beyond what you have already mastered, you will never grow."

- Ralph Waldo Emerson

RESUMO

O modelo de *Cox proportional hazards* é um modelo de sobrevivência semi-paramétrico utilizado em análise de regressão para dados censurados. O modelo original tem sofrido alterações, com a inclusão de novas extensões que permitem maior flexibilidade e a análise de um conjunto mais alargado de dados. Entre as recentes extensões, incluem-se: a análise de variáveis dependentes do tempo, múltiplas observações de eventos, *tied events* e estratificação de variáveis dependentes do tempo.

A sobrevivência é um fator determinante na ecologia de populações e na biologia da conservação. Este tipo de estudos só é possível com recurso a técnicas de telemetria, que tornam possível a aquisição de informação contínua. Existem vários problemas associados a este tipo de análise quando aplicada a animais em estado selvagem, muitas vezes ignorados pelos investigadores. Os mais típicos consistem na influência que o equipamento de seguimento tem no animal e no stress induzido pela marcação. Estes e outros problemas tornam a análise de sobrevivência de animais selvagens um desafio.

O sisão (*Tetrax tetrax)* é uma ave ameaçada na Península Ibérica classificada como Vulnerável (Cabral et al., 2005; Madroño et al., 2004) em Portugal e catalogada como *Near Threatened* pela *International Union for Conservation of Nature* (IUCN) a nível global (Collar et al., 1994). Tem estado em declínio acentuado na Europa, especialmente em Itália, França e Espanha, apresentando uma tendência desconhecida em Portugal. Trata-se de uma ave estepária adaptada a meios agrícolas abertos extensivos e a pastagens. Entre os principais fatores de ameaça à espécie, destacam-se: a perda e fragmentação do *habitat* devido à intensificação agrícola nos solos mais produtivos, o abandono agrícola nos solos menos produtivos, a construção de infraestruturas (linhas elétricas, vedações, estradas) e as alterações climáticas.

Neste contexto, a identificação e análise da forma como diferentes fatores influenciam a mortalidade do sisão e a distinção entre as diferentes causas de mortalidade constitui um contributo relevante para o desenvolvimento e aplicação de medidas que promovam a conservação desta espécie.

Com este trabalho pretendeu-se aplicar a análise de sobrevivência – normalmente utilizada em estudos de medicina, engenharia, economia e sociologia – a um estudo ecológico, usando *software* estatístico comum, geralmente utilizado por investigadores desta área.

Neste trabalho foi utilizada uma amostra de 143 indivíduos capturados e seguidos na Península Ibérica, com o objectivo de: (1) identificar as principais causas de mortalidade natural e antropogénica e quantificar a sua importância relativa; (2) modelar o efeito de quatro variáveis: sexo, uso do solo,

época e região bioclimática na sobrevivência do sisão, usando o estimador de Kaplan-Meier e o Modelo *Extended Cox Proportional Hazards Model for Time-Dependent Variables.*

A mortalidade antropogénica teve uma prevalência maior do que seria expectável para uma espécie protegida. Cerca de 17% da população ibérica morre anualmente devido a causas humanas, uma maior percentagem que a morte anual por causas naturais (15%). Estes valores de mortalidade antropogénica, associados a uma fraca produtividade do sisão, mais nítida em áreas com pior estado de conservação de *habitat*, levam à falta de sustentabilidade da espécie, pondo em risco a sobrevivência de uma ave com estatuto de Quase Ameaçada. A colisão com linhas elétricas foi, até hoje, a causa de mortalidade antropogénica com maior destaque. Com este estudo, concluímos que cerca de 4% da população da Península Ibérica morre anualmente por colisão com linhas elétricas e cerca de 1% por atropelamento. Para a população de Portugal, tinha sido estimada anteriormente uma mortalidade anual de 1,5% por linhas elétricas (Silva et al., 2010b). No entanto, a causa de morte antropogénica com maior prevalência foi a caça, com cerca de 12% da população a morrer anualmente por esta causa.

Não foram evidenciadas diferenças entre a proporção de mortes dentro e fora da época de caça, sugerindo que esta variável não influencia a proporção de mortalidade por cada uma das causas. Não foi encontrada uma relação entre o sexo e a morte dentro ou fora da época venatória.

Dentro da morte por causas naturais, foi apenas identificada a morte por predação. Mamíferos e rapinas tendem a predar o sisão aproximadamente na mesma proporção, ainda que tenham sido identificados 5% de casos em que o predador não foi identificado.

Aplicámos com sucesso o modelo de Cox a um objeto de estudo ecológico, conseguindo eliminar o máximo de *bias* associado e identificar os principais fatores que influenciam a sobrevivência do sisão. A região bioclimática revelou-se o fator com maior influência na mortalidade da espécie. Regiões mais secas e quentes tendem a ter um maior risco de mortalidade, provavelmente devido à escassez de vegetação e insetos em épocas de seca, desencadeando uma menor disponibilidade de alimento. Concluímos que fêmeas de sisão têm um maior risco de mortalidade que os machos. Estas são as únicas responsáveis pelos cuidados parentais, despendendo energia tanto na postura como na alimentação das crias. Este é, portanto, um período de grande vulnerabilidade para as fêmeas, que se pode estender após a época de reprodução, e que pode afetar o seu *fitness* de inverno.

Uma vez que a intensificação agrícola é o fator mais associado à ameaça da espécie, seria expectável observar um impacto negativo deste uso do solo na mortalidade do sisão. Contudo, tal não se verificou, tendo este factor apresentado pouca influência em comparação com a região bioclimática e o sexo. Sobretudo durante os anos de seca, o sisão está mais dependente de culturas

intensivas (com maior disponibilidade de alimento) durante a época de pós-reprodução e inverno, havendo registos de movimento dos mesmos de áreas extensivas para áreas intensivas durante estas épocas do ano. Assim, admite-se a existência de uma relação entre o uso do solo e a época, que influencie o comportamento destas variáveis. A época foi a variável com menor impacto na mortalidade do sisão.

O nosso trabalho demonstra quão mal compreendidas e desconhecidas eram algumas ameaças à população do sisão na Península Ibérica. Uma redução da mortalidade antropogénica terá um grande impacto na viabilidade desta espécie, particularmente em zonas com menor produtividade da mesma. Alterações na legislação das épocas venatórias e medidas de sensibilização poderiam reduzir o problema de caça associado, sendo um fator que afeta anualmente quase 12% da população. A adaptação da rede elétrica em termos de localização e *design* de linhas também deve ser considerada prioritária.

A análise de sobrevivência usando o modelo de Cox foi aplicada com sucesso a um caso de estudo ecológico, e revelou ser capaz de acomodar os problemas associados ao seguimento de animais selvagens. Foi igualmente aplicada com sucesso a análise de causas específicas de mortalidade para esta espécie, utilizando o estimador de Heisey e Fuller, o que possibilitou o cálculo das taxas de mortalidade anuais para causas de morte de origem natural e antropogénica.

Devem ser tomadas medidas para orientar o trabalho de campo especificamente para este tipo de análise, principalmente no que diz respeito à obtenção de uma data de mortalidade mais precisa. Um maior esforço de identificação de carcaças, juntamente com o uso de emissores que permitam medir parâmetros fisiológicos para determinar o estado do indivíduo, seriam soluções que contribuiriam para atenuar este problema. Neste trabalho conseguimos testar a influência do peso dos emissores e do tipo de tecnologia de seguimento na sobrevivência do sisão e verificámos que emissores com peso abaixo de 6,76% do peso corporal não tiveram influência significativa na sobrevivência. O uso de diferentes tecnologias de seguimento também não teve influência ao nível da mortalidade.

Palavras-chave: Modelo Cox proportional hazards, análise de sobrevivência, dados censurados, conservação, seguimento de aves, *Tetrax tetrax*, estimador de Heisey and Fuller.

ABSTRACT

The Cox proportional hazards model has become the most widely used method for regression analysis for censored data and is frequently used to model survival data. The introduction of new extensions to the original model like time-dependent covariates, multiple observations, multiple time-scales, tied events and time-dependent strata improved it and made it more widely applicable. Survival estimation is an important aspect of population ecology and conservation biology, with telemetry making possible the acquisition of continuous survival information. There are often problems associated with this type of analysis in ecological studies of free-ranging animals, that are frequently disregarded by researchers, such as the influences of the transmitter's weight, induced stress in handling the species, or interval-censored death dates, which make survival studies on free ranging animals a challenge.

In this study, we aim to apply survival analysis - frequently used in medicine, engineering, economics and sociology - to an ecological study, using a standard statistical software, widely used by researchers of this area. We have successfully applied this approach to a case study and managed to address potential bias sources and identify the main factors that influence Little Bustard's (*Tetrax tetrax*) survival. Furthermore, we have also computed cause-specific mortality rates for this species, using the Heisey and Fuller estimator.

Keywords: Cox proportional hazards model, survival, censoring, ecological, tracking, Heisey and Fuller estimator.

INDEX

INTRODUCTION

Survival analysis consists in the analysis of data in which the outcome variable of interest is time until an event occurs. It is frequently used in medical research for analyzing subject treatment responses, but is also used in other areas to study different events like equipment failures (engineering), stock market crashes (economics) and births or retirements (sociology).

In ecological studies, survival analysis is most commonly used to model animal survival rates (Murray and Patterson, 2006). Although this type of analysis has not been very explored in the past, development and assessment of survival methods applied to free ranging animals has been increasing as a focus point in many studies (Pollock and Winterstein, 1989; Lebreton et al., 1992; Murray, 2002; Murray and Patterson, 2006) with significant improvements in the development of software for the analysis of complex survival functions (Williams et al., 2002). This has led to a recent increase in the use of survival analysis methods in conservation biology studies (Gauthier et al., 2001; Ward et al., 2004; Haines et al., 2005; Bender et al., 2007; Hupp et al., 2008; Le Gouar et al., 2008; Ma and Bechinski, 2008; Persson et al., 2009; Evelsizer et al., 2010; Domingues et al., 2012; Buuveibaatar et al., 2013; Melis et al., 2013; Olson et al., 2014).

Survival analysis and the assessment of mortality causes in wildlife are essential to analyze and quantify the main pressures that affect individual mortality and species population trends, contributing to conservation strategies of threatened species. Comparatively to other demographic parameters of wildlife populations, survival is difficult to estimate (McCallum, 2000) due to animal capture and monitoring difficulties that lead to small sample sizes and uncertainly about the fate of large part of the tracked individuals. Nevertheless, the increasing use of remote tracking devices and the improvements made in terms of capture techniques has facilitated survival assessment for free-ranging animals (Krebs, 1999; Kenward, 2001; Millspaugh and Marzluff, 2001), leading to an increased number of survival studies applied to ecology.

Current survival analysis procedures are capable of dealing with key data analytical problems like censoring. Censoring is very common in radio-tracking studies and occurs when we do not know an individual's exact survival time for different reasons: the date of death is uncertain, the individual is "lost to follow-up", i.e., it's signal may be lost during the tracking with no carcass found, or the individual is still alive at the end of the study. To estimate survival probability at a given time, in addition to the risk set at that time, the information of censored individuals is included up to the time of censorship, rather than excluded from the start.

Kaplan-Meier estimate as a tool to model survival

The computation of survival over time can be carried out using the Kaplan-Meier estimate (Kaplan and Meier, 1958), also known as product limit estimator. The Kaplan-Meier estimate of the survival function, $S(t)$, is the non-parametric maximum likelihood estimate of $S(t)$ and describes the probability of a subject surviving beyond time t:

$$S(t) = P(T > t) = 1 - F(t)$$

This analysis holds three assumptions: (1) individuals that are censored have the same survival prospects as those that continue to be followed; (2) survival probabilities are the same for subjects recruited early and late in the study; (3) the death happens at the time specified (Goel et al., 2010).

Assuming we are following individuals in short intervals of time, and defining:

n_j = number of individuals observed and surviving at time *j*, when deaths (but not losses) at *t* itself are subtracted off;

δ_j = deaths observed in the interval (u_{j-1}, u_j);

the survival probability for any particular time interval is given by:

$$S_j = \frac{n_j - \delta_j}{n_j}$$

(adapted from Goel et al.,2010)

And consequently, the product-limit estimate of $S(t)$ is given by the multiplication of the successive probabilities:

$$\hat{S}(t) = \prod_{j=1}^{k} \frac{n_j^{'}}{n_j} \text{ , with } u_k = t \text{ , } n_j^{'} = n_j - \delta_j$$

(Kaplan and Meier , 1958)

where $n_j^{'}$ is the number under observation just after δ_j deaths.

The estimates obtained can be expressed as a stepwise survival curve that can be defined as the probability of an individual surviving in a given length of time while considering time in many small intervals (Altman, 1992).

One downside of this estimate is that it is only capable of dealing with one explanatory variable at a time. When we want to consider the effect of several explanatory variables simultaneously, Cox Proportional Hazards Model is the right approach for estimating survival.

Extended Cox Proportional Hazards Model for Time Dependent Variables

Cox Proportional Hazards Model (Cox, 1972) is a semi-parametric model used for analyzing survival data. The formula consists in that the hazard (probability of failure) at time *t* is the product of the baseline hazard function, $h_0(t)$, and the exponential of the linear sum of the effect parameters:

$$h(t, X) = h_0(t) \times e^{\sum_{i=1}^{p} \beta_i X_i}$$

The baseline hazard function describes how the risk of death changes over time at baseline levels of the covariate, and the exponential expression describes how the hazard varies in response to the explanatory variables.

This model must hold two important assumptions: (1) Non-informative censoring assumption states that censored individuals must not be related to the probability of occurrence of the event; (2) Proportional hazards assumption is based on the fact that the effect of a covariate does not change over time. Herewith, the baseline function is an expression of time, whereas the exponential expression involves the variables but not the time - the variables are then called time-independent.

When considering variables that do change its value over time, we call them time-dependent and the model is renamed Extended Cox Model:

$$h\big(t, X(t)\big) = h_0(t) \times e^{\left[\underbrace{\sum_{i=1}^{p1} \beta_i X_i}_{\textit{time-independent}} + \underbrace{\sum_{j=1}^{p2} \delta_j X_j(t)}_{\textit{time-dependent}}\right]}$$

(Kleinbaum and Klein, 2005)

Although the value changes over time, there is only one coefficient for each time-dependent variable. Thus, there is only one value that has an effect in the hazard, measured at time t.

The Little Bustard as a case study

The Little Bustard *Tetrax tetrax* (Linnaeus, 1758) is the only species of the genus *Tetrax* and belongs to the family Otididae. It had originally a Palaearctic distribution (Cramp and Simmons, 1980) extending from the Iberian Peninsula and Morocco, to Kyrgyzstan and extreme northwest China (del Hoyo et al., 1996), but became extinct as a breeding species in late 19th and 20th centuries in many countries of Central and Southern Europe (Cramp and Simmons, 1980; Schulz, 1985, 1987; Goriup, 1994). Today, its breeding distribution is fragmented and concentrated in two main regions: the first one centred in south-eastern European Russia and Kazakhstan, and the second, and most important one, in the Iberian Peninsula, southern France, Sardinia and Morocco (Cramp and Simmons, 1980; del Hoyo et al., 1996; Palacín and Alonso, 2009) (Fig. 1). Over the last decades the population has been declining in most of its range. In France, for example, between the 80's and late 90's the population almost became extinct with an estimated decline of 92% (Jolivet, 1997). The Iberian Peninsula is thought to be the main stronghold of the western European population (Iñigo and Barov, 2010).

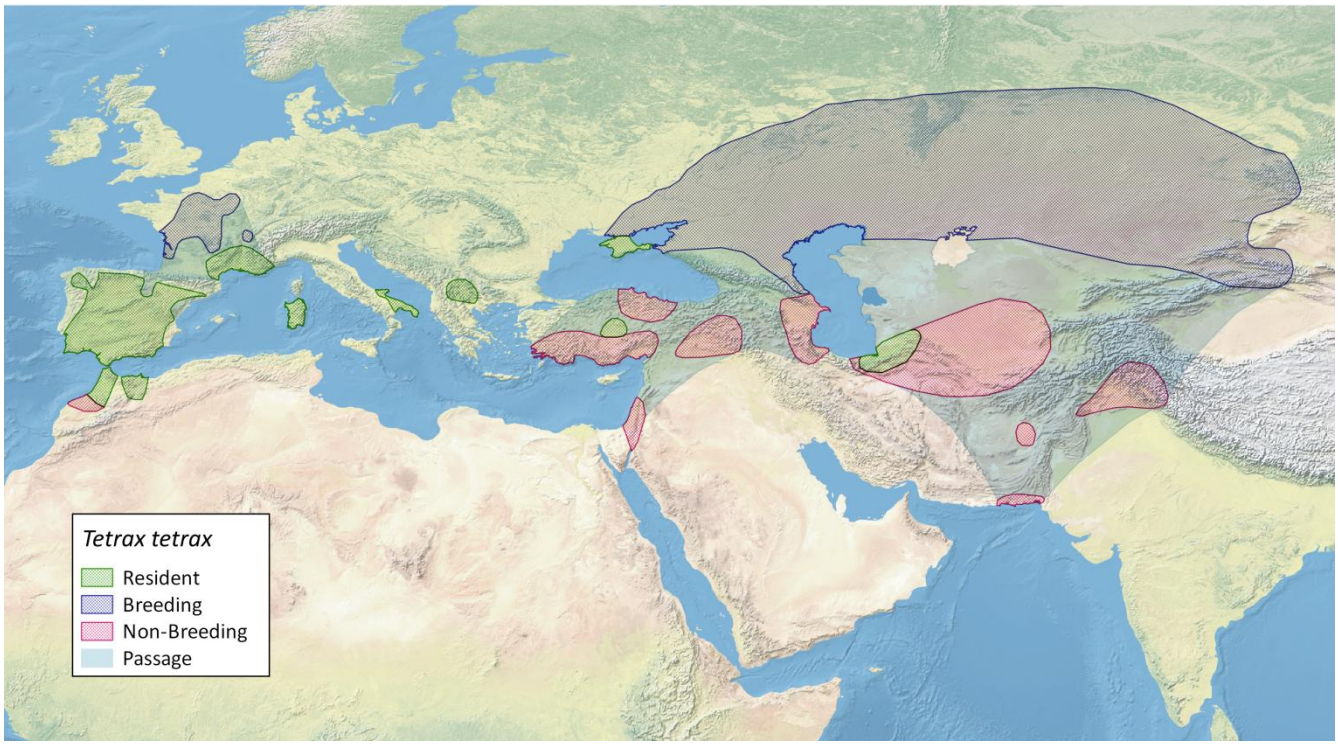


Figure 1 - World distribution map of the Little Bustard (BirdLife International and NatureServe, 2014).

The Little Bustard is currently classified as globally Near Threatened by the International Union for Conservation of Nature (IUCN) (Collar et al., 1994) and Vulnerable in the Iberian Peninsula (Madroño et al., 2004; Cabral et al., 2005; Iñigo and Barov, 2010). The estimated population in Spain is now about 40.000-85.000 breeding males (García de la Morena et al., 2006) in contrast with 100.000-200.000 in the 90's (de Juana and Martínez, 1996). In Portugal, the population is estimated to be around 17.500 breeding males (Silva and Pinto, 2006). Furthermore, the Little Bustard is considered an umbrella species (Silva et al., 2010a) since the European Union Directive on the Conservation of Wild Birds (79/409/EEC) has classified it as priority species for conservation and defined Special Protection Areas (SPA) for its conservation.

As a grassland bird, it is adapted to open extensive agricultural areas with low intensity farming (Cramp and Simmons, 1980). In western Europe its main habitat consists not only of extensive cereal farmland (Martínez, 1994; Silva et al., 2004, 2007; Morales et al., 2006b), but also of fallow land and extensive pastureland during the breeding season (Martínez, 1994; Salamolard and Moreau, 1999; Delgado and Moreira, 2000; Wolff et al., 2002a, 2002b). This species diet consists primarily of green plant leaves but also includes arthropods (Jiguet, 2002). It is mainly based on Leguminosae and Cruciferae during winter (Cramp and Simmons, 1980; Martínez, 1994), and the consumption of invertebrates is especially important during post-breeding season (Jiguet, 2002).

The Little Bustard performs regular movements in the Iberian Peninsula at the end of the breeding season, from the nesting sites towards areas with greater food availability.

The main causes of Little Bustards decline are related to habitat loss and fragmentation, population fragmentation and habitat degradation, mainly caused by intensive farming. Intensive farming is usually characterized by the establishment of monocultures, the use of chemical additives and heavy mechanisation. This type of agriculture increased in the last decades (Chamberlain and Fuller, 2000; Siriwardena et al., 2000) resulting in a reduction of extensive agricultural habitats (Donázar et al., 1993; Bignal and McCracken, 1996) and habitat degradation, consequently leading to the decline of farmland species (Schulz, 1985; Blanco et al., 1998; Bouma et al., 1998; Chamberlain and Fuller, 2000; Donald et al., 2001; Martínez and Tapia, 2002; Sanderson et al., 2005; Santos and Suárez, 2005). In the Iberian Peninsula, permanently irrigated lands are the predominant type of intensive farming. They are associated to crops that rely on irrigation infrastructures and, depending on the type of crop, can lead to a partial or total loss of habitat for the species. Together with the abandonment of agricultural activity and the decreasing use of crop rotation systems, intensive farming is believed to be the main reason for Little Bustard decreasing numbers, due to decreased habitat extent and quality (Goriup, 1994; Morales et al., 2005b, 2006b; García et al., 2007).

Other factors responsible for the Little Bustard's population decline include collision with infrastructures, hunting and predation.

What we know about Little Bustard mortality causes

Predation is the most common natural cause of death for Little Bustard (Schulz, 1987). Its preference for hill tops (Silva et al., 2004) and low vegetation (Martínez, 1994; Moreira, 1999; Salamolard and Moreau, 1999) can be considered an anti-predatory strategy (Silva et al., 2010b). Maintaining distance of tall over-head lines, where avian predators perch, can be also a protective measure to become less vulnerable to predation (Silva et al., 2010b). However, no information is available about Little Bustard predators, but potential species include the red fox (*Vulpes vulpes*), hen harrier (*Circus cyaneus*), peregrine falcon (*Falco peregrinus*) and Iberian imperial eagle (*Aquila adalberti*).

Bioclimatic regions can have also influence on this species behavior. Drier and hotter climates lead to less productive soils and consequently to fewer food resources (Delgado and Moreira, 2010), possibly the affecting the survival of the Little Bustard, in particular of juveniles. Alongside that, seasons are also a possible factor of interference, with higher temperatures occurring in post-breeding season, when the Little Bustard have to spend energy when moving to more productive soils (Silva et al., 2007), probably increasing its mortality risk. Differences between survival rates between sexes are

also a possibility, since there are several studies that report a biased sex ratio towards males (Inchausti and Bretagnolle, 2005; Morales et al., 2008; Faria et al., 2012).

Human-caused deaths are poorly studied, but the construction of infra-structures such as power lines, fences and roads has been considered the main threat (Silva et al., 2010b; Alcazar, 2013). This kind of structures is on ongoing construction and, in addition to causing habitat fragmentation by partly blocking the birds movements, are thought to be a significant source of direct mortality by collision (Neves et al., 2005). In Portugal it is estimated that about 134 Little Bustards die every year by collision with power lines (Infante et al., 2005). Little is known about the effect of illegal hunting of the species in the Iberian Peninsula, but, given the number of hunters active during autumn and winter all over the region, this is suspected to be an important cause of mortality. And even if it is not an important source of direct mortality, it could be a major factor of disturbance (Gauger, 2007).

Existent ecological studies on Little Bustard (de Juana and Martínez, 1996; Jolivet and Bretagnolle, 2002; Inchausti and Bretagnolle, 2005; Morales et al., 2006a; De Juana, 2009; Delgado et al., 2009; Palacín and Alonso, 2009; Silva et al., 2010b, 2014b; Moreira et al., 2012a) do not report any specific information about what is the impact of anthropogenic directly caused deaths (e.g., power line collision, hunting, vehicle collision) in comparison with deaths by natural causes (e.g., predation, starvation, hyper- and hypothermia). Moreover, none of the studies reference mortality rates associated with environmental pressures that influence survival through time. It is then essential to study the importance of these factors in terms of mortality risk. That information would allow population modelling under different land use and climatic scenarios and be a major contribute to the development and application of conservation strategies for this species.

Using a sample of over 100 little bustards captured, tagged and followed in the Iberian Peninsula, the aims of this work are: (1) to identify the main causes of natural and anthropogenic deaths, and quantify their relative importance; (2) to model the effect of four variables: sex, land cover, seasons and bioclimatic region on Little Bustard's survival, using Kaplan-Meier estimate and the Extended Cox Proportional Hazards Model for Time-Dependent Variables.

CHAPTER1:

Analyzing causes of little bustard mortality in the Iberian Peninsula and modelling factors driving natural deaths

ABSTRACT

The Little Bustard (*Tetrax tetrax*) is facing a number of threats in the Iberian Peninsula - the region considered to be the stronghold of the Western Europe population of the species. Understanding what is causing this species' decline is a key step towards reverting this tendency. Knowing survival and cause-specific mortality rates is essential to make decisions about future management of the population.

Here, we use Cox Proportional Hazards Model to evaluate what are the main environmental and anthropogenic threats of the Little Bustard in the Iberian Peninsula, using 10 years of tracking data. This work proved to be key in identifying new threats and assessing the importance of previously known ones. Previous studies suggest that this species decline is mainly caused by recent changes in agricultural practices. However, we concluded that 17.1% of the Little Bustard's mortality is caused by direct anthropogenic sources, mainly through hunting. Bioclimatic region is the main environmental pressure, suggesting that low precipitation and temperature extremes are possibly connected with the decline of this species. We observed different mortality tendencies between sexes, with females having a higher mortality risk than males, which can explain the biased sex ratio reported in previous studies. Agricultural practices did not have the expected impact on survival. Conservation strategies should focus on reducing hunting zones in classified areas and further studies on survival of the Little Bustard should be focused on temperature extremes.

Keywords: survival; cause-specific mortality; Iberian Peninsula; *Tetrax tetrax*; hunting; anthropogenic deaths; tracking data; Cox proportional hazards model.

INTRODUCTION

Little Bustard (*Tetrax tetrax*) had originally a continuous Palaearctic distribution (Cramp and Simmons, 1980), but has suffered an accentuated decline in the late 19th century. Currently it occurs on two major breeding areas: one centred in Kazakhstan and European Russia and another in the Iberian Peninsula, Southern France, Sardinia and Morocco (Cramp and Simmons, 1980; del Hoyo et al., 1996; Palacín and Alonso, 2009).

The Iberian Peninsula is thought to be the main stronghold of the western European population (Iñigo and Barov, 2010). This species population has, however, declined from 100.000-200.000 in the 90's (de Juana and Martínez, 1996) to 40.000-85.000 breeding males in Spain (García de la Morena et al., 2006) and it seems to be keeping this declining tendency (Morales et al., 2006a; De Juana, 2009). In Portugal, the population is estimated at 17.500 breeding males (Silva and Pinto, 2006) with an unknown trend. It is currently classified as globally Near Threatened by the International Union for Conservation of Nature (IUCN) and as Vulnerable in the Iberian Peninsula (Cabral et al., 2005, Madroño et al., 2004) and it is considered an umbrella species since the European Union Directive on the Conservation of Wild Birds (2009/147/EC) has classified it as priority species for conservation and defined Special Protection Areas (SPA) for its conservation.

As a grassland bird, the Little Bustard is well adapted to open extensive agricultural areas with low intensity farming (Cramp and Simmons, 1980), which are especially important during the breeding season (Martínez, 1994; Moreira, 1999; Delgado and Moreira, 2000; Morales et al., 2006b). The adults mainly feed on green plants (Jiguet, 2002), mostly Leguminosae and Cruciferae (Cramp and Simmons, 1980; Martínez, 1994), while chicks depend almost exclusively on arthropods during the first weeks of life (Jiguet, 2002).

A number of factors could be contributing to Little Bustard's decline, but the main threat is considered to be agricultural intensification which leads to habitat loss or degradation (Goriup, 1994; Morales et al., 2005b, 2006b; García et al., 2007). In Iberian landscapes, agricultural intensification leads to the decreasing use of crop rotation systems and suppression of fallow land, which is a key breeding habitat for the Little Bustard (Martínez, 1994; Morales 2005; Moreira et al., 2012). In France, nest destruction during harvesting is also reported (Inchausti and Bretagnolle, 2005).

Little Bustard death causes

Predation is thought to be the most common natural cause of death for Little Bustard (Schulz, 1987). Potential predator species include the red fox (*Vulpes vulpes*), hen harrier (*Circus cyaneus*), peregrine falcon (*Falco peregrinus*) and Iberian imperial eagle (*Aquila adalberti*). Its preference for hill tops (Silva

et al., 2004), low vegetation (Martínez, 1994; Moreira, 1999; Salamolard and Moreau, 1999) and keeping distance from overhead lines, can be an anti-predatory strategy (Silva et al., 2010b).

The Little Bustard is also probably vulnerable to climatic changes. Precipitation is associated with higher densities of Little Bustard due to positive effects on vegetation growth and arthropod availability (Delgado and Moreira, 2010). Predicted changes in the climate of the Iberian Peninsula indicate a decline in overall precipitation and an increase of the mean temperature. The combination of lower rainfall and higher temperatures lead to dry fields and less vegetation, and consequently decreasing the abundance of plants and arthropods (Delgado et al., 2009), putting in jeopardy the survival of adults, but particularly of juveniles, that depend almost exclusively on insects during their first days of life (Jiguet, 2002).

Collision with power lines is one of the most significant identified anthropogenic causes of mortality, the Little Bustard being one of the birds most susceptible to it (Bevanger, 1998; Janss, 2000). It is estimated that about 1.5% of the Portuguese population dies annually due to overhead power lines (Silva et al., 2010b). In addition to causing direct mortality by collision, the ongoing construction of power lines, fences and roads is likely to be causing habitat loss. Another source of non-natural mortality is illegal hunting, although, the extent to which this might be affecting Iberian populations is unknown. Hunting may not only result in mortality but it may also cause disturbance (Gauger, 2007), which disturbs Little Bustards, possible leading to some additional collisions with infrastructures.

The first goal of this study was testing for differences in survival between sexes. We hypothesized that females had a higher mortality risk comparing to males, since there are several studies that report a biased sex ratio towards males (Inchausti and Bretagnolle, 2005; Morales et al., 2008; Faria et al., 2012). We also compared differences in terms of mortality risk between two land types: non-irrigated arable land (extensive agriculture) and permanently irrigated land (intensive agriculture), hypothesizing that intensive agriculture would have a higher mortality risk due to lack of habitat suitability. We tested if seasons had influence in Little Bustard's mortality risk throughout the year. It was to expect that the post-breeding season had the highest risk of mortality, by the fact that the Little Bustard loses energy in the breeding season, being more susceptible to predation afterwards. Finally, we tested bioclimatic regions influence on Little Bustard's survival.

An understanding of the main causes for population decline is required for sound decision-making aiming at a species recovery. How different factors affect survival is therefore key for outlining recovery plans of threatened species (Le Gouar et al., 2008). Even though many of the anthropogenic mortality factors threatening this species have been described, the impacts of these factors on the overall population are greatly unknown. Furthermore, existing ecological studies on Little Bustard do

not report mortality rates associated with environmental pressures that influence survival through time (de Juana and Martínez, 1996; Jolivet and Bretagnolle, 2002; Inchausti and Bretagnolle, 2005; Morales et al., 2006a; De Juana, 2009; Delgado et al., 2009; Palacín and Alonso, 2009; Silva et al., 2010b, 2014b; Moreira et al., 2012a). It is then essential to study the importance of these factors in terms of mortality risk. That information would allow population modelling under different management scenarios and be a major contribution to the development and application of conservation strategies specific for this species.

The main objectives of this paper are: (1) to identify the causes of anthropogenic and natural deaths of the Little Bustard, and their relative prevalence; (2) to determine how and what factors influence natural mortality in the Iberian Peninsula. To answer the first question, we gathered all the available mortality data from telemetry studies of individuals tracked in the Iberian Peninsula and divided and quantified them by death cause. To model factors driving natural deaths we gathered Little Bustard tracking data from individuals which died from natural causes from two regions of the Iberian Peninsula (Alentejo/Extremadura and Catalonia) and modelled the role of sex, land cover, seasons and bioclimatic region in survival.

METHODS

Study Areas

The study was carried out in the Iberian Peninsula, within several Spanish autonomous regions (Catalonia, Aragon, Castilla-La Mancha, Castile and León and Extremadura) and the Portuguese region of Alentejo, covering important areas of the species distribution of Iberia (de Juana and Martínez, 1996; Iñigo and Barov, 2010). The data collected is mostly concentrated in Catalonia and Alentejo/Extremadura (Fig. 1 and Fig. 2). In the Northeastern areas of the Peninsula, agriculture is more intensive with smaller fields and more irrigation (Lapiedra et al., 2011). Conversely, in the Southwestern regions of Iberia the landscape is characterized by larger fields and considerable amounts of grasslands (Moreira et al., 2012b).

The Iberian Peninsula is characterized by four types of bioclimatic regions according to Köppen–Geiger climate classification system (Peel et al., 2007)(Fig. 3). This climate classification system defines distinct types of climates using average monthly values for precipitation and air temperature. Southwestern Iberia has a temperate climate with dry or hot summer (Csa) which consists in a dry summer and average temperature in the hottest month above 22 °C. On the other hand, Northeastern region has a more diverse climate, but is mainly characterized by a temperate climate without a dry season and with a temperate summer (Cfb). The other two predominant climates of this region are the

cold steppe (BSk) and temperate with dry or temperate summer (Csb). Cold steppe is a dry climate where, on average, evapotranspiration exceeds precipitation, and has average annual temperature below 18 °C. Csb is also a temperate climate with dry summer, but with four months or more with average temperatures above 10 °C.

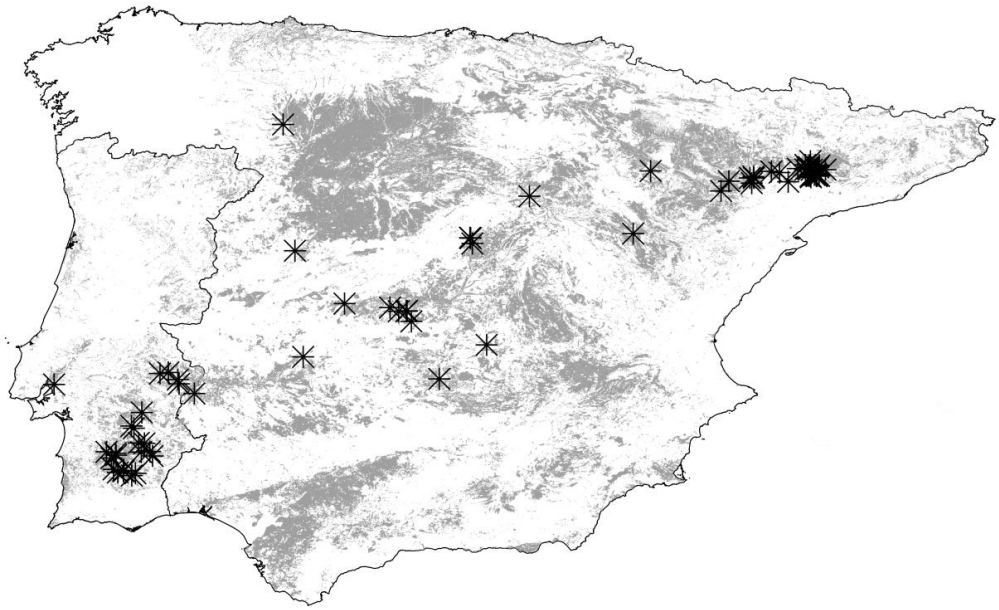


Figure 1. Asterisks indicate the location of dead individuals recorded between 2001 and 2012 used in the cause-specific analysis. The areas in light grey represent the potential habitat for the Little Bustard, representing the land uses most frequented by the species.

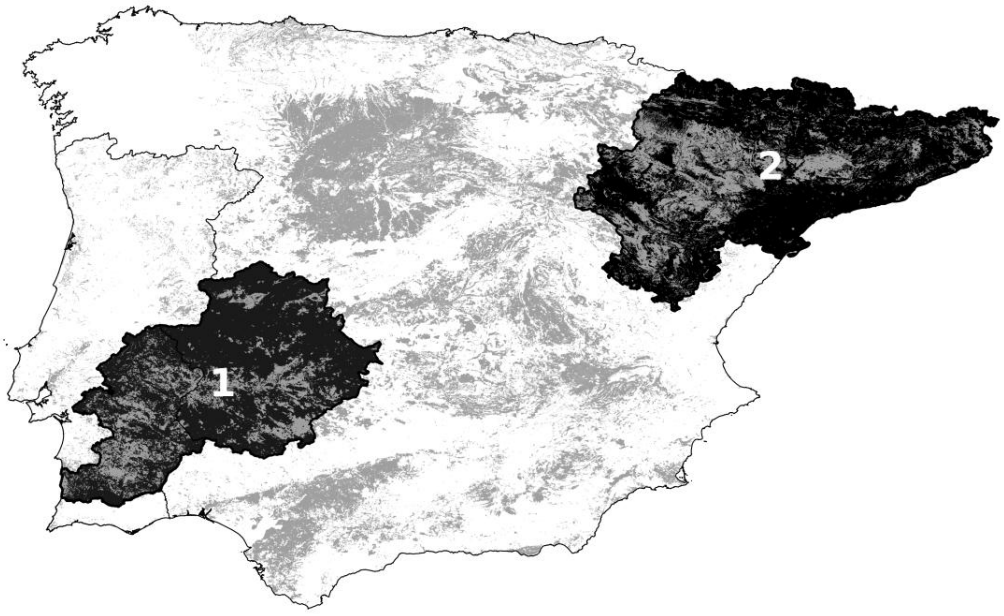


Figure 2. Location of the two study areas (in dark grey), within the Iberian Peninsula: 1 - Southwestern region (Alentejo/Extremadura); 2 - Northeastern region (Catalonia/Aragon). The areas in light grey represent the potential habitat for the Little Bustard, representing the land uses most frequented by the species.

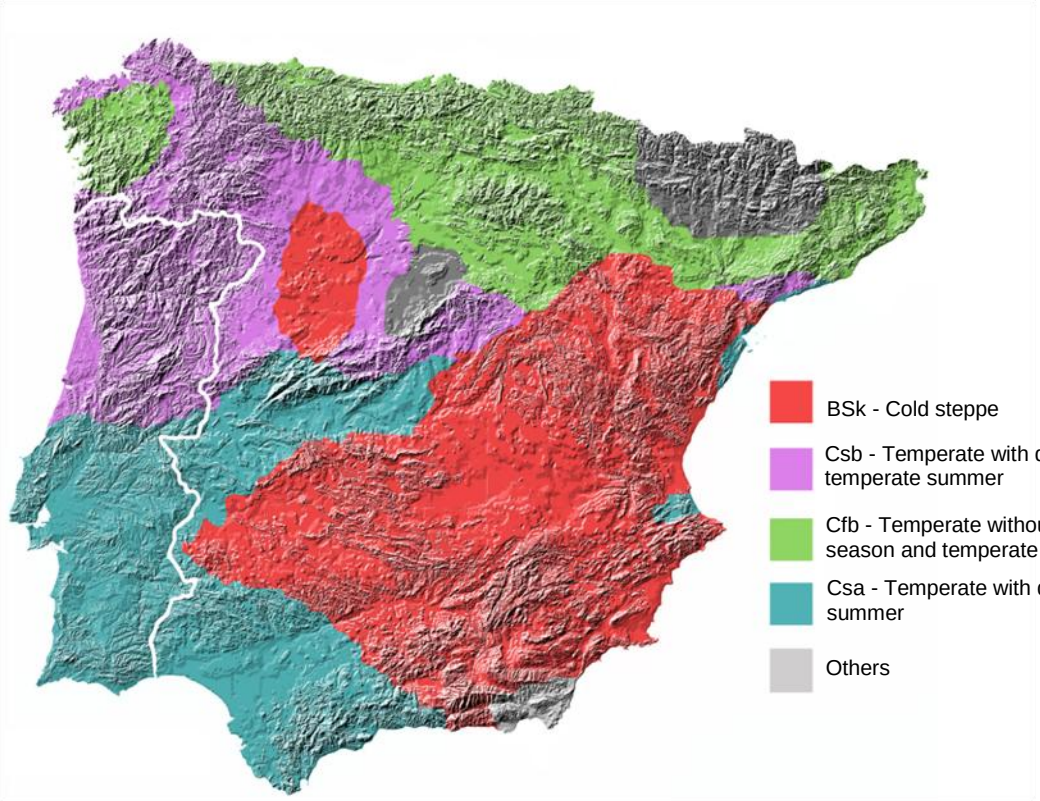


Figure 3. Iberian Peninsula Köppen-Geier climate classification.

Data collection

We collected mortality data of 143 birds that were radio-tracked in several areas of the Iberian Peninsula (Fig.1 and Fig. 2), from 2001 to 2014, over a total period of 13 years. From this overall database, specific datasets were used accordingly with requirements of the different objectives of this study.

In Southwestern Iberia, the capture was made with a stuffed female used as a decoy, with trap loops around it. The individuals that were attracted and fell in the traps were fitted with harnesses with transmitters attached. In Northeastern Iberia, males were also captured with loops and females were captured with the funnel trap method (Ponjoan et al., 2010). Captured individuals were followed on a daily basis in Southwestern Iberia and on a biweekly basis in Northeastern Iberia until the signal was lost or indicated immobility for a considerable period of time.

Assessing mortality causes

To analyze cause-specific mortality we collected data from 139 tagged individuals from several areas of the Iberian Peninsula (Fig. 1). When a tracking signal was lost or indicated immobility for a long period of time, the individual's last position was searched and when a feather spot or carcass were located, the probable cause of death was designated. The cause of death was always accessed by

recovery of the carcass or by local evidences, and categorized as human-caused or natural. We defined probable natural deaths as ecological and environmental pressures (e.g. predation or starvation) and probable anthropogenic deaths as direct human-caused deaths (e.g. hunting or collision). For example, if only the harness was found with bullet marks on it, or the individual was found with dog bite marks, the cause was considered to be hunting. If the bird was found underneath overhead transmission power lines or dead in a road, the registers were collision with power lines or cars. Censored individuals were not considered to be mortalities (Pollock and Winterstein, 1989). Probable deaths were therefore classified as one of the following causes: predation, collision with overhead transmission power lines, vehicle collision and hunting. Only the former was considered natural causes, and it was possible to distinguish predation by birds of prey from predation by mammals. When in the presence of broken carcass bones, or feathers still attached to the remains, the probable predator was considered to be a mammal. In contrast, if the carcass was left whole, with feathers around it, a bird of prey was the probable predator. The individuals status was set to censured when in doubt of the individual's death. The day of death/disappearance was assumed to be 50% of the time between the date of the last register alive and the date when the carcass was found.

Factors driving natural deaths

To analyze natural mortality we used a subset of 67 adult Little Bustards mainly from two regions of the Iberian Peninsula: Alentejo/Extremadura and Catalonia/Aragón, from now on referred as Southwestern Iberia (1) and Northeastern Iberia (2) (Fig. 2). On the contrary of the former dataset, that also included individuals that died from anthropogenic causes, this dataset only included individuals which have died from natural causes or had been lost to follow-up. From 2001 to 2013, between April and mid-July, the individuals were marked and followed with different tracking technologies (Table 1).

Table 1. Means and standard deviations of the number of birds, tracking days per region and tracking methods.

Region	n	Tracking days	Males	Females	VHF Tracking	PTT Doppler	PTT GPS
Northeastern Iberia	41	26270	13	28	26	0	15
Southwestern Iberia	26	9415	23	3	0	7	19
μ	33,5	17842.5	18	15.5	13	3,5	17
σ	10.61	11918.3	7.1	17.7	18.4	4.9	2.8
Total	67	35685	36	31	26	7	34

Radio telemetry is used to track individuals in real time, from distance. Each individual is attached with a transmitter that sends very high frequency (VHF) signals that can be identified in the site with antennas and receivers. It is a relatively low cost, well tested technique which uses lightweight transmitters that can remain active for extended periods of time. By recording and mapping the

animal's location from multiple angles, its position can be estimated with triangulation rules. Although VHF signals travel enormous distances, they can be blocked due to topography, water or heavy vegetation (Poole, 2003). This technology also depends on the person's ability to work with the antennas and receivers and the effort that is made to locate the animal. Without that, the technique has low performance and can easily mislead the study. PTT Doppler satellite telemetry uses platform transmitter terminals (PTTs) to emit ultra-high frequency signals that are identified by polar orbiting meteorological satellites with Argos systems attached. This satellites sweep the earth surface registering the PTTs signals. An animal location is determined by the perceived change in frequency that results from the movement of a transmitter and receiver - like in the Doppler effect. This technology can obtain locations variable error between 250 and 2000 m, depending on the quality of the location (Collecte Localisation Satellites, 2014). PTT GPS technology combines the calculation of locations via Global Positioning System (GPS) and the transmission of this information to the Argos receivers on meteorological satellites. A GPS location has only a few meters of error and usually a high frequency data acquisition.

Data analysis

Capturing and handling of birds can lead to capture myopathy (Williams and Thorne, 1996). The Little Bustard is particularly susceptible to myopathy (Marco et al., 2006; Ponjoan et al., 2008). This disease can affect the bird's mobility up to 11 days after capture (Ponjoan et al., 2008) and consequently turning it vulnerable to predation. To avoid confounding effects between natural deaths and capture myopathy, the period of time between capturing handling and releasing of the birds was reduced to a minimum and all bird mortality that occurred up to 25 days after capturing and tagging, was eliminated.

Data homogenization is also an important step to reduce bias. Since we were dealing with different data collection methods, there was a need to adapt the data grain. PTT GPS daily tracking data was thereby transformed in biweekly data so we could analyse it conjointly. All the in-between daily locations were deleted.

Testing the influence of tracking devices

Because we used VHF tracking, PTT Doppler and PTT GPS tracking technologies in this study, it was essential to test for survival differences between birds followed with different technologies. To access that, we carried out a Kaplan-Meier analysis (Kaplan and Meier, 1958), and the Log-Rank Test (Harrington and Fleming, 1982) was used to compare the distributions. To do this analysis, we used data from Northeastern Iberia, as it was the only area with representative numbers of GPS and radio-tracking technologies (Table 1).

Transmitter's weight can also influence animal survival, and consequently can potentially affect the study final results (Wilson and McMahon, 2006; Brooks et al., 2008; Casper, 2009). Backpack

transmitters, like the ones used in our study, have the advantage of not affecting the bird's balance (Irvine et al., 2007). In relation to transmitter weight, it is recommended it be under 5% of the body weight for harness mounts (Kenward, 2001). The medium weights of the backpacks was of 4,54 (σ ±0.69) of the weight of the bustards. To test whether the weight of the technology influenced the survival of the tracked birds we also used Kaplan-Meier estimate and the Log-Rank Test.

Assessing mortality causes

Descriptive statistics was used to identify the relative prevalence of anthropogenic and natural deaths in the population. The Heisey and Fuller estimator (Heisey and Fuller, 1985) was used to compute annual mortality rates of the Little Bustard for each type of causing factor, using the software MICROMORT 1.3 (Heisey and Fuller, 1985). We evaluated the hunting indirect pressure by disturbance, testing if the mortality frequencies of the different death causes were affected by the hunting season. The seasons were divided based on hunting periods in the Iberian Peninsula: hunting season (1 September - 28 February) and non-hunting season (1 March - 31 August), based on knowledge of legal hunting periods on land within the study areas. The frequencies were computed excluding all individuals whose exact death day was not known, to avoid bias related with hunting seasons. To compare mortality frequencies we used the Fisher's exact test, using the *fisher.test* function in R software. We also divided the data between sexes to test if they were differently affected by both seasons, comparing mortality frequencies in and out of the hunting periods.

Factors driving natural deaths

We selected four variables that could have influence on Little Bustard's survival: sex, season, land cover and bioclimatic region.

The year was divided in three different periods of four months each: Breeding (March to June), Post-breeding (July to October) and Winter (November to February). This divisions were made to comprehend the season periods of Southwestern Iberia and Northeastern Iberia, since there is a small difference in the start and end dates between the two regions.

Land use data was obtained from Corine Land Cover (European Environment Agency) and processed in Geographic Information System (GIS) using Quantum GIS 2.0 (Quantum GIS Development Team 2012).

To find the estimates of the survival function for the environmental variables that possible influence Little Bustard's survival, multivariate statistical analyses was performed. We quantified mortality risks for the Little Bustard with the Extended Cox Proportional Hazards Model for Time-Dependent Variables (Andersen and Gill, 1982; Therneau and Grambsch, 2000; Kleinbaum and Klein, 2005)

using the *survival* package (Therneau and Grambsch, 2000; Therneau, 2014) of the statistical programming language R (R Development Core Team 2014). We tested for an effect of sex, land use, season and bioclimatic regions, using those factors as covariates on the global model. *Dredge* function from the MuMIn package (Barton, 2014) was used to generate a set of models with all combinations of the terms in the global model. We compared the relative performance of the alternative models using Akaike's Information Criterion (AIC; Akaike, 1974; Burnham and Anderson, 2002) and chose models with $\Delta AIC < 5$ (Burnham et al., 2010). Full model averaging based on AIC (Burnham and Anderson, 2001; Lukacs et al., 2009) was performed and the importance of the covariates was estimated. To deal with ties between events (Xin et al., 2013), the Efron approximation was used for being more accurate and computationally efficient than the usually used Breslow method (Therneau and Grambsch, 2000). To visualize the main patterns in the averaged model, fitted values were plotted against each of the explanatory variables in the model. As all variables were categorical, the mean and standard errors of the fitted values were estimated for each category. Prior to model selection, we assessed to which extent the assumption of proportional hazards was met, using *cox.zph* function (Grambsch and Therneau, 1994).

RESULTS

Assessing mortality causes

The weight of the tracking device did not affect the survival of Little Bustards (χ^2 = 0.6, 1 df, p=0.436), neither did the tracking method (χ^2 = 0, 1 df, p=0.926).

We documented 62 mortality events from all causes over the course of the study, after censoring individuals whose transmitter stopped working. Anthropogenic death causes represented 41.9% of the Little Bustard's detected mortality (Fig. 4). Hunting was the most common source of anthropogenic mortality (32.3%), followed by power line collision (6.5%) and vehicle collision (3.2%). The only identified natural cause of mortality was predation, which was responsible for 58.1% of the registered mortality. Birds of prey were the main identified predators with a presence of 55.6%, and mammals correspond to 38.9% of the predation events. Cases where the identified cause was predation but in which it was not possible to recognize the predator corresponded to 5.6%.

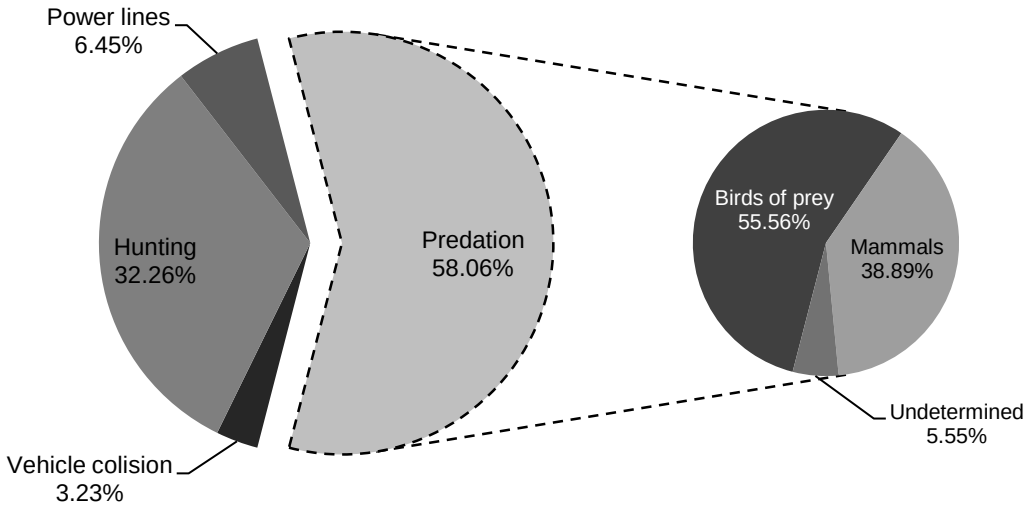


Figure 4. Percentage of the different identified cause specific deaths during the 12 years of study (n=62 individuals).

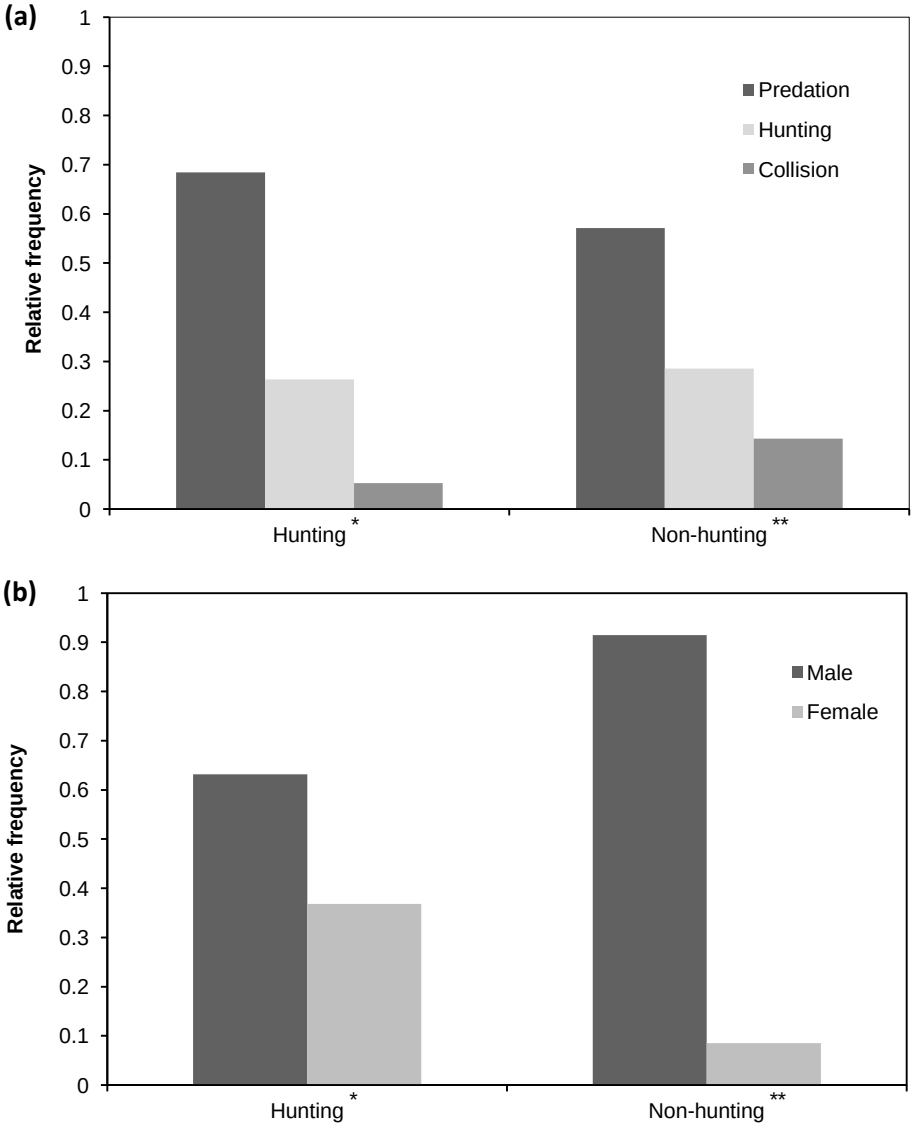
Annual cause-specific mortality rates are presented in Table 2. Yearly, 17.1% of the Little Bustards died from anthropogenic causes, while 15.4% died from predation. Hunting represented 11.7% of the deaths, followed by power line collision with 4.3% a year and vehicle collision with 1.1%.

Table 2. Annual survival and cause-specific mortality rates estimated using Heisey and Fuller method (1985) for the Little Bustard (*Tetrax tetrax*), in the Iberian Peninsula. Annual rates were computed based on a 12 year period (2001 to 2012).

	Death cause	Mortalities	Annual Rates
Survival			0.697
Natural	Predation	36	0.154
Total		36	0.154
Anthropogenic	Vehicle collision	2	0.011
	Hunting	20	0.117
	Power line collision	4	0.043
Total		26	0.171

The mortality frequencies inside and outside the hunting season were computed using a subset of 40 individuals. The cause-specific and sex-specific relative mortality frequencies according to the hunting period are presented in Fig. 5. Predation, hunting and power lines collision were the sources of mortality found. There were no significant differences in the prevalence of death causes between hunting and non-hunting periods ($p=0.718$). Sex and hunting season did not present any correlation between them ($p=0.301$).

Figure 5. (a) Cause-specific relative mortality frequencies for hunting and non-hunting periods (b) Male and female relative mortality frequencies inside and outside the hunting period.



* 1 Sep - 28 Feb

** 1 Mar - 31 Aug

Factors driving natural deaths

According to Burnham et al. (2010), models where ΔAIC is in the 2–7 range have some support and should rarely be dismissed. We have then considered all models with ΔAIC<5 , resulting in a selection of 7 models with Akaike weights summing approximately 99% confidence set (Table 3). Bioclimatic region was the covariate with strongest support, having a relative importance equal to 1 (Table 4), followed by sex, land cover, and finally, season.

Table 3. Summary of Cox proportional hazards models estimating survival rates of the Little Bustard, during the course of the study, 2001-2014. AIC weights sum up to 0.99 and indicate the relative likelihood of a given model being the most appropriate.

Covariates	df	AIC	ΔAIC	w
Region + Sex	4	194.28	0.00	0.39
Region + Land Cover + Sex	6	195.59	1.30	0.20
Region	3	195.68	1.40	0.19
Region + Land Cover	5	197.63	3.35	0.07
Region + Season + Sex	6	197.75	3.47	0.07
Region + Season	5	198.93	4.64	0.04
Region + Land Cover + Season + Sex	8	199.20	4.92	0.03

Table 4. Relative importance of the covariates after model averaging, calculated as the sum of the AIC over all of the models in which the parameter of interest appears.

Covariates	Region	Sex	Land Cover	Season
Relative variable importance	1.00	0.70	0.31	0.14
Nº of containing models	7	4	3	3

The full model averaged regression coefficients showed that the temperate climate with dry or hot summer (Csa), characteristic of Southwestern climate, has a higher relative mortality risk than Cfb, which covers the majority of the Northeastern study area. BSk has the second higher risk of mortality, followed by Csb. The results are expressed in Table 5.

Table 5. Full model-averaged coefficients for factors driving natural deaths.

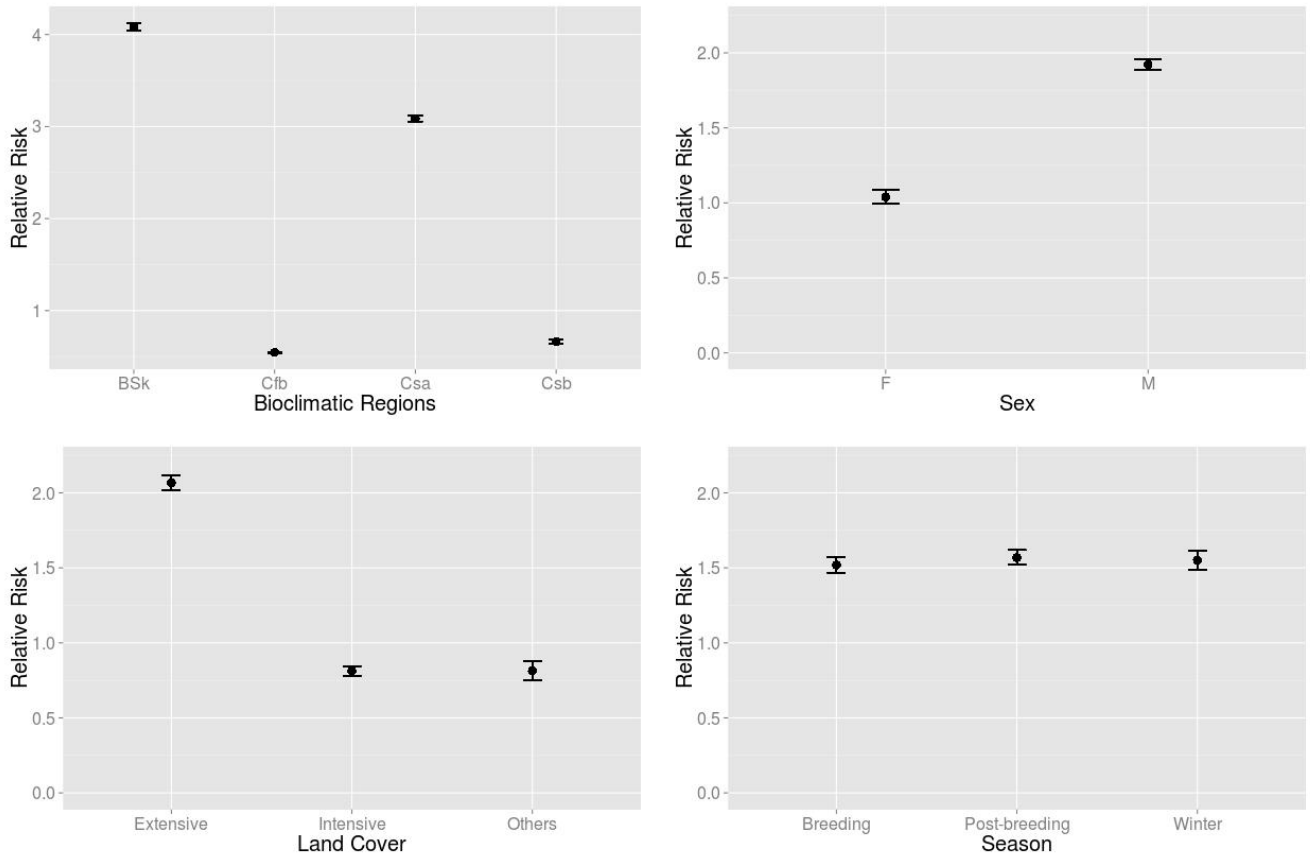
Covariates	Estimate	Std. Error
BSk climate	0	-
Cfb climate	-1.726	1.147
Csa climate	0.410	1.246
Csb climate	-1.570	1.477
Female	0	-
Male	-0.727	0.661
Extensive	0	-
Intensive	-0.243	0.494
Others	0.055	0.283
Breeding	0	-
Post-breeding	-0.015	0.424
Winter	0.055	0.399

Differences were observed between the mortality of females and males. Females seem to have a greater risk of mortality than males. Land cover use did not have a significant influence on mortality, although extensive agricultural land uses showed higher risk of mortality than intensive agricultural

land uses and other land uses. Seasons practically showed no differences between them. Cox proportional hazards assumption was met for every variable (Appendix A).

When plotting the fitted values to visualize the net effect of the variables (Fig. 6), sex was the only variable showing a different tendency than the model coefficients, with males showing a higher mortality than females.

Figure 6. Relative mortality risks of each variable: bioclimatic regions, sex, land cover and season, after doing model average and computing the mean of the fitted values. Standard errors are also plotted.



DISCUSSION

Is anthropogenic mortality sustainable?

Anthropogenic death causes had greater prevalence than anticipated for a protected species, representing ca. 42% of the registered mortality events. Our work estimates that ca. 17% of the Iberian population die every year due to human-caused deaths, which is higher than annual mortality by predation (ca. 15%), the main natural cause of mortality. It should be noticed however that this result only expresses adult annual mortality. Given that only 54% of the chicks survive to became adult (Schulz, 1987), total annual population mortality (considering both juvenile and adult together) will be higher. Productivity is a key demographic parameter for the viability of Little Bustard populations and, if favorable productivity is maintained, the Little Bustard's population may not decline in spite of these estimated mortality rates (Inchausti and Bretagnolle, 2005; Morales et al., 2005a; Delgado et al., 2009). In areas with poor habitat quality like the Northeastern Iberia (Lapiedra et al., 2011), a biased sex ratio towards males occurs (Inchausti and Bretagnolle, 2005; Morales et al., 2005a, 2008), and this results in a decrease in productivity due to female shortage (Tarjuelo et al., 2013). Therefore, degraded sites with populations presenting low breeding productivity such as the ones recorded in Northern Spain and Western France (Inchausti and Bretagnolle, 2005; Morales et al., 2005a; Delgado et al., 2009), are vulnerable due to natural and anthropogenic mortality.

Iñigo and Barov (2010), in the European Union action plan for the Little Bustard, state that the main threats to the species are entirely connected with agricultural practices. Anthropogenic death causes are poorly studied and are mainly attributed to collision with power lines (Silva et al., 2010b, 2014b), with no references to hunting. We found that poaching contributes substantially to the overall mortality; 11.7% of the population dies every year from hunting.

Previous studies estimated that collision with overhead power lines cause an annual mortality of over 1.5% of the Portuguese Little Bustard population (Infante et al., 2005; Silva et al., 2010b). Our work now estimates a mortality almost three times higher, with 4.3% of the population dying per year due to power line collision. We also found that 1.1% die every year by collision with vehicles, which was an unknown threat up to now. However, this estimate was based only on two casualties, and thus requires validation. The Little Bustard has been described to avoid the vicinity of transmission lines (Silva et al., 2010b) as well as roads and human structures (Suárez-Seoane, 2002; Silva et al., 2004; Osborne and Suárez-Seoane, 2006). The ongoing habitat loss and degradation in the Iberian Peninsula and the growing expansion of the power line grid can be forcing the species proximity to these structures.

As for predation, even though birds of prey were responsible for most of the observed cases of mortality caused by predation, this difference was not sufficiently marked to ascertain statistically if they are more important predators than mammals.

There were no differences between mortality frequencies inside and outside hunting periods. This suggests that the Little Bustard is subject to the same amount of pressure by predation, power lines collision and hunting, inside and outside the hunting period. There was also not found a relation between sex and hunting period. From the 40 mortality events recorded, eight were hunting cases found in non-hunting periods, indicating that hunting is occurring also outside the general hunting season, although four of them coincide with the hunting period of *Columba palumbus* and *Streptopelia turtur* in Portugal or the half-closed season in Spain, a hunting season with restrictions that usually starts in mid-August.

Bioclimatic region and sex explain most of the natural mortality

Bioclimatic region is the environmental factor with the greatest influence on Little Bustard's mortality rates, with Csa presenting the higher risk and Cfb the lowest. These two climates are, respectively, the characteristic climates of the Southwestern and Northeastern study areas. The Southwestern grasslands in Iberia (Csa) are dominated by a dry climate with high temperatures in summer, while Northeastern Iberia (Cfb) does not have a marked dry season and has milder temperatures. Higher temperatures associated with low rainfall lead to dryer fields and less food resources, with likely negative impacts on the species. The results corroborate the conclusions of previous studies which found that higher densities of the Little Bustard are associated with higher precipitation, due to increased vegetation growth and arthropod availability (Delgado and Moreira, 2010). Although poorly represented in our data, BSk was also found to be associated to a high mortality risk. It is a dry climate, with mild hot summers and cold winters, supporting the same conclusion. Temperature seems to play a crucial factor in Little Bustard's survival (Delgado et al., 2011). Having a higher surface-to-volume ratio than, for example, the Great Bustard (*Otis tarda*), the Little Bustard is described to have a lower tolerance to extreme temperatures, and is more sensitive to temperature changes (Delgado et al., 2011).

Our results suggest that females have a higher risk of mortality than males. Inchausti and Bretagnolle, (2005) reported a higher annual sex-specific survival rate for males in western France, although the difference was not statistically significant. During the breeding season, females have to spend energy with egg laying and satisfying the requirements of the offspring (Cramp and Simmons, 1980), and in some cases producing a second clutch. Females solely ensure parental care, which extends until after the breeding season (Cramp and Simmons, 1980). As a result, they may reach a lower physical condition, which can lead to a lower tolerance to winter temperatures and a greater exposition to

predators, thus affecting their winter fitness. Males, on the other hand, have time to store the energy needed to migrate to the wintering sites after breeding. We did not find cases of female deaths due to crop harvesting. Fitted values show a opposite pattern compared with the model averaging coefficients (for a similar case, see Águas et al., (2014)), probably because there is a confounding effect between this variable and the bioclimatic region. In fact, only 12% of the tagged individuals in the Southwestern Iberia (where the mortality risk is higher) were females, in contrast with the Northeastern Iberia, with 68% tagged females.

The habitat in Northeastern Iberia has experienced, since the mid-thirties, agricultural intensification and a decrease of fallow land; its Little Bustard population is experiencing a decline (Lapiedra et al., 2011). On the contrary, the Southwestern area presents better preserved habitats. In spite of the higher mortality risk associated with a drier and hotter climate, it maintains some areas with high Little Bustard densities (García de la Morena et al., 2006; Silva and Pinto, 2006). Areas with low habitat quality are usually associated with populations with a sex ratio biased towards males, while the sex ratio in regions with good habitat tends to be balanced (Silva et al., 2014a). The decline of this species in low habitat quality sites was, until now, attributed to crop harvesting and lack of food resources for the offspring (Inchausti and Bretagnolle, 2005). Adding to that, females have to expend more energy to feed and are consequently more vulnerable than males. We hypothesize that in Northeastern Iberia, the low fecundity rate and overall reproductive success (Tarjuelo et al., 2013) does not compensate natural and anthropogenic mortality.

Land cover had a lower impact on survival when compared with both bioclimatic region and sex. Intensive agriculture is believed to be the main cause of the Little Bustards decline (Goriup, 1994; Morales et al., 2005b, 2006b; García et al., 2007), and thus it was expected that land cover types associated with agricultural intensification, such as intensive farmland mosaics, would have a higher associated mortality risk. Nevertheless, we did not identified this pattern. Although extensive agriculture is crucial during the breeding season, intensive agriculture may provide abundant food availability that could be important for post-breeding and winter survival, especially during drought years. In the Southwestern Iberia, in these seasons, the Little Bustard performs movements from non-irrigated farmland to more intensive agricultural sites, some of which with irrigated land uses (Silva et al., 2007). The risk of mortality could eventually not differ between extensive and intensified sites, however there could be a relation between the effect of land use and season.

Season has a small impact on survival of the Little Bustard, having the least importance in the final model, with a maximum difference between relative mortality risks of 0.015. However, it is possible that a regional confounding effect may be affecting the results. The study was conducted at an Iberian Peninsula scale, and thus it is expected that the Southwestern study area, being the hottest and driest

region, has a higher mortality in summer, and therefore seasons could have a more evident effect at a regional scale. In the Northeastern Iberia, the climate is not as dry and hot, being the seasonal effect less evident.

Data limitations and assumptions

It is important to note that we are assuming that the identifications of the causes of death are reliable, although it is a probable death cause, with absence of necropsy. In addition, we also assume that the probability of detecting anthropogenic death is the same as in detecting natural death.

To examine population dynamics, we rely on marked individuals. We have shown that different tracking technologies and transmitter weights under 6.76% of body weight did not influence Little Bustard survival. We want to note that, although we tried to eliminate all bias related to tagging and tracking techniques, we cannot distinguish survival between marked individuals and non-marked individuals.

Conservation implications

Our work shows how poorly understood and previously unknown threats are affecting the survival of the most important western Little Bustard European population. Reducing anthropogenic mortality can have a major impact on the species viability particularly with populations that show low breeding productivity. Awareness campaigns and law enforcement at key conservation areas could reduce poaching, that alone affects almost 12% of the population annually.

Anthropogenic caused deaths seem to have higher importance than what was initially foreseen in this species, which has a threatened conservation status and is subjected to conservation measures. A tighter control during hunting seasons, together with the enforcement of hunting legislation and possibly the creation of additional no hunting zones in classified areas, should be major contributions to the decrease of anthropogenic mortality. Low awareness of the species conservation status is a problem among the general population and hunters, and should be promoted.

The unexpected high impact of collision with transmission power lines, highlights the importance to adapt the overhead electric power line network to conservation needs. This may include the relocation of existing hazardous power lines and the routing of new ones away from areas with greater collision risk (Silva et al., 2014b). New power line designs, that minimizes collision, can also be considered (Silva et al., 2010b, 2014b; Alcazar, 2013). Legislation should be drawn up at national level to ensure the integration of these preventive measures into the design of new power lines nationwide and not limited to the Natura 2000 network.

Taken together, our results suggest that climate and sex are the main factors affecting Little Bustard's survival. This species decline may be accentuated by the recent changes in climate, characterized by decreasing precipitation, temperature rise and frequency of heat waves. We hypothesize that in addition to rainfall, temperature could be affecting Little Bustard's survival, and that should be considered in future survival studies. Survival modeling under different climatic conditions could be the next step for understanding the Little Bustard climatic requirements.

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DISCUSSION

Conservation and development of management programs on threatened bird species, as well as species recovery plans, crucially benefit from information derived from survival studies on free-ranging animals (e.g., Lebreton et al., 1992; Beissinger and Westphal, 1998; McCallum, 2000; Reed et al., 2002; Le Gouar et al., 2008). These kind of studies is key to assess the relevance of the main threats. It can also provide a valuable understanding of population dynamics, as the probability of survival may vary with individual characteristics and as a function of biotic and abiotic environmental variables (Lebreton et al., 1992).

Survival data require specialized analytical techniques, and field studies are not always implemented to take into account all the assumptions associated with this kind of analysis. Accordingly, an important concern is that survival rates derived from ecological studies have associated bias and lack of precision (McCallum, 2000; Fox, 2001; Winterstein et al., 2001; Williams et al., 2002; Zens and Peart, 2003). An effort should be made to improve the planning of field work, especially to obtain a more precise time of death. This could be achieved by increasing the field work effort when searching for carcasses, or by using tracking devices with accelerometers and sensors that measure physiological parameters (Grant et al., 1971; Butler and Woakes, 1979). The development of new, lighter and low cost devices it is also essential to obtain more data, since the overall available devices are expensive, not allowing the tagging of a large number of animals.

Telemetry is the most reliable technology used in wildlife survival estimation, but has some constrains that turn robust survival estimation in wildlife studies challenging (Murray, 2002). It is a common practice to assume that transmitters do not affect subject survival, and consequently adding bias to the study (White and Garrott, 1990; Winterstein et al., 2001). In our case-study we managed to test the influence of transmitter weight, tracking method and eliminate the presence of deaths by myopathy. Transmitter weight under 6.76% of body weight and the use of different tracking methods did not have influence on Little Bustard's survival, not adding bias to the study. Probable deaths by myopathy where rejected from the study.

Telemetry studies have user defined frequency of monitoring observations, which can lead to uncertainty in the timing of the death event. Daily or biweekly monitoring should be adequate for unbiased survival studies, while greater time intervals make it unreliable (Murray, 2002). As we used data from multiple sources and consequently with different frequencies of monitoring observations (daily and biweekly), we had to homogenise the data to the lowest frequency. This was a good compromise and allowed us to standardize the data to a greater scale, and also qualify it, by removing cases where the data acquisition had errors .

Recently, wildlife survival estimation had significant improvements in the development of software for the analysis of complex survival functions (Williams et al., 2002). Although there are many available software products that perform survival analysis, such as MARK (White and Burnham, 1999), SAS (Allison, 1995), SPSS (IBM Corp, 2013), XLSTAT-Life (Addinsoft, 2014) or Stata (StataCorp, 2009), R (R Development Core Team 2009) is a free and open-source software that allow for high data flexibility and provides interfaces to other programs such as QGIS (Quantum GIS Development Team 2012) or GRASS-GIS (Neteler and Mitasova, 2008). It also provides the user with very active and engaged community that provides high quality support, allowing the user to solve conceptual problems quicker.

Overall, survival analysis using Cox proportional hazards model was successfully applied to an ecological case-study and proved capable of accommodating problems resultant from the estimation of survival rates of free-ranging animals like time-dependent covariates, tied events and interval-censored deaths. We found that bioclimatic region is the most important variable influencing Little Bustard's survival, higher mortality being associated with dryer climates and higher temperatures. This knowledge might be crucial for planning future studies, focused on climatic effects on this species. We also found that females have a higher risk of mortality than males. This is probably due to energy carry over effects of the breeding and post-breeding season, given that parental care is provided solely by the female (Cramp and Simmons, 1980). This problem is possibly aggravated in low habitat quality areas, where habitats do not provide the sufficient amount of food in post-breeding season (Silva et al., 2007). Although it was initially foreseen that land use was the main driver of survival, this was not confirmed. Dispersion due to inadequate habitat leads to an extra energy expense that can lead to higher mortality probability. Season was the factor with the least impact in mortality.

Cause-specific mortality rates were also successfully computed using Heisey and Fuller estimator (Heisey and Fuller, 1985), with MICROMORT 1.3. The software was found to have low flexibility and provide a difficult graphical interpretation. We found that anthropogenic mortality has almost the same impact as mortality by natural causes, probably not being sustainable at short-term, especially when associated with low productivity. Hunting was also found to be the main direct human-caused threat, followed by collision with power lines, with a much higher prevalence than what was initially expected.

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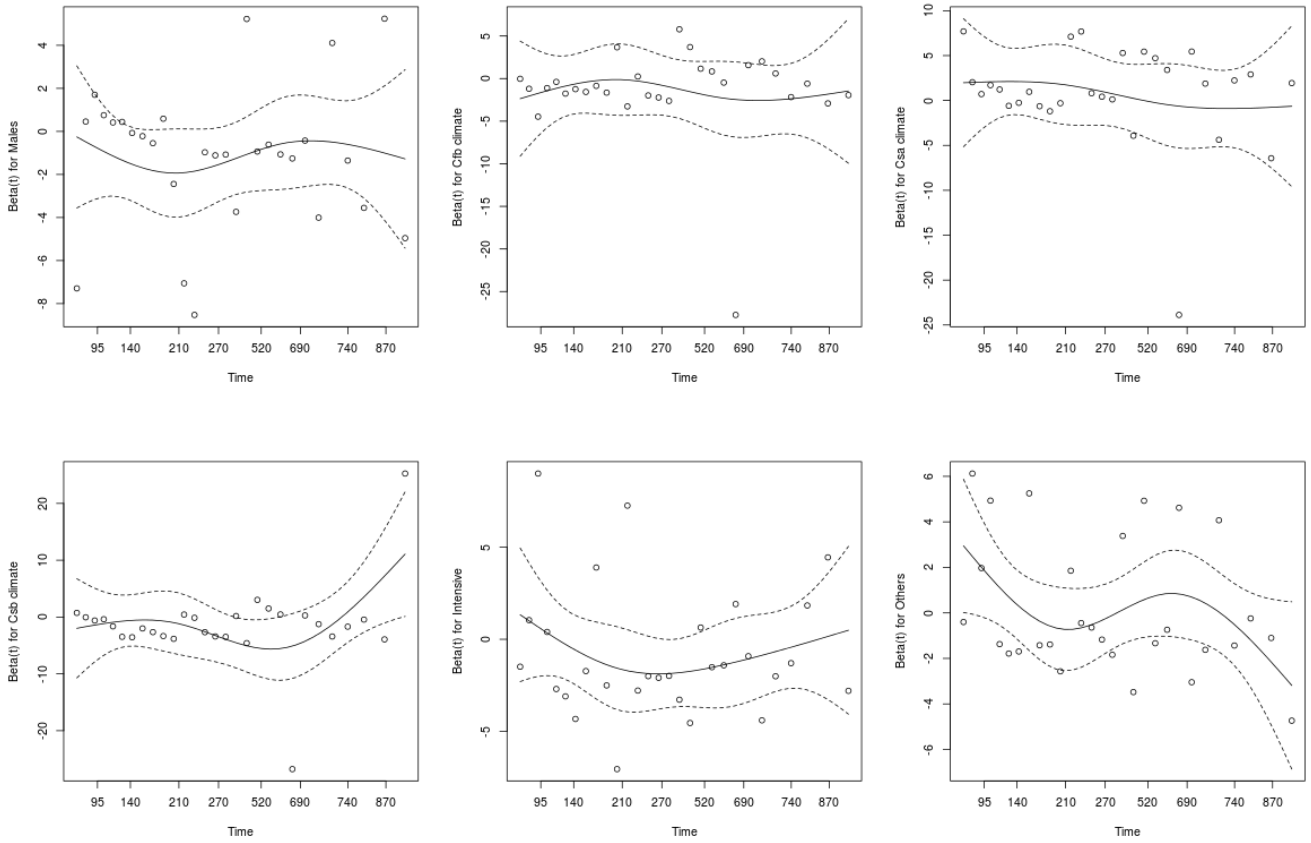
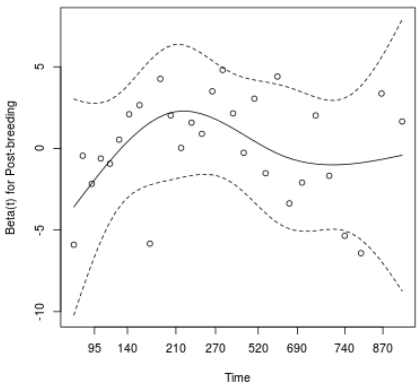
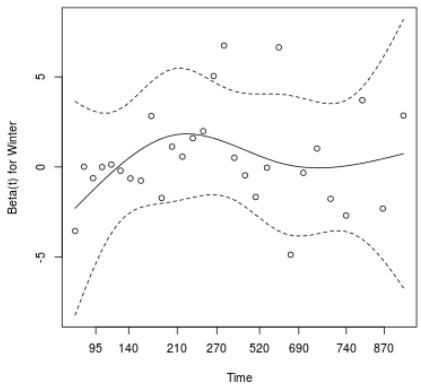
APPENDICES

Appendix A. Proportional hazards assumption results for the Cox regression model fit. The column rho is the Pearson product-moment correlation between the scaled Schoenfeld residuals and log(time) for each covariate. The last row contains the global test for all the interactions tested at once. The residuals for individual predictors are plotted below.

Covariates	rho	chisq	p
Male	0.048	0.076	0.783
Cfb climate	-0.073	0.123	0.726
Csa climate	-0.199	0.908	0.341
Csb climate	0.159	0.587	0.444
Intensive	-0.054	0.094	0.759
Others	-0.272	2.583	0.108
Post-breeding	0.010	0.001	0.976
Winter	0.090	0.059	0.809




Beta(t) for Post-breeding
5
0
-5
-10
95
140
210
270
520
690
740
870
Time


Beta(t) for Winter
5
0
-5
95
140
210
270
520
690
740
870
Time