UNIVERSIDAD DE CÓRDOBA

DEPARTAMENTO DE ZOOLOGÍA

Recovering a keystone species in a biodiversity hotspot: the European Rabbit (*Oryctolagus cuniculus*) in Sierra Morena (Spain)

TESIS DOCTORAL

José Manuel Guerrero Casado

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Recuperación de una especie clave en un área de alta biodiversidad: el caso del conejo europeo (*Oryctolagus cuniculus*) en Sierra Morena (España)

Recovering a keystone species in a biodiversity hotspot: the European Rabbit (*Oryctolagus cuniculus*) in Sierra Morena (Spain)

TESIS DOCTORAL

Doctorando: José Manuel Guerrero Casado

VºBº Director: Francisco Sánchez Tortosa

CÓRDOBA, 2013

TÍTULO DE LA TESIS: Recuperación de una especie clave en un área de alta biodiversidad: el caso del conejo europeo (*Oryctolagus cuniculus*) en Sierra Morena (España).

DOCTORANDO/A: José Manuel Guerrero Casado

INFORME RAZONADO DEL/DE LOS DIRECTOR/ES DE LA TESIS

La tesis se ha enmarcada dentro del Proyecto de Mejora de Hábitat del Buitre Negro en la Provincia de Córdoba, ejecutado por la Consejería de Medio Ambiente de la Junta de Andalucía. El objetivo del proyecto era recuperar las poblaciones de conejo a gran escala mediante la construcción de cercados donde los conejos eran liberados y la mejora de hábitat alrededor de los mismos. Esta tesis ha mejorado sensiblemente varios aspectos relativos a esta técnica que permite recuperar las poblaciones de conejo a gran escala. Debido a que la escasez de conejo es uno de los mayores problemas de conservación en España, los resultados son de gran utilidad para futuros programas de recuperación.

Como paso previo, el primer capítulo de esta tesis hace una revisión exhaustiva del conocimiento necesario para mejorar el éxito de las repoblaciones conejos, siendo una revisión muy útil para futuras repoblaciones. Esta revisión ha sido recientemente aceptada para su publicación en la revista *Animal Biodiversity and Conservation.*

Los dos siguientes capítulos de la tesis, tratan de identificar qué factores mejoran la producción de conejo en dichos cercados de repoblación, obteniéndose importantes resultados con gran aplicación práctica, los cuales han derivado en la publicación de dos artículos en las revistas *Acta Theriológica* (Vol. 58: 415-418) y *World Rabbit Science* (Vol. 21: 193-199).

El capítulo 4 pretende identificar qué factores influyen en el establecimiento de las poblaciones de conejo alrededor de los cercados, evaluando la eficacia de estas como método para establecer poblaciones de conejo a gran escala. Los resultados son de gran utilidad para los programas de recuperación de especies amenazadas como el lince ibérico o el águila imperial que son llevados a cabo en Sierra Morena, los cuales pretenden recuperar las poblaciones de conejo, ya que estos depredadores dependen del conejo para su supervivencia. Este capítulo ha sido aceptado para su publicación en *Journal for Nature Conservation*.

Finalmente, como la tesis se enmarca en un proyecto para la conservación del buitre negro, en el último capítulo se han identificado qué factores son importantes para la colonia de buitre negro en la Sierra de Hornachuelos, creando un mapa potencial para su futura dispersión. Este mapa es de gran utilidad para acciones locales de manejo y conservación de esta especie. El capítulo ha sido recientemente aceptado para su publicación en la revista *Bird Study*.

Por lo tanto, debido a que todo el contenido de la tesis ha sido publicado o aceptado en revistas científicas indexadas en el *Journal Citation Report*, considero que la tesis cumple todos los requisitos de calidad para su presentación.

Por todo ello, se autoriza la presentación de la tesis doctoral.

Córdoba, 28 de Octubre de 2013

Firma del/de los director/es

Fdo.: Francisco Sánchez Tortosa

Esta tesis ha sido presentada como compendio de publicaciones. Los siguientes trabajos han sido publicados en revistas incluidas en los tres primeros cuartelis según el *Journal Citation Reports*:

Título: *The short-term effect of total predation exclusion on wild rabbit abundance in restocking plots*
Autores: Guerrero-Casado, J., Ruiz-Aizpurua, L., Tortosa, F.S.
Revista: Acta Theriológica. Vol. 58: 415–418. 2013.
Base de Datos: Journal Citation Reports.
Área temática: Zoology
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Lugar que ocupa/nº Revistas del Área Temática: 85 / 151

Título: *Factors affecting wild rabbit production within extensive breeding enclosures: How we can optimize efforts?*
Autores: Guerrero-Casado, J., Ruiz-Aizpurua, L., Carpio, A.J., Tortosa, F.S.
Revista: World Rabbit Science. Vol. 21: 193. 2013.
Base de Datos: Journal Citation Reports.
Área temática: Agriculture, Dairy & Animal Science.
Índice de impacto (2012): 0.62
Lugar que ocupa/nº Revistas del Área Temática: 34 / 54

Título: *Restocking a keystone species in a biodiversity hotspot: recovering the European rabbit on a landscape scale*
Autores: Guerrero-Casado, J., Carpio, A.J., Ruiz-Aizpurua, L., Tortosa, F.S.
Revista: Journal for Nature Conservation, 2013.
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Base de Datos: Journal Citation Reports.
Área temática: Biodiversity Conservation
Índice de impacto (2012): 1.535
Lugar que ocupa/nº Revistas del Área Temática: 18 / 40

Título: *Modelling the nesting-habitat of the cinereous vulture (Aegypius monachus) on a fine scale for conservation purposes.*
Autores: Guerrero-Casado, J., Arenas, R., Tortosa, F.S.
Revista: Bird Study, 2013.
http://dx.doi.org/10.1080/00063657.2013.849657
Base de Datos: Journal Citation Reports.
Área temática: Ornithology
Índice de impacto (2012): 1.017
Lugar que ocupa/nº Revistas del Área Temática: 10 / 22

Para todo aquel aficionado a monterías que no haya visitado Sierra Morena, debe hacer cuanto le sea posible para conocer tan hermosas cuanto accidentadas montañas (…) Aquello es un pequeño Pirineo; sus continuos precipicios, sus fragosísimos barrancos seguidos de inaccesibles y escarpadas alturas, aquellos terroríficos cauces del Névalo, Benaljarafe, Bembézar, Pajarón y otros, erizados de enormes y picudos peñascos, cautivan mi alma, y desde que allí puse los pies no quiero otro cazadero".

Antonio Covarsí, Trozos venatorios y prácticas cinegéticas (1911).

Un monte sin conejos es un espacio yermo donde linces y águilas imperiales no tienen cabida, del mismo modo que campiñas pobladas con perdices de colores y sierras profanadas por quimeras por cuernos hipertrofiados alertan del peligro inminente de la extinción del cazador responsable.

Juan Mario Vargas, Perdices de colores.

GENERAL INDEX

ABSTRACT

The European rabbit (*Oryctolagus cuniculus*) is a multifunctional keystone species in Mediterranean ecosystems, with an additional high economical value as a consequence of the hunting activities. However, the rabbit population in Iberia has undergone a sharp decline as a consequence of habitat loss, over-harvesting, and the outbreak of myxomatosis in the 1950's decade and rabbit haemorrhagic disease in the late 1980's. A huge conservation effort has therefore been made to boost wild rabbit populations, notwithstanding no method for the widespread recovery of wild rabbit populations in the long-term has yet been developed. This is a major concern in these areas in which the wild populations remain at very low densities and their endangered predators still coexist (such as in Sierra Morena, sourthen Spain), and the development of a management tool that will permit the recovery of wild rabbit populations in these areas is therefore urgent. In the light of these considerations, the principal aim of this thesis is to assess wether the restocking of rabbits in extensive enclosures combined with habitat management around them for further colonisation can be considered as effective tools to promote new wild rabbit populations on a large-scale, both temporally and spatially.

As a previous step, the scientific knowledge on rabbit restocking was reviewed with the aim of summarising all the factors that affect restocking success, highlighting those that improve overall success and establishing future research perspectives in accordance with the IUCN guidelines for re-introduction.

The first two experimental works attempt to identify which factors enhance the abundance of wild rabbits in these restocking enclosures. In this respect, this thesis shows that rabbit abundance in the first weeks after release can be improved by excluding aerial predation through the use of roofed enclosures. Over a longer period, the rabbit

abundance/production in these enclosures can be significantly enhanced by selecting plots with appropriate cover availability and structures in which to built warrens, and it would be advisable to concentrate the restocking effort by ensuring that the restocking plots are close to each other, thus avoiding isolated enclosures in order to scatter the impact of aerial predation.

In turn, the following chapter shows that restocking in enclosures combined with an intensive habitat improvement around them can be considered as an effective tool with which to promote wild rabbit populations on a large-scale, since rabbit abundance increased significantly after release, and some areas managed attained the threshold value needed to support stable lynx presence in Sierra Morena. However, rabbit abundance was strongly influenced by scrub coverage and the habitat management treatment, and it would be advisable to increase habitat complexity and heterogeneity through the use of artificial warrens and brush piles in low cover areas, and to create pastures patches via scrub clearing in dense scrubland.

All the actions involved in this thesis are included in a program to improve the habitat for the Cinereous Vulture. The last chapter of this thesis tries to identify which factors affect the Cinereous Vulture nesting-habitat selection, creating a fine scale predictive map (50-m resolution) for local conservation applications. Nests of Cinereous Vultures were found to be located farther from roads, villages and the edge of vegetation patches, in large patches containing high cork oak cover with steep slope and lower solar radiation. Less than 8 % of the study area was predicted to be suitable as regards these criteria potentially allowing critical conservation areas to be identified. If a better conservation benefit is to be ensured, Cinereous Vulture conservation programmes should consider both the strict protection of their breeding habitats and the recovery of wild rabbit populations (and their potential habitat) in the foraging area.

RESUMEN

El conejo europeo o conejo de monte es una especie clave en los ecosistemas mediterráneos, a lo cual se le añade un gran valor económico proveniente de la actividad cinegética. Sin embargo, las poblaciones de conejo en la Península Ibérica han sufrido un acusado declive como consecuencia de la pérdida de su hábitat óptimo, la sobreexplotación cinegética y la aparición de la mixomatosis en la década de los 50 y posteriormente la enfermedad hemorrágico vírica a finales de los 80. Por lo tanto, un gran esfuerzo conservacionista se está llevando a cabo para mejorar las poblaciones de conejo, a pesar de la cual no hay un método que permita la recuperación del conejo a gran escala. Esto es uno de los mayores problemas de conservación en aquellas áreas en las cuales las poblaciones de conejo permanecen a bajas densidades y sus depredadores amenazados todavía existen (como es el caso de Sierra Morena, sur de España), y por lo tanto es necesario el desarrollo de una herramienta de gestión que permita la recuperación del conejo en estas regiones. De acuerdo con esto, el principal objetivo de esta tesis es evaluar si las repoblaciones de conejo en los cercados combinado con el manejo del hábitat alrededor de los mismos para favorecer la colonización por parte de los conejos podría ser una medida efectiva para promover nuevas poblaciones de conejo a gran escala y a largo plazo.

Como paso previo, el conocimiento científico sobre las repoblaciones de conejo fue revisado con el propósito de resumir todos los factores que influyen en el éxito de las repoblaciones, destacando todos aquellos que mejoran la supervivencia de los animales liberados. Además se establecieron las nuevas perspectivas de la investigación sobre esta materia de acuerdo a las directrices de la UICN.

Los dos primeros experimentos versan sobre los factores que mejoran la abundancia de conejo en los cercados de repoblación. En primer lugar, esta tesis muestra que la abundancia de conejo durante las primeras

semanas tras la suelta puede ser mejorada a través de la exclusión de la depredación mediante el uso de cercados techados. En un período de tiempo más largo, la abundancia o producción de conejo en estos cercados se puede mejorar significativamente seleccionando lugares con una cubierta de matorral adecuada y diversas estructuras que permitan la construcción de madrigueras, y es totalmente recomendable concentrar los esfuerzos asegurándose de que todos los cercados de repoblación estén cercanos entre sí, evitando la construcción de cercados aislados con el fin de diluir el impacto de la depredación aérea.

El siguiente capítulo muestra que las repoblaciones en estos cercados combinado con un intensa mejora del hábitat alrededor de los mismos puede ser considerada una herramienta efectiva mediante la cual establecer poblaciones de conejo a gran escala, ya que la abundancia de conejo incrementó considerablemente tras las repoblaciones, alcanzándose en algunas de las zonas intervenidas el umbral de densidad de conejo necesario para soportar poblaciones estables de lince ibérico en Sierra Morena. Sin embargo, es importante resaltar que la abundancia de conejo está fuertemente influenciada por la cobertura de matorral y el tratamiento de mejora de hábitat realizado, siendo recomendable el aumento de la complejidad y la heterogeneidad mediante el uso de madrigueras artificiales y cúmulos de ramas prudentes de la podas en áreas con escasez de refugio y la creación de pastizales en manchas densas de monte a través del desbroce del matorral.

Debido a que todas las acciones includias en estas tesis están enmacardas en el *Proyecto para la mejora de hábitat del buitre negro en la provincia de Córdoba*, el último objetivo de esta tesis es identificar qué factores caracterizan el hábitat de nidificación del buitre negro, creando un mapa predictivo a fina escala (50-m de resolución) para su aplicación en los programas locales de conservación. Los nidos de buitre negro se encuentran en lugares apartados de carreteras, pueblos y del borde del parche de vegetación donde están ubicados, en grandes parches de vegetación con una alta cobertura de alcornoque y en zonas con pronunciadas pendientes y baja insolación, ubicándose los nidos agrupados entre sí.

Menos del 8 % fue identificada como potencialmente apropiada para el establecimiento de futuras parejas reproductoras, siendo necesaria su estricta protección. Por lo tanto, para conseguir un mejor estatus de conservación, los programas de conservación del buitre negro deberían conservar tanto las áreas de nidificación así como recuperar las poblaciones de conejo (y su hábitat potencial) en el área de campeo de la especie.

INTRODUCTION

INTRODUCTION

BIOLOGY OF THE SPECIES: A PLASTIC SPECIES

The European rabbit or wild rabbit (*Oryctolagus cuniculs* L.) is a successful coloniser, owing to its great ability to adapt to different environments, which makes the rabbit an extremely ecologically plastic species (Lees & Bell 2008). Although different types of habitat may be suitable for rabbits due to their high phenotypic plasticity, two elements are essential for their survival and reproduction: food and shelter. Rabbits are herbivores which depend on the availability of proteins in the grassland for their reproduction (see below), since the breeding season starts when the females consume more than a threshold value of protein in their diet (Villafuerte et al., 1997). Rabbits prefer to feed on legumes and highly nutritious annual grasses owing to their high protein levels (Soriguer 1988), but the re-ingestion system of their pellets (Kuijper et al., 2004) enables them able to exploit other less nutritious plants during periods of food shortage (Ferreira & Alves 2009). On the other hand, given that the rabbit is a staple prey for many predators, particularly in its native range (Delibes & Hiraldo 1981; Delibes-Mateos et al., 2008a), the shelter availability (mainly scrubland) is fundamental in providing them with places in which to escape from said predators, leading to positive overall results. Hence, the most appropriate areas for this species are those with a high availability of both resources, and the rabbits therefore attain their highest densities in mosaic landscapes and ecotones, in which the food and shelter are distributed in a complex manner (Lombardi et al., 2003; 2007), thus allowing to them optimize their spatial behaviour, since they are able to easily access both high-quality food in the pastures and to shelter in the scrubland (Lombardi et al., 2007). Other factors such as altitude, slope, climate conditions and soil hardness also affect the wild rabbit's distribution and abundance (Trout et al., 2000; Calvete et al., 2004a).

The other biological characteristic that allows the rabbits to colonize a wide range of habitats is their high reproduction rate resulting from their low age of sexual maturity, their short gestation period, the concurrent of gestation and lactation at the same time, and their ability to adapt their reproduction activity to local conditions and food availability (Tablado et al., 2009). For instance, in southern Spain, the gestation length is about 30 days, with a mean litter size of 4 offspring/female, which reach sexual maturity at 4 months (Soriguer 1981a; Gonçalves et al., 2002). Environmental factors (mainly temperature and rainfall) have a strong influence on the onset and length of the breeding season (Gonçalves et al., 2002), since the testicular function is strongly correlated with temperature, and the ovarian development is positive influenced by vegetation biomass and protein availability, which are directly correlated with rainfall (Villafuerte et al., 1997; Gonçalves et al., 2002). As a consequence, rabbit populations in Mediterranean environments have pronounced intra and inter annual oscillations depending on the environmental conditions, with the reproductive season that commonly lasts from November to June, with a higher breeding activity between March and June, when the majority of females are pregnant and/or lactating (Soriguer 1981b; Gonçalves et al., 2002).

Gregariousness, social grouping, territoriality and social dominance are integrant parts of the biology of the wild rabbit (Ruiz-Aizpurua 2013), which influences breeding and the survival of the individuals within the group (Mykytowycz and Goodrich, 1974). Wild rabbit social groups consist of hierarchies (Mykytowycz, 1958), with separate linear ranking orders for bucks and does, whose bucks and does defend their warrens and territories and exclude the subordinate individuals during the breeding season (Mykytowycz, 1958, Mykytowycz & Gambale, 1965). One critical element in the maintenance of social relationships is that of warrens, which are central to rabbit's biology. Warrens are essential as regards protection against predators, refuge against climatic extremes, the establishment of social ties, breeding, and improving juvenile survival, thus allowing the colonisation of less suitable habitats (Parer & Libke, 1985).

Another important advantage is their behavioural plasticity, since rabbits can modify their spatial and temporal patterns according predation pressure and environmental conditions (Villafuerte et al., 1993; Moreno et al., 1996; Villafuerte & Moreno 1997). During the day rabbits avoid open areas in order to minimise aerial predation, while at night rabbit do not use the areas close to scrub cover, which are used as hunting areas for mammalian carnivores (Moreno et al., 1996). Also, Villafuerte et al., (1993) found seasonal changes of diary activity probably as a consequence of antipredatory strategies and environmental conditions. With regard to spatial behaviour, rabbits can also modify their home range according to food resources and shelter (Lombardi et al., 2007; Barrio 2010).

TAXONOMIC STATUS AND DISTRIBUTION

The European rabbit is a small mammal belonging to *Leporidae* family, of the *Lagomorpha* Order. Its original distribution after the last ice age included Spain, Portugal, western France and northern Africa. In fact, the first discovery of *O. cuniculus* in the fossil records in the Iberian Peninsula dates from more than half a million years ago (López-Martínez 2008). After the glacial period, two relict populations were confined in its original range, one in southern Spain and another in the north-east of Spain and the south of France, which evolved into two separate evolutionary lines (Branco et al., 2000; Carneiro et al., 2010). The confined line in the south originated the subspecies *Oryctolagus cuniculus algirus*, which is constrained to south-western Iberia, while the northern line originated the subspecies *O.c. cuniculus*, which is distributed towards the Northeast, with a parapatry distribution in the centre of Iberia where they also hybridize (Branco et al., 2000; 2002; Carneiro et al., 2010). These subspecies are different as regards body size, sexual maturation and litter size (Gonçalves et al., 2002, Ferreira 2011). The subspecies *O. c. cuniculus* is thought to be the descendant of early domestic rabbits released into the wild (Gibb 1990), and is now the subspecies that has been introduced throughout Europe and worldwide (Smith & Boyer 2008).

Like many game species, the European rabbit has been introduced into many countries for hunting purposes, although, the beginning of the expansion of rabbits started in Romans times, when they were spread throughout Europe as a favourite food of the Romans (Gibb 1990). Current distribution includes most Western European countries, including the United Kingdom and Ireland, Australia, New Zealand, Chile, Argentina and many islands worldwide (Thompson & King 1994). A special mention should be given to the rabbit populations in Australia, where this species is considered to be an agricultural pest and one of the most important conservation problems.

A MULTI-FUNCTIONAL KEYSTONE SPECIES

Rabbit is considered to be a multifunctional keystone species in Iberia, because of it is crucial in maintaining the organisation, functioning, and diversity of the ecological community (Delibes-Mateos et al., 2008a). Indeed, the Mediterranean ecosystem is sometimes called a "rabbit ecosystem", to reflect the historical abundance and essential role of rabbits (Ward 2005). Rabbit grazing increases habitat heterogeneity and plant species richness (Delibes-Mateos et al., 2008a); it acts as an important seed dispersal agent (Malo et al., 1995); its latrines and faecal pellets improve soil fertility and plant growth (Petterson 2001), providing food resources for many dung beetles species (Sánchez-Piñeiro & Ávila 1991); and the burrows are used as a refuge and breeding sites for many species of vertebrates (Gálvez et al., 2008). In point of fact, rabbits play an important role as *ecosystem engineers* owing to their ability to modify their habitat (Gálvez et al., 2008).

However, the most important role is as a key prey for more than 30 predator species (Delibes & Hiraldo 1981; Delibes-Mateos et al., 2008a), many of which are classified as *Critically Endangered, Endangered, Vulnerable* or *Near Threatened* by the IUCN red list (Table 1). Since the rabbit is a high energetic resource with relative low catchability costs, the majority of large raptors and mammalian carnivores consume a high proportion of rabbits when they reach high densities (Delibes & Hiraldo 1981; Delibes-Mateos 2008a). Some of these predators, such as the Eagle owl (*Bubo bubo*) or the Golden Eagle (*Aquila chrysaetos*), are facultative, and they consume a high proportion of rabbits in areas of high rabbit densities; others species are opportunist and prey mainly on juveniles; others scavengers such as the Cinereous Vulture (*Aegypius monachus*) consume large amounts of rabbit as carrion (Costillo 2007); and there are two rabbit-specialist predators, the Iberian lynx (*Lynx pardinus*) and the Spanish Imperial Eagle (*Aquila adalberti*). For both species, the rabbit represent more than 88 % of their diet (Ferrer & Negro 2004). Indeed, the Iberian lynx and the Spanish Imperial Eagle emerged as separated species from the Eurasian lynx (*Lynx europeus*) and the Eastern Imperial Eagle (*Aquila heliaca*) in the Pleistocene refuge as a consequence of their specialisation in rabbit consumption (Ferrer & Negro 2004). What is more, the scarcity of rabbits is one of the main causes of the decline of these super-specialist predators (Ferrer & Negro 2004).

Lastly, in Iberia the rabbit has provided a food resource of great importance for hominids (Fa et al., 2013). In spite of the recent decrease in rabbit abundance (see below), this animal is still the main small game species in Spain, Portugal and France (Delibes-Mateos et al., 2008a; Letty et al., 2008; Ferreira et al., 2010), small game hunting being an important economic activity in rural areas.

TRENDS OF WILD RABBIT POPULATIONS IN IBERIA: THE POPULATION CRASH AND THE SPATIALLY UNEVEN RECOVERY

Historically, wild rabbit abundance has been extremely high. Interestingly, the origin of the name Hispania comes from *i-shepham-im*, an expression used by the Phoenicians which means “Hyrax coast or island”, since they confused rabbits with these animals. Moreover, in

Roman times, the Emperor Augustus sent a Roman legion to the Balearic Islands to clear the land of rabbits since they severely damage buildings (Rogers et al., 1994).

Unfortunately, the rabbit population in Iberian Peninsula has undergone a sharp decline as a consequence of several harmful factors. The population decline probably started at the beginning of the 20th century as a consequence of optimal habitat loss and fragmentation (Ward 2005). On one hand, the intensification of agricultural practices in huge areas have simplified the landscape, removing patches and edges of natural vegetation (Fernández-Alés et al., 1992), thus decreasing the habitat quality and food availability for rabbits in these areas (Delibes-Mateos et al., 2010). On the other hand, the rural exodus in mountain areas, and the subsequent abandoning of traditional agricultural and cattle grazing activities has led to a significant increase in scrub coverage (Fernández-Alés et al., 1992) and an important loss of habitat heterogeneity and complexity, which has been detrimental to rabbit density in the mountainous areas of southern Spain (Delibes-Mateos et al., 2010). As a result of this, the most appropriate habitats for the wild rabbit have declined in Andalusia over the last decades (Delibes-Mateos et al., 2010).

Others harmful factors such as a high hunting pressure (Angulo & Villafuerte 2003), the interespecific competition with big game species (Lozano et al., 2007; Cabezas-Díaz et al., 2011), the intensification of livestock farming (Ward 2005), the climatic change (Tablado & Revilla 2012) or urbanisation and infrastructure development (Delibes et al., 2000) have already been cited as additional negative factors for rabbit populations.

However, the most detrimental factors for the wild rabbit in Iberia and France have been the outbreak of two viral diseases: myxomatosis in the 1950s (Muñoz 1960) and rabbit haemorrhagic disease (RHD) at the end of the 1980s (Villafuerte et al., 1995). Some estimations have calculated that rabbit populations within the natural range may have declined by an estimated 95% since 1950 (Ward 2005). Myxomatosis was first introduced into Europe in France in 1952 by a farmer keen to rid rabbits from his land, and it was first detected in Spain in 1953, and over 90% of rabbits in Iberia were subsequently killed by the disease (Muñoz 1960; Ward 2005). This virus is transmitted by fleas and mosquitoes, has a greater impact on the younger than on adults, and the infection is more frequent in summer. Unfortunately just when rabbit populations appeared to be recovering from myxomatosis, another viral diseases arrived: rabbit haemorrogahic disease (RHD), thus jeopardising their populations. This virus arrived in Spain in 1989, and further devastated Iberian rabbit populations causing mortality rates of 55-75 % (Villafuerte et al., 1995). Indeed, Spain's wild population was estimated to be half of what it had been before the arrival of this disease (Blanco & Villafuerte, 1993). Unlike myxomatosis, RHD has a greater impact on adults, spreads mainly by direct contact, and is more prevalent in winter (Calvete et al., 2002). Both viral diseases are still a major regulating factor of wild rabbit populations (Ferreira et al., 2009; Cotilla et al., 2010), and both diseases are currently enzootic to wild rabbit populations, with recurring natural outbreaks causing mortalities every year (Mutze et al., 2002).

After the initial population crash resulting from RHD, the recovery of wild rabbit populations in Spain has varied greatly between locations, since in some areas rabbit number have now reached high densities that cause agricultural damage (Barrio et al., 2010; 2012), while in contrast the rabbits remain at very low densities in extensive areas (Delibes-Mateos et al., 2009a). These different tendencies are a consequence of multiple factors and their interactions. Put simply, the highest rabbit population recovery has been recorded in areas with lower hunting pressure (Williams et al., 2007); with soft soils that allow warrens to be dug (Delibes-Mateos et a., 2008b); with a high proportion of rabbit-friendly habitats (Calvete et al., 2006); and on intensively managed hunting estates on which the rabbit is considered to be the main game species (Delibes-Mateos et al., 2008b).

Several authors have hypothesized that the "predator pit" phenomenon has prevented the recovery of rabbit populations in the Iberian Peninsula

(Trout & Titterson 1989; Moreno et al., 2007). This hypothesis supports the idea that the high predation pressure on low prey density populations will keep rabbit numbers stable but at low densities, thus preventing the recovery of rabbit numbers to those which existed before the arrival of RHD, particularly in areas of high density and a diversity of predators. Fernández-de-Simón (2013) has recently shown some evidences to support the theory that some opportunist predators such as the red fox (*Vulpes vulpes*) may limit and regulate the recovery of wild rabbit populations, owing to its density dependent predation response and the negative relationship of rabbit population growth with fox predation.

This dramatic decline has led to the rabbit's categorization as a *Vulnerable* species in Spain (Villafuerte & Delibes-Mateos 2007), and *Near Threatened* in both Portugal (Cabral et al., 2005) and throughout the world (Smith & Boyer 2008) in the light of IUCN criteria.

THE SITUATION OF WILD RABBIT POPULATIONS IN MY STUDY AREA: SIERRA MORENA

Sierra Morena is a large 400-km long mountain chain, which is situated in the north of the Andalusia region (southern Spain). The typical landscape is characterised by Mediterranean scrubland which is dominated by *Cistus spp., Pistacia spp., Erica spp., Rosmarinus spp., Phyllirea spp., and Quercus spp.;* mature oak (*Quercus rotundifolia*) and cork oak (*Quercus suber*) forests; pine plantations of *Pinus pinea* and *Pinus pinaster*; and pastures together with scattered oaks (oak-savannah called *dehesa*). The altitude range oscillates between 200 and 1300 m.a.s.l.; the climate is the typically Mediterranean, with a mild wet winter and a warm dry summer, the mean annual rainfall being about 500-750 mm. The main economic exploitations are cork extraction, livestock farming, marginal agriculture and the extraction of wood and pinecones. However, the crisis as regards these traditional resources has led to a quantitative change in the exploitation of natural resources, being the harvest of big game species the principal economic support in this region at the present time (Mulero-Mendigorri 2013).

Sierra Morena contains 6 Natural Parks; many areas have been designated as Special Areas of Conservation (SACs) and Special Protection Areas (SPAs) for the Nature 2000 Network; and it is part of the Biosphere Reserve knon as *Dehesas de Sierra Morena*. As a result of the good status of the mature vegetation, the particular topographic conditions, and the low human populations, this area contains a high diversity of reptiles, birds and mammals (Junta de Andalucía, 2003), some of which, such as the Iberian lynx, the Spanish Imperial Eagle, the Golden Eagle, the Iberian wolf (*Canis lupus signatus*), the Black Stork (*Ciconia nigra*) or the Cinereous Vulture, are considered to be flagship species. Rabbits play an essential role in the maintenance of this high biodiversity, since some of these emblematic species partly depend on the rabbit for their survival.

Table 1: Presence of the rabbit in the diet of the main predators in Sierra Morena. Adapted from Delibes-Mateos et al., (2008a). The minimum and maximum percentage on rabbit in the diet and the IUCN category according to the Andalusia or Spanish red list are shown.

Predators	Rabbi in the diet (%)	IUCN category
Mammalian		Andalusia / Spain
Iberian lynx (*Lynx pardinus*)	77 – 99	EN / CR
Red fox (*Vulpes vulpes*)	0 - 96	NE / NE
Egyptian mongoose (*Herpestes inchneumon*)	22 – 80	NE / NE
Wild cat (*Felis silvestris*)	0 – 64	NE / NT
European badger (*Meles meles*)	0 – 62	NE / NE
Wolf (*Canis lupus signatus*)	0 – 44	CR / NT
Stone marten (*Martes foina*)	0 - 20	NE / NE
Common genet (*Genetta genetta*)	3 – 11	NE / NE
Birds of prey		
Spanish imperial eagle (*Aquila adalberti*)	27 - 88	CR / EN
Cinereous vulture (*Aegypius monachus*)	24 - 71	EN / VU
Eagle owl (*Bubo bubo*)	17 - 67	NE / NE
Common buzzard (*Buteo buteo*)	0 - 66	NE / NE
Golden eagle (*Aquila chrysaetus*)	13 - 63	VU / NT
Booted Eagle (*Hieraaetus pennatus*)	2 - 60	NE / NE
Black kite (*Milvus migrans*)	1 – 57	NE / NT
Bonelli's Eagle (*Aquila fasciata*)	13 - 51	VU / EN
Tawny owl (*Strix aluco*)	8 – 38	NE / NE
Red Kite (*Milvus milvus*)	8 - 29	CR / EN
Northern Goshawk (*Accipiter gentilis*)	12 – 22	NE / NE

CR = critically endangered; EN = endangered; VU = vulnerable; NT = near threatened; NE = no evaluated. Sources: Atlas y Libro Rojo de los Mamíferos Terrestres de España; Libro Rojo de Aves de España; Libro Rojo de los Vertebrados Amenazados de Andalucía.

Although Sierra Morena has had no long-term monitoring programmes which can categorically state that the rabbit population has collapsed in this area, some technical reports have shown a current low density. In Córdoba province, the current average values for rabbits in this region are estimated to be 0.095 individuals/hectare and 0.21 animals per kilometre surveyed (Junta de Andalucía, 2009). These values are noticeably lower than those attained in agricultural areas of the same province (7 individuals/kilometre; Barrio et al., 2009a). Very low hunting yields are similarly currently recorded for this species in contrast with other areas of Andalusia (Farfán et al., 2004).

Nevertheless, Sierra Morena has traditionally been an important area for the wild rabbit in the past, as is shown by the good hunting yields reported in many municipalities in the middle of 20th century (Delibes-Mateos et al., 2009b). The substantial land-use changes that occurred in this area from the second half of the last century (Fernández-Alés et al., 1992) have significantly decreased favourabile areas for the wild rabbit in this region (Delibes-Mateos et al., 2010). The rabbit abundance in Sierra Morena has traditionally been correlated with zones with a high proportion of sparse scrubland and oak-savannahs with appropriate scrub cover. However, in many areas the sparse scrubland has been replaced with large patches of dense scrubland as a consequence of the abandoning of traditional cattle grazing (Fernández-Alés et al., 1992). In contrast, the intensification of livestock farming and crops in the oak-savannahs has decreased the scrub layer (Fernández-Alés et al., 1992), thus making this habitat unsuitable for the wild rabbit (Delibes-Mateos et al., 2010). Additionally, the replacement of mature Mediterranean scrubland with monospecific pine plantations in thousands of hectares (Fernández-Alés et al., 1992) has been considered as an additional harmful factor in this area, since the density of pine mass is so high that it prevents the growth of scrubland and pastureland.

In this scenario, after the collapse of rabbit populations, rabbit numbers may have remained at a low density in the best suitable patches in Sierra Morena (Figure 1). These isolated and fragmented populations are much more vulnerable to statochastic phenomena and the likelihood of extinction (Wilcox & Murphy 1985; Virgós et al., 2003), and are more vulnerable to outbreaks of disease (Cotilla et al., 2010). Furthermore, in these low density areas the high predation of rabbits resulting from the diversity of the predator community may more effectively avoid their recovery to pre-RHD levels. The great increase in wild boar (*Sus scrofa*) and red deer (*Cervus elaphus*) in this area is also noteworthy (Vargas et al., 2007; Delibes-Mateos et al., 2009b), and may also affect the European rabbit through food competition and habitat alteration (Côte et al., 2004; Lozano et al., 2007; Cabezas-Díaz et al., 2011).

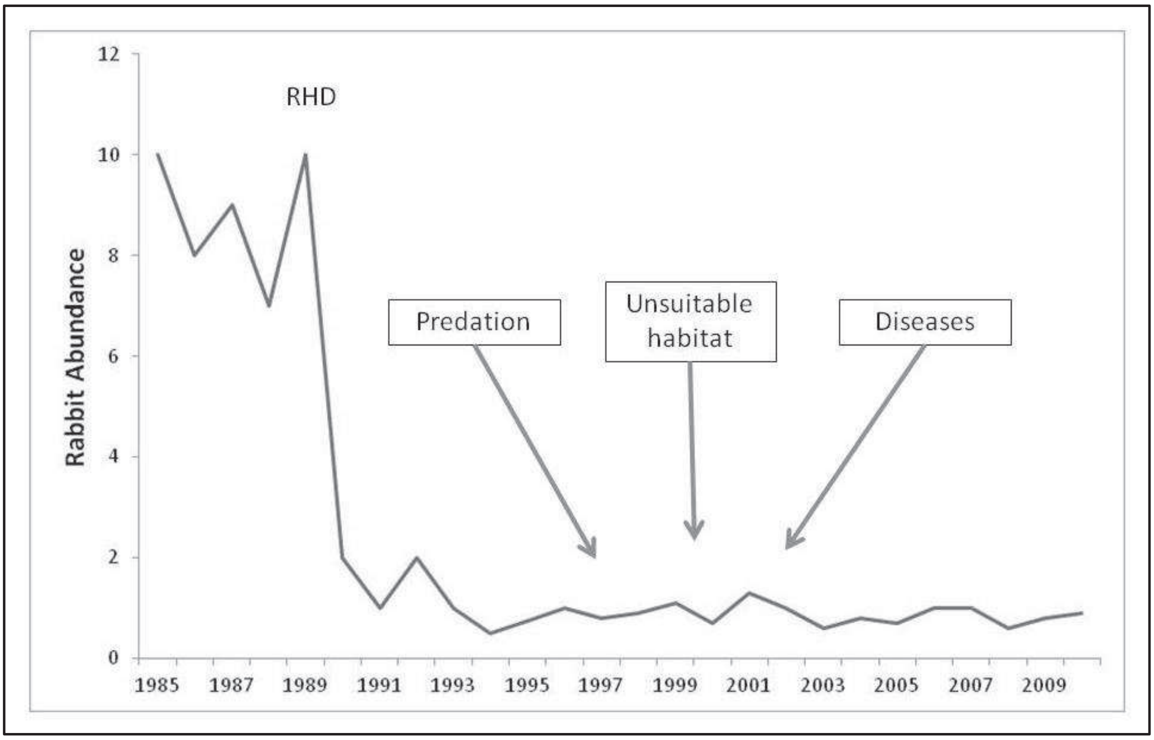


Figure 1: Hypothetical trends of rabbit populations in Sierra Morena and harmful factors that prevent their recovery. Based on Moreno et al., (2007).

This population crash may have had a detrimental effect on the predator's community that occurs in Sierra Morena. For instance, the scarce food availability as a consequence of the rabbit's decline has been suggested as a reason for the decline of the cinereous vulture, thus making the recovery of its natural populations necessary in order to enhance food resources (BirdLife International 2012). Furthermore, this large vulture is highly affected by human disturbance, poisoning and habitat loss (Donazar et al., 2002; Madroño et al., 2004), and it is therefore crucial to identify suitable areas in which harmful factors are not present. Currently, the breeding areas for this species in Andalusia have been dramatically reduced, and it is now confined to remote areas of Sierra Morena (Dobado & Arenas, 2012).

THE MANAGEMENT ACTIONS WITH WHICH TO RECOVER RABBIT POPULATIONS

This abrupt fall in rabbit populations may have had important cascading effects on the functioning of the Iberian-Mediterranean ecosystem, with serious ecological and economic consequences (Delibes-Mateos et al., 2008a), particularly for their predators (Ferrer & Negro 2004). But not only for their predators, since owing to their multi-functional roles, their scarcity could be considered as a major problem for the conservation of the Iberian ecosystem (Delibes-Mateos et al., 2009a). The rabbit decline has, meanwhile, also had economical effects, since the number of hunted rabbit significantly decreased after the population crash (Delibes-Mateos et al., 2009a), and this may have affected economic support in rural areas.

For these reasons, wildlife managers, conservations, researchers and hunters carry out a huge conservation effort to boost wild rabbit populations over the last few decades. In fact, Angulo (2003) showed that 95 % of the hunting estates in Andalusia applied management strategies to improve small-game populations, the strategies most frequently used being the habitat management, a reduction in hunting pressure, predator control and vaccination, although rabbit restocking has significantly increased after the population collapse in France, Spain and Portugal (Delibes-Mateos et al., 2008c), where around half a million rabbits are translocated each year (Letty et al., 2008).

Reduction in hunting pressure: reducing the hunting pressure on rabbits has been positively correlated with rabbit recovery after the arrival of RHD (Williams et al., 2007), and hence, this management tool is appropriate to boost wild populations. In fact, about 75 % of the hunters have implemented some restrictions to avoid over-harvesting (Angulo & Villafuerte 2003), through a reduction in hunting days, hunters per day, number of hunted animals, and/or increasing the area of the hunting reserve (Angulo 2003). Furthermore, a sector of hunters claims for a change in the hunting timing in order to avoid over-exploitation, although there is no consensus among researchers to advise regarding this topic (Angulo & Villafuerte 2003; Calvete et al., 2005).

Habitat management: this practice is generally considered to be an appropriate tool by which to boost rabbit density (Catalán et al., 2008; Ferreira et al., 2013). Although management practices must be performed according the habitat features in each case, as a general rule it should be targeted with the aim of increasing the habitat heterogeneity, and creating habitats with high shelter and food availability. In summary, in areas with low refuge availability, natural shelter can be easily increased by building artificial warrens (Fernández-Olalla et al., 2010) or heaps of wood branches (Rouco et al., 2008), whereas in areas with dense scrubland scrub clearing should be applied to create feeding areas and reproduce traditional landscapes (Moreno & Villafuerte 1995). Moreover, the establishment of high quality food (pastures or crops) and water points may also increase the carrying capacity (Ferreira & Alves 2009). Notwithstanding that these measures are difficult to apply in huge areas, small spatial management units of around 1 ha will provide the resources that rabbits need at the spatial scale of their home ranges (Lombardi et al., 2007).

Predator control: Since predation has been considered one of the main problems in small game species management in general (Reynolds & Tapper 1996), attempts to decrease mortality have been made through the use of non-selective traditional predator control, which could have compromised the endangered predators species (Reynolds & Tapper 1996). Although more than 80% of small game estates in Central Spain control foxes (Delibes-Mateos et al., 2013), empirical evidence whit which to better understand the real effect of this practice in Iberian Peninsula is still lacking. Furthermore, the effectiveness of predator removal depends on numerous parameters such as the method used, the bait, the place, the abundance of target species, or the staff's experience (Ferreras 2009). Alternatively, the management actions should minimise the predation rate rather than concentrating efforts on predator removal. For instance, increasing habitat heterogeneity and quality in order to minimize predation risk; benefitting preys' overall physical condition in order to reduce their vulnerability; or favouring the conservation of the top predators which can control the smaller generalist predators, could decrease the predation rate without the use of lethal methods (Palomares et al., 1995; Ferreras 2009).

Diseases control: Myxomatosis and RHD are still a major regulating factor of wild rabbit populations (Ferreira et al., 2009, Cotilla et al., 2010), and hence vaccination should be a useful management tool to control both diseases. Nevertheless, despite the fact that the effectiveness of vaccination has been frequently discussed (Calvete et al., 2004b, 2004c, Calvete 2006, Guitton et al., 2008, Ferreira et al., 2009), most rabbit conservation programmes include vaccination as a frequently tool (Angulo 2003). Although the vaccine has positive effects on the individuals in the short-term (Cabezas et al., 2006), a long-lasting positive effect of a single vaccination at the population level in wild rabbits remains untested (for more details see Chapter 1).

Restocking: The popularity of this management practice was evidenced by Delibes-Mateos et al., (2008c), who showed that rabbit translocations were carried out at least once during the 2000s on 43 % of hunting estates in Central-Southern Spain with the purpose, in the majority of cases, of hunting (92%) in contrast with those with conservation goals (8 %). In part, this popularity may be owing to the rapid increase in rabbit abundance which may be reached in the short-term by restocking activities, thus obtaining a rapid amount of hunting bags or rapid food availability for the endangered predators. However, translocation success requires the achievement of several steps: the survival of the released rabbits, their settlement in the release area and their successful reproduction in the release area (Letty et al., 2008), if the final objective is to be attained: the establishment of a self-sustaining population (Griffith et a., 1989). According to these authors, most rabbit translocations have failed in this final goal, since several scientific works have recorded very low success rate, as a consequence of the short-term mortality after release (Calvete et al., 1997). However, the importance of rabbit restocking in the recovery plans for the species signifies that this topic will be discussed in more detail in Chapter 1.

OBJETIVES AND STRUCTURE

In spite of the fact that half a million rabbits are translocated each year in France, Portugal and Spain (Letty et al., 2008), there is no clear common protocol that can be applied in rabbit translocations. Furthermore, some management tools are discussed by researchers and wildlife managers, and there is no consensus about them. **Hence, the Chapter 1 of this thesis reviews the scientific literature currently available as regards rabbit restocking, highlighting the techniques and the factors that improve restocking success, and discussing the main gaps that still remain as regards optimizing the restocking processes and establishing the perspectives for future research efforts.**

To avoid the aforementioned short-term mortality, the establishment of semi-extensive rabbit captive breeding enclosures has become a widely used management tool in predators' conservation projects over the last few decades (Ward 2005; Ferreira & Delibes-Mateos 2010). The role of fences is that of reducing mortality by terrestrial predators and the initial dispersal movements, and then keeping the rabbits confined in order to maintain a population density which is sufficiently stable and high to withstand the combined effects of diseases and predators. However, little is known regarding the impact of aerial predation on rabbit survival in the short-term, and wildlife managers hardly ever carry out measures to tackle the problem of avian predation. **The purpose of the Chapter 2 is therefore to study the short-term effect of aerial predation exclusion in rabbit restocking enclosures.**

These enclosures are also used as *in situ* farms to produce healthy and genetically pure rabbits (Arenas et al., 2006), which could have a great ability to adapt to the local conditions. The high rabbit abundance attained in the fenced plots makes it possible to utilise the rabbits from fenced plots as colonisers of surrounding areas (Rouco et al., 2008). In spite of the high cost, the role of some important issues such as enclosure size, slope, habitat attributes, the effect of predation, the number of rabbits released, or the optimal habitat management around them is still unknown. **Consequently, the third goal (Chapter 3) is to address which factors enhance the production of wild rabbits in the *in situ* extensive enclosures.**

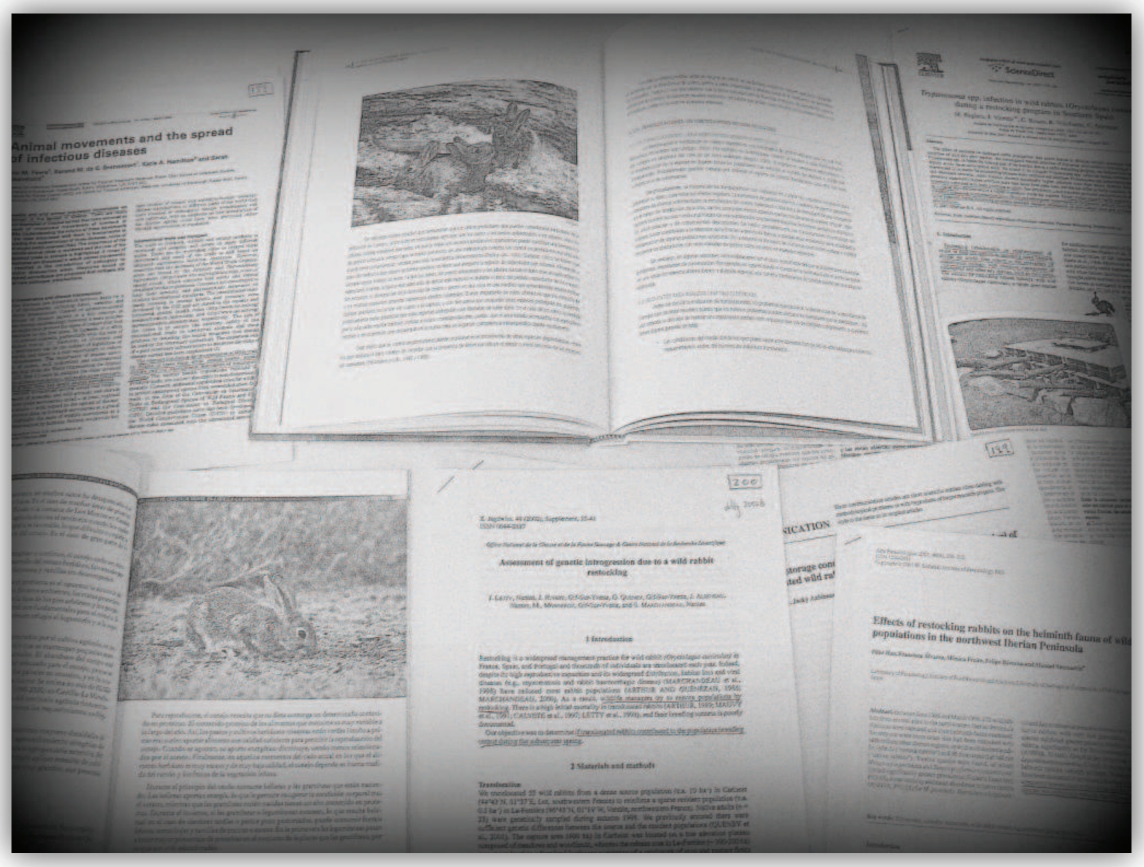
Release into enclosures plus the habitat management around them could be powerful tools with which to establish wild populations in very low density areas in which others management practices are insufficient in themselves owing to the fact that the founder population is too small. The conservation of endangered predators depends on the recovery of rabbit populations in their home ranges, and it is therefore vital to establish a reliable management tool to recover wild rabbit populations in these areas. **In the light of these considerations, the fourth objective (Chapter 4) is to assess the effectiveness of restocking in enclosures plus habitat management as a conservation technique to establish wild populations on a landscape scale and in the long-term, and to evaluate the habitat management that should be promoted around them.**

Conservation of endangered predators in Sierra Morena is linked with the recovery of wild rabbit populations. T**he last goal (Chapter 5) is to identify which factors affect the Cinereous Vulture nesting-habitat selection, creating a fine scale predictive map (50-m resolution) for**

local conservation applications. The increase of rabbit abundance affect on the spatial distribution of cinereous vulture's nest is also discussed.

At the end of the thesis three is a general discussion in which the main finding of the different chapters are summarised and the cost-effectiveness of the rabbit recovery plan in the study area is discussed.

CHAPTER 1


Animal movements and the spread
of infectious diseases
Assessment of genetic introgression due to a wild rabbit
restocking
Effects of restocking rabbits on the helminth fauna of wil
populations in the northwest Iberian Peninsula

CHAPTER 1

European rabbit restocking: a critical review in accordance with the IUCN (1998) guidelines for re-introduction.

José Guerrero-Casado[1,2], Jérôme Letty[3] & Francisco S. Tortosa[1]

Abstract

European rabbit restocking is one of the most frequent action in hunting estates and for conservation projects in Spain, France and Portugal, where rabbit is a keystone species. The aim of this work is to review current knowledge as regards the establishment of rabbit restocked population in accordance with the IUCN (1998) guidelines for re-introduction in order to identify gaps in knowledge and highlight the techniques that improve the overall success rate. Eight of the 17 selected items of these guidelines were identified as partly studied or unknown, including important items such as the management and release of captive-reared wild rabbits, the development of transport and monitoring programs, the application of vaccine programs, and post-release long-term studies. Researchers should therefore concentrate their efforts on bridging these knowledge gaps, and wildlife managers should consider all the factors reviewed herein with the aim of establishing accurate management guidelines for subsequent rabbit restocking programs.

Keywords: lagomorphs, hunting management, *Oryctolagus cuniculus*, translocation, wildlife management.

Resumen

Repoblaciones de conejo europeo: una revisión crítica según las directrices de la IUCN (1998) para las re-introducciones.- Las repoblaciones de conejo europeo son una de las medidas más empleadas en los cotos de caza y en proyectos de conservación en España, Francia y Portugal, donde el conejo es una especie clave. El objetivo de este trabajo fue revisar el conocimiento actual sobre los factores que afectan el establecimiento de las poblaciones de conejo introducidas según las directrices de la IUCN (1998), a fin de identificar lagunas de conocimiento y destacar las técnicas que mejoran el éxito de las mismas. Ocho de los 17 puntos seleccionados de estas directrices fueron identificados como desconocidos o parcialmente estudiados, incluyendo importantes aspectos como el efecto sobre las poblaciones de origen, el manejo y la suelta de animales criados en cautividad, el desarrollo de planes de transporte y monitoreo, la aplicación de vacunas, y estudios post-suelta a largo plazo. Por lo tanto, los investigadores deben de concentrar sus esfuerzos en suprimir esta falta de conocimiento, y los gestores deben de considerar todos los factores que aquí revisamos, con el objetivo de establecer una guía definitiva para las futuras repoblaciones de conejo.

Palabras clave: gestión cinegética, lagomorfo, manejo de fauna silvestre, *Oryctolagus cuniculus,* translocaciones.

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Introduction

The translocation of animals with the aim of conservation is becoming more frequent around the world as a consequence of the emergency regarding the loss of biodiversity, but most often translocation success proved to be problematic (Griffith et al., 1989, Armstrong & Seddon, 2008). As many reintroduction attempts failed the IUCN edited guidelines for re-introductions (IUCN, 1998) with the aim of establishing the knowledge needed to ensure that reintroductions would achieve their intended conservation goal. Nevertheless, in many translocation programs many questions still remain unanswered (Armstrong & Seddon, 2008). This also concerns the European rabbit (*Oryctolagus cuniculus*) in France, Portugal and Spain, where likely around half a million rabbits are translocated each year in order to promote the recovery of natural populations and to improve hunting stocks (Arthur, 1989, Calvete et al., 1997, Letty et al., 2008).

Nevertheless, a very low restocking success rate was often recorded, mostly due to a low survival after release, in traditional restocking in which a large number of rabbits are simultaneously released with no other wildlife management measure (Calvete et al., 1997). Wildlife managers have consequently begun to place importance on different management tools to improve rabbit survival. Several works have evidenced the effectiveness of some of these tools, such as soft-release or habitat management, whereas other strategies like vaccination or quarantining are controversial. Furthermore, Ferreira & Delibes-Mateos (2010) have suggested that recommendations made by researchers have not been fully employed by wildlife managers and hunters, thus often leading to a decrease in rabbit translocation success. This also reveals the need for a clear protocol to apply in rabbit translocations. Hence, we have reviewed the current knowledge of rabbit restocking in accordance with the IUCN guidelines for reintroductions, highlighting the techniques and the factors that improve translocation success, and discussing the main gaps that still remain in order to establish the perspectives for future research.

Data source

The IUCN guidelines for re-introduction have been used as a reference guide to review the current knowledge of rabbit restocking, since this report establishes the items and the steps that restocking programs must take. These guidelines define a re-introduction as an attempt to establish a species in an area that was once part of its historical range, but from which it has been extirpated or become extinct; a translocation as a deliberate and mediated movement of wild individuals or populations from one part of their range to another; and restocking as an addition of individuals to build-up an existing population. Whatever the case, the IUCN establishes a unique guideline for the re-introductions, restocking or reinforcement and translocations, and in accordance with literature on rabbits and Armstrong & Seddon (2008), we hereafter use the terms restocking and translocation as general and synonym terms.

The IUCN guidelines are divided into three main sections: (1) pre-restocking activities, (2) planning, preparation and restocking phases, and (3) post-release activities, and we have therefore analyzed the knowledge concerning rabbit restocking in accordance with these sections. Following the suggestion of Armstrong & Seddon (2008), this paper is mostly focused on the population level, and particularly on the factors and management measures affecting the critical stage of the establishment of a reintroduced population. Therefore, for this study, only items concerning the biological, ecological and practical monitoring aspects of a restocking that scientific literature should document have been selected. Once established, the subsequent persistence of the population depends on general factors of ecology requirements and classical wildlife management. A total of 17 out of 52 items described in the IUCN guidelines were eventually selected (Table 1). An issue has been deemed to be partly studied if there was only a theoretical or a not fully developed approach, or there is no consensus about it. We reviewed the papers that address rabbit restocking in the available scientific literature by use of the three principal web engines: Google Scholar™, ISI Web of Knowledge® and Scopus®, in which the following words were searched in the following combinations: "rabbit" OR "Oryctolagus" AND "restocking" OR "translocations". Furthermore, in order to address each item involved in rabbit restocking (e.g. habitat management, vaccines, quarantine or stress) additional searches were performed following the same method. We also searched for data about these topics in PhD theses, books and technical reports. Eight of the 17 items in the IUCN guidelines were identified as being poorly studied or unknown, which are summarized in Table 1 and discussed in the following sections.

Table 1: Selected items of IUCN guidelines for reintroduction programmes, with the references that support the information and summary and/or observation for rabbit in each case.

Item	Knowledge	Summary/Observations	References
Pre-restocking activities			
Taxonomic status	Yes	Avoid the mixing of the two subspecies and perform genetic analyses	(Branco et al., 2000, Delibes-Mateos et al., 2008c)
Status and biology of wild population	Yes	Population crash after the appearance of viral diseases. Many populations remain at low density	(Marchandeau et al., 2000, Delibes-Mateos et al., 2009a, Ferreira et al., 2010)
Habitat requirements	Yes	Positive effect of habitat quality. No studies on the importance of local ecological adaptations	(Moreno et al., 2004, Villafuerte & Castro, 2007)
Identification of previous causes of decline	Yes	Mainly viral diseases and habitat loss	(Villafuerte et al., 1995, Delibes-Mateos et al., 2009a)
Wild population	Partly	No studies on training in captivity. Possible genetic,	(Arenas et al., 2006, Piorno,

management in captivity	studied	behavioural and ecological problems with hybrids domestic rabbits.	2007a, Guerrero-Casado et al., 2013b)
The release of wild rabbit reared in captivity	Partly studied	No studies on restocking success. Genetic introgression of hybrid rabbits in wild populations.	(Piorno, 2007a, Sánchez-García et al., 2012)
How to minimize the infection rate	Yes	Treat for external and internal parasites before release	(Cabezas & Moreno, 2007, Rouco et al., 2008)
Planning preparation and restocking phases			
Identification of success indicators	No	No suitable guidelines	
Design a monitoring program	Partly studied	No consensus about a standardised monitoring protocol.	(Fernández-de-Simón et al., 2011)
Health of release stock	Yes	Positive effect of good body condition and negative impact of stress	(Calvete et al., 2005b, Cabezas et al., 2007, 2011)
Vaccination	Partly studied	Disagreements on its effectiveness. Positive effect of animals released with high natural antibody concentration.	(Calvete et al., 2004b, Guitton et al., 2008, Ferreira et al., 2009)
Quarantine	Yes	Negative effect on animal's physiological condition.	(Moreno et al., 2004, Calvete et al., 2005b)
Transport plan	Partly studied	No detailed guide. No demonstrated effect of crowding and long transports.	(Letty et al., 2003, Letty et al., 2005)
Release strategy	Yes	Positive effect of soft-release, habitat management	(Calvete & Estrada, 2004,

		and predator exclusion	Rouco et al., 2008, Cabezas et al., 2011)
Post-release activities			
Demographic, ecological and behavioral studies	Partly studied	Restocked rabbit's show the same behaviour in the long-term as wild individuals.	(Rouco et al., 2011b, Ruiz-Aizpurua et al., 2013)
Long-term adaptations	Partly studied	Low breeding contributions. No studies on the interactions with the resident congeners.	(Letty et al., 2002a)
Investigation of mortalities	Yes	Mainly predation, environmental novelty and stress. Survival and dispersal after release in short-term are well documented	(Calvete et al., 1997, Letty et al., 2008, Cabezas et al., 2011)

Pre-restocking activities: biological knowledge

The first step in a restocking program is to determine the source population from which the rabbits will be captured. This question is particularly relevant in the Iberian Peninsula, in which two rabbit subspecies coexist: *Oryctolagus cuniculus algirus*, and *O.c.cuniculus* (Branco et al., 2000). Nevertheless, Delibes-Mateos et al., (2008c) found *algirus* rabbits in localities within the *cuniculus* subspecies range, and vice-versa, as a consequence of past translocations, since in most cases rabbits are released regardless of their genetic lineage. These subspecies have differences in body size, sexual maturation and litter size (Gonçalves et al., 2002, Ferreira, 2011), which could affect the success of rabbit translocations, with unknown ecological and demographic consequences. Wildlife managers should therefore avoid the mixing of subspecies by identifying the genetic lineage of the rabbits by means of DNA analysis, and both the donor and the receiving populations must be located within the geographic range of the corresponding genetic lineage (Delibes-Mateos et al., 2008c). Furthermore, at the metapopulation level, although the impact of rabbit extraction on the donor population has not been empirically tested, it should also be considered, since excessive captures of individuals may lead to the decline of the donor population (Cotilla & Villafuerte, 2007). Finally, rabbits are captured in donor populations by means of ferreting or gill and trap nets (Cotilla & Villafuerte, 2007), but no scientific studies have tested the effect on sex ratio or age class selectivity, survival rate or stress degree (but see Cowan 1984).

The interest in the captive rearing of wild rabbit for release purposes as an alternative to capturing wild individuals has increased over the last two decades (Arenas et al., 2006). Indeed, the proportion of captive-reared rabbits released in Spain might exceed 50% of the total number of wild rabbits released (Sánchez-García et al., 2012). All the same, the success of restocking operations using captive-reared rabbit remains untested, and genetic, epidemiological and behavioural problems could be expected when hybrids between wild and domestic lineages are reared in captive intensive systems, as occur on some farms in France and Spain (Rogers et al., 1994, Piorno, 2007a). Moreover, although Arenas et al., (2006) reported the management techniques that improve the reproduction of wild rabbits in captivity, the development of training techniques in captive environments that enhance rabbit restocking success is still unknown. However, it would appear to be possible to recreate wild-like environmental conditions (food availability, soil type and aerial predator pressure) in suitable extensive breeding enclosures in situ to attain appropriate rabbit behaviour for release purposes, and a greater ability to adapt to local conditions (Guerrero-Casado et al., 2013b). The sustainability of such captive-rearing populations and relevant factors to consider for translocation success such as body condition, behaviour and age of captive-reared individuals could be studied more. Problems of genetic drift of selection (domestication) could also occur in captivity in the mid-term with a genuine wild lineage of rabbits. It could be worth to better assess this issue due to the importance of wild rabbit captive rearing.

With regard to habitat requirements, although different types of habitat may be suitable for rabbits owing to a high phenotypic plasticity, release areas must provide the possibility of digging numerous burrows to allow a rapid increase in both population size and viability, shelters and covers to escape from predators, and grazing areas for food supply. Release into an optimal habitat is expected to increase rabbit survival and to limit dispersal movements (Calvete & Estrada, 2004, Moreno et al., 2004). On the other hand, it should be expected that when the release and capture areas have similar ecological characteristics, rabbits would adapt better to the new environment, by means of pre-adaptations to landscape, soil, flora or parasites type (Letty et al., 2008), and it is therefore advisable that donor population should be located as close to the target area as possible (Villafuerte & Castro, 2007). However, there is clearly a gap in the knowledge concerning the possible importance of adaptations to local ecological conditions on restocking success.

Planning, preparation and restocking phases

According to scientific literature, the crux of the translocation problem is the high mortality during the first weeks after release, and the interaction among the main factors affecting rabbit survival: predation, environmental novelty and stress (Calvete et al., 1997, Moreno et al., 2004, Letty et al., 2008). The translocated animals may display a high activity during the first days after release. Since they are introduced into a novel habitat, they are disorientated, do not know where to feed, rest or seek refuge from predators, and may also explore the area, possibly in order to find their usual landmarks or return to their previous home range (Letty et al., 2002b, Letty et al., 2008). Indeed, Letty et al., 2008 concluded that the main problem for the survival of translocated rabbits may be the environment novelty, since rabbits do not experience a significant mortality after handling and release in their familiar environment (Letty et al. 2003).

Stress is an inevitable component of restocking programs, since the process of translocation involves multiple stressors: (1) capture and handling, (2) captivity or some form of prolonged restraint, (3) transport, and, (4) permanently and likely above all, release into an unfamiliar environment (Letty et al., 2007, Teixeira et al., 2007, Dickens et al., 2010). Therefore, this succession of stressors could make translocated animals chronically-stressed, which may have a strong negative impact on animal physiological condition (Cabezas et al., 2007), thus increasing their vulnerability to predation or diseases. Hence, in these phases, stress levels should be controlled by reducing handling and physical restraint (Letty et al., 2005), avoiding crowding, decreasing time between capture and release, and facilitating a more rapid access to high quality food (Calvete et al., 2005).

Furthermore, eye damage, fractures, bites and wounds (Rouco, 2008) and even sporadic death (Letty et al., 2005) have been reported during the transport phase. A specific guide should therefore be provided to specify all the points related to rabbit capture, transport and handling, with special emphasis on minimizing stress and ensuring animal welfare, since there is often little effort to reduce losses during these stages (Calvete et al., 1997). However, in a wild rabbit translocation, transport and handling stress may only induce temporary negative effects compared to those induced by the permanent change of area (Letty et al., 2003). Finally, there isn't evidence of the positive effect of the use of tranquillizer on survival, despite of the strong physiological stress experienced by the rabbits (Letty et al., 2000).

Various release strategies aimed at minimizing the aforementioned problems and improving rabbit's survival have been developed over the last decade (soft-release, habitat management, or predation exclusion). For instance, the progressive acclimatisation of the translocated rabbits to the new environment in soft-release fenced warrens is designed to prevent initial exploratory movements and predation mortality just after release, when the animals are more vulnerable, and hence, acclimatization highly increases the rabbit survival rate in the short-term (e.g. 82 % Calvete & Estrada, 2004; 87 % Rouco et al., 2010). Although this gain in survival is not always clear, and sometimes only temporary (Letty et al., 2000, Letty et al., 2008), a long-lasting acclimatisation period is advisable to increase early survival and to decrease dispersal, particularly in poor habitats (Calvete & Estrada, 2004), thus favours the settlement in the release area, leading to increased initial breeding stock and overall restocking viability (Letty et al., 2008, Rouco et al., 2010).

More recently, the establishment of in situ extensive rabbit captive breeding enclosures has been widely used in predator conservation projects in order to enhance rabbit availability (Ward, 2005, Ferreira & Delibes-Mateos, 2010), since it may be definitely a very effective way of establishing a new population. The role of fences is not only to reduce mortality caused by terrestrial predators and dispersal movements, but also, by establishing a captive in situ breeding stock, to allow the breeding of young individuals which could naturally disperse to settle in the surrounding areas (Letty et al., 2006, Rouco et al., 2008, Guerrero-Casado et al., 2013a). Anyway, given that rabbits translocated into a new environment are very vulnerable to predation in the short-term, soft-release or long-lasting acclimatization in predator-free enclosures should provide an effective solution to minimise the impact of predation without concentrating efforts on removal of predators. It is also possible to reduce the impact of predation by selecting areas with a high shelter availability (Calvete & Estrada, 2004) or by increasing it through habitat management (Cabezas et al., 2011) and avoiding the simultaneous release of excessive number of animals in a small area in order to avoid the attractiveness of the area to predators (Moreno et al., 2004).

Habitat management is another widespread practice in rabbit restocking, which is considered to be very effective (Catalán et al., 2008, Ferreira & Alves, 2009). If the rabbit release takes place in a sub-optimal habitat, habitat management should occur prior to release in order to create feeding habitats and provide shelter through scrubland management and/or the construction of artificial warrens where refuge for rabbits is scarce (Ferreira et al., 2013). Thereby, rabbit abundance and their survival rate is significantly higher when the translocation is carried out in areas improved by the creation of pastureland and provision of artificial warrens (Cabezas & Moreno, 2007, Cabezas et al., 2011). In particular, the construction of artificial warrens into which rabbits can be released is a common practice that will also enhance the availability of shelter and breeding sites. Put simply, it is preferable to build many small warrens rather than a few large warrens (Rouco et al., 2011a), these should be enough close to each other (Barrio et al., 2009b) to allow the foundation a small population, and located in adequate food and shelter coverage areas, and preferentially made with tubes (Fernández-Olalla et al., 2010). Detailed guidelines on how to perform habitat management can be found in several technical documents (Anomynous, 2003, Ferreira & Alves, 2006, San-Miguel, 2006, Guil, 2009).

Disease risk constitutes other threat that may jeopardize wild rabbit translocations, and many translocation therefore include the vaccination of rabbits against myxomatosis and RHD virus (Delibes-Mateos et al., 2008c), despite the fact that its effectiveness in the field is controversial. Despite some possible short-term negative effect, an overall positive effect of vaccination has been recorded in free-ranging rabbit populations (Cabezas et al., 2006, Calvete, 2006, Guitton et al., 2008, Ferreira et al., 2009). Vaccination in translocation may have some drawbacks: its short-term negative effect may negatively affect the physiological condition of rabbits and increase early mortality risks (Calvete et al., 2004); the immune response depends on body condition and may be decreased by the stress induced by translocation (Cabezas et al., 2006); the vaccine may even cause an immunosuppressive effect in individuals with a poor physiological condition (Calvete et al., 2004); and its effectiveness may be reduced in immunized individuals or, for RHD, in case of a significant evolution of the virus (Le Gall-Reculé et al., 2011). On the other hand, a translocation is a rare case in which vaccination may be relevant, and indeed, it could be crucial for population establishment in case of subsequent disease outbreak. Vaccination campaign effectiveness should be high since it is possible to vaccinate the whole "population" (released individuals), although a subsequent booster is not feasible, thus a long-lasting positive effect of a single vaccination and of the related immunity in wild rabbit seems questionable. Anyway, the question of the exact impact of vaccination on the fitness of translocated rabbits, and of the related cost benefit ratio for restocking success, partly remains to be properly and experimentally addressed.

Given that the long-term survival is positively correlated with the antibody concentrations before release (Cabezas et al., 2011), as an alternative to vaccine, Rouco (2008) proposed releasing rabbits with naturally high antibody concentrations. This might be feasible in the wild, since just after the annual outbreak of diseases, most individuals have natural antibodies in high density rabbit populations (Cotilla et al., 2010). Thus, vaccinations is unnecessary when restocking with such individuals (Calvete, 2006), and vaccination protocols would be only necessary if donor populations have low antibody prevalence (Cabezas et al., 2006). However, the precise monitoring of antibody prevalence in wild populations may not be easy to carry out in the field.

In turn, in some restocking programs rabbits are kept in quarantine for several days to ensure the vaccine's effect and to control that animals do not incubate the viral diseases. However, if the probability that an animal incubates the diseases during quarantine period is very low, and if quarantine does not reduce the risk of introduction of a virus, a quarantine period would not be necessary (Rouco 2008). Also, this captivity period induces stress, loss of body mass, abortion in pregnant females and other possible physiological disorders (Calvete et al., 2005b). Indeed, this management tool does not generally increase restocking success (Calvete & Estrada, 2004), except by simply controlling clearly diseased or injured animals (Calvete et al., 1997).

In the same way, restocking operations are shown to be a potential means of introducing pathogens into resident populations. For instance, rabbit translocations favour the spread of sarcoptic mange (Navarro-González et al. 2010), a higher Tripanosoma spp. infection rate (Reglero et al. 2007) and a higher prevalence in the host's helminth fauna (Haz et al. 2001). Hence, all rabbits should be treated for external and internal parasites before release in order to minimise the possibility of disease and parasite transmission. As regard the health of the stock released, selecting animals with a good body condition (a good index of fat, without traumatic injuries, no cachectic animals, and without high parasite levels) enhances the probability of survival (Calvete et al., 2005b, Cabezas et al., 2006).

The effects of release timing, sex ratio, age, and number of rabbits on restocking success have been suggested by some authors. For demographical reasons, the release of rabbits before the breeding season could lead to higher population growth (Cotilla & Villafuerte, 2007), whereas releasing rabbits during breeding season (when social stress is high) might lead to an increased agonistic behaviour and direct competition among rabbits (Moreno et al., 2004). However most of releases are carried out during the reproductive timing, when the availability of wild rabbits is higher (Moreno et al., 2004, Cotilla & Villafuerte 2007). The timing of the release could also affect the translocation success if, for instance, the impact of predation depends on the season (the availability of food and cover differs among seasons). Regarding the age, the model of Cotilla &Villafuerte (2007) indicated that success would be maximized by releasing only adult rabbits (at least 4 month-old). But, in fact, the fitness of individuals to translocation may differ between adult breeders and full-grown juveniles, which might be less affected by translocation than adults and better able to adapt to the new situation (Mauvy et al., 1991, Letty et al., 2008). Hence, it is necessary to clarify the optimal age of release animals. As a general rule, it is advisable to release rabbits in optimal number and natural sex ratio with the objective of attaining viable population dynamics after release (e.g. 1:1 Moreno et al., 2004; Cabezas & Moreno 2007).

Post-release activities

Little is known with regard to demographic, ecological and behavioural long-term adaptations in released populations, although some works have suggested that restocked rabbits exhibit the same behaviour in the long-term as wild individuals (Rouco et al., 2011b, Ruiz-Aizpurua et al., 2013). Indeed, social behaviour can also affect restocking success (Ruiz-Aizpurua, 2013), although Letty et al., (2006, 2008) did not record a clear difference in the survival rate between individuals released in familiar groups (captured in the same warren) and unfamiliar groups, suggesting that the translocation process destabilises previous social relationships. Earlier studies have also suggested a low breeding contribution of introduced individuals during the first months after release (Letty et al., 2002a), and hence, those points should be clarified in further researches in order to understand the behaviour of the rabbits released and their interactions with the resident congeners.

Finally, it is necessary to identify short- and long-term indicators that could permit an assessment of the outcome of the translocation in agreement with aims and objectives. Similarly, many rabbit translocation are not carefully monitored (Cabezas & Moreno, 2007), and, it might therefore be relevant to create a somewhat standardised monitoring protocol with which to collect reliable data on restocking success (rabbit abundance method, temporal and spatial scale, etc.). Among the existing indices, wildlife managers could monitor rabbit abundance by using a methodology based on transect counts or pellet counts (Fernández-de-Simón et al., 2011), before and after release to correctly assess the restocking effectiveness.

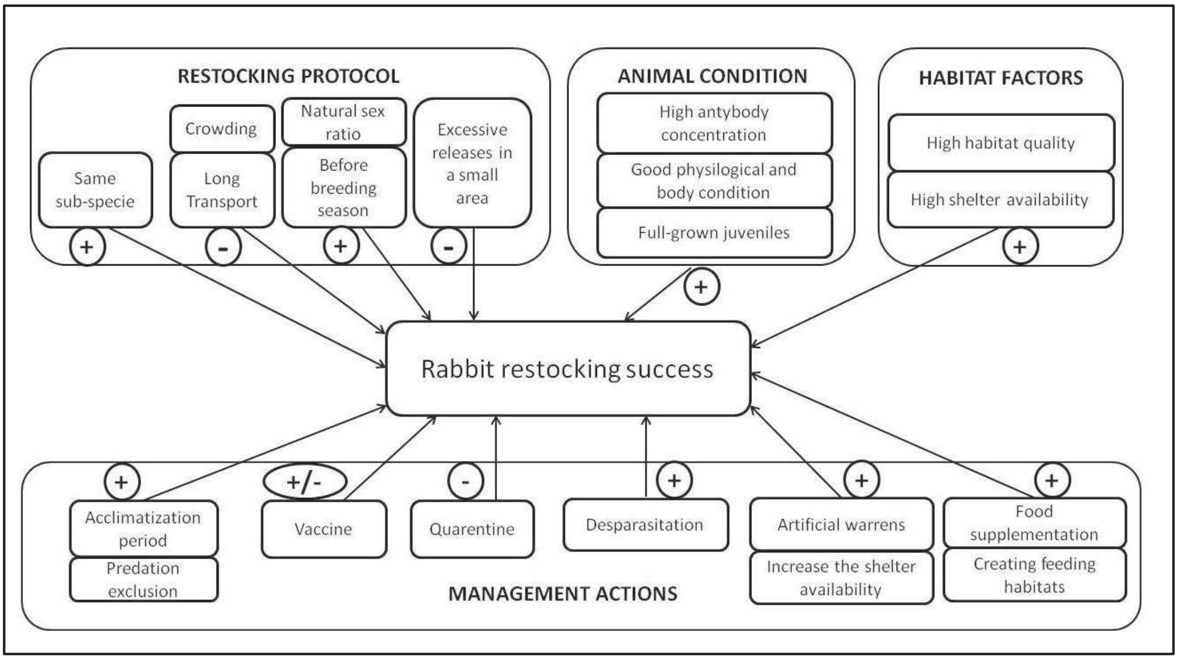

Figure 1: Summary of the factors that affect wild rabbit restocking success. The symbols + and – indicate positive or negative relationships with the restocking success.

Conclusions

In conclusion, despite the importance of rabbit restocking activities, 8 items that the IUCN guidelines for re-introduction considered important for the establishment of restocked animals remain partly unanswered. Therefore, conservationists, hunters, wildlife managers and researchers should concentrate their efforts on bridging these knowledge gaps and attempting to implement the scientific recommendations with the aim of establishing accurate management guidelines for subsequent rabbit restocking. We accordingly suggest that the overall success rate would be improved by: a long-lasting acclimatization period; selecting a high quality habitat and/or enhancing its carrying capacity with artificial warren, food supplementation or scrub management; avoiding the mixing of the two subspecies; selecting animals with a good body condition and antibody concentration; reducing predation risk and stress;

releasing full-grown rabbits before breeding season, in a natural sex ratio; and avoiding the simultaneous release of an excessive number of animals in a small area (Figure 1).

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CHAPTER 2

CHAPTER 2

The short-term effect of total predation exclusion on wild rabbit abundance in restocking plots

José Guerrero-Casado[1], Leire Ruiz-Aizpurua[1] & Francisco S. Tortosa[1]

Abstract

About half a million rabbits are translocated in south-western Europe every year with conservation and hunting purposes. However, the success of traditional rabbit restocking is generally extremely low, and this has been attributed to short-term predation by mammalian carnivores. Hence, the recent recovery programs have tackled the problem of terrestrial predators with the use of exclusion fences, but no additional measures have been employed to avoid aerial predation. In this study, we have therefore conducted a field experiment to test the short-term effect of total predation exclusion in rabbit restocking enclosures, comparing rabbit abundance in plots which are only accessible to raptors (top open plots) and plots which are accessible to neither carnivores nor raptors (top closed plots). The results showed that the top closed plots had higher rabbit abundance in short-term, and the highest differences in rabbit abundance between two kinds of fences was attained in the first two weeks. We therefore conclude that the top closed plots were an effective tool to increase rabbit abundance during the first weeks after release through the exclusion of raptor predation.

Keywords *Oryctolagus cuniculus*; predator exclusion; rabbit conservation; raptors; restocking.

Resumen

Efecto a corto plazo de la exclusión absoluta de la depredación sobre la abundancia de conejo en los cercados de repoblación.- Alrededor de medio millón de conejos son translocados cada año en el suroeste de Europa con fines conservacionistas o cinegéticos. Sin embargo, el éxito de las repoblaciones realizadas por el método tradicional es generalmente muy bajo, lo cual ha sido atribuido a la depredación a corto plazo por los carnívoros. Por lo tanto, los programas de recuperación del conejo han abordado el problema de la depredación con el uso de cercados de exclusión de carnívoros terrestres, pero no se han empleado otras medidas para evitar la depredación aérea. En este estudio, nosotros hemos realizado un experimento para comprobar el efecto a corto plazo de la depredación aérea en cercados de repoblación de conejo, comparando la abundancia de conejo en cercados solo accesibles para las rapaces (abiertos) con cercados que no eran accesibles ni para las rapaces ni para los carnívoros terrestres (cerrados). Los resultados mostraron que los cercados cerrados tuvieron una mayor abundancia de conejo a corto plazo, y que las mayores diferencias en la abundancia de conejo entre los tipos de cercados fueron alcanzadas durante las dos primeras semanas. Por lo tanto, nosotros concluimos que los cercados cerrados por arriba fueron una herramienta efectiva para incrementar la abundancia de conejo durante las primeras semanas después de la suelta a través de la exclusión de la depredación por parte de rapaces.

Palabras clave: conservación del conejo; exclusión de la depredación; *Oryctolagus cuniculus*; repoblación.

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Introduction

Within its native range, around half a million European wild rabbits (*Oryctolagus cuniculus*) are translocated each year in order to boost rabbit population after their decline (Letty et al. 2008). Nevertheless, several scientific studies (Calvete et al. 1997; Letty et al. 2002b) show a very low success rate in rabbit restocking. In lagomorphs, some works have highlighted that the crux of the problem is the high short-term mortality (Calvete et al. 1997), as a consequence of predation by terrestrial carnivores, environmental novelty and stress (Calvete et al. 1997; Moreno et al. 2004; Letty et al. 2008; Misiorowska & Wasilewski 2012). Because of this, to prevent the impact of predation and rabbit dispersal after release, the construction of predator exclusion fences, within which the rabbits are released, is one of the most frequent actions in recent rabbit recovery programs (Ferreira & Delibes-Mateos 2010; Ward 2005).

Although some authors have suggested that the impact of raptors on rabbit enclosures could be very high and may therefore decrease restocking efficiency (Rouco et al. 2008; Cabezas et al. 2011), wildlife managers hardly ever carry out measures to tackle the problem of avian predation, other than providing more shelter. Then, in this study we aim to test the short-term effect of total predation exclusion in rabbit restocking enclosures. This is done by comparing the most common plots, which are only accessible to birds of prey, with those which are accessible to neither mammalian predators nor raptors. To date, this is the first study that reported the effect of total predator exclusion in a rabbit translocation program.

Material and Methods

Field work was carried out in central Sierra Morena, in southern Spain, where 6 of the main raptors that consume rabbit (Delibes-Mateos et al., 2007) were present in the study area: the Spanish imperial eagle (*Aquila adalberti*), the golden eagle (*Aquila chrysaetos*), the eagle owl (*Bubo bubo*), the bonellis eagle (*Aquila fasciata*), the booted eagle (*Hieraetus pennatus*) and the buzzard (*Buteo buteo*). Five fences were built close to each other in an area of 3 ha, three of which had no top net and were therefore only accessible to raptors (top open plots), and two enclosures with no access to either raptors or terrestrial carnivores (top closed plots), which were also equipped with a fence on the roof to prevent birds of prey from gaining access. All the fenced plots were of 0.5 ha, had the same number of artificial warrens (5 per plot), and had similar vegetation, structure and cover.

All the plots were fenced 0.5 m below the ground and 2m above the ground with two electric wires and a floppy overhang to exclude terrestrial carnivores (Moseby & Read 2006). Additionally, water and food were supplied ad libitum during the study period. Twenty five adult rabbits were released in each plot inside the artificial warrens in February 2010, in the same sex ratio: 4 females and 1 male in each warren (total: 20 females and 5 males per plot). All the rabbits were captured with the use of ferrets in a high rabbit density area in the south of the Córdoba province, and they were immediately transported to the release fences (in the north of the Córdoba province) with no vaccines, no confinement period or quarantines.

Rabbit abundance was estimated through the use of pellet counts at fixed sampling sites in 0.5-m2 circular sampling (Fernández-de-Simón et al., 2011). Within each plot 20 fixed points (4 x 5 grids) were set 20m from each other, where pellets were removed in all visits to ensure that only fresh pellets of less than 1 week old were counted. To standardize all pellets counts, a pellet abundance index (PAI) was estimated in each sampling site by dividing the number of pellets at each counting station by the number of days since the last count (Rouco et al., 2011b). The counts were repeated on a weekly basis for six weeks after release. To evaluate the effect of top fences, we used generalized linear mixed models (GLMM), where pellet abundance index in the counting points was the dependent variable, with Poisson error distribution and logit link function. "Treatment" (closed and open plots) and "week" (each weekly pellet count) were regarded as fixed factors; and "plot" (each individual plot) and "sampling site" (each counting point) were included as random factors. Finally, to compare rabbit abundance between weeks of sampling and treatment, we used Bonferroni's test for pairwise multiple comparisons within the mixed-model analysis. We used Spss 20.0 software (IBM corp. Chicago, U.S.A.) to perform the mixed models.

Results

We found a significant effect of treatment (Table 1), the "week" factor also had a statistically significant effect (Table 1), but the interaction between "treatment" and "week" was not significant (treatment x week, Table 1). Bonferroni pairwise test showed that rabbit abundance was higher in closed top plots than open top plots (P<0.05) at all times after release (Figure 1). The rabbit abundance was different from the first to the third week in both kind of plots (Bonferroni, P<0.05), this being more stables since the fourth week, when the rabbit abundance remained constant (Bonferroni, P> 0.05).

Table 1: Results of the generalized linear mixed models (GLMM) of the effect of treatment (closed and open top plots) and week on the rabbit abundance.

Variables	*F*	$d.f._{n,}$ $d.f._{d}$	*P*
Intercept	35.82	11, 288	<0.001
Treatment	16.86	1, 288	<0.001
Week	75.59	5, 288	<0.001
Treatment x Week	0.26	5, 288	0.932

d.f.n = degrees of freedom of numerator; d.f.d =degrees of freedom of denominator.

Discussion

Our results show that the top closed plots had higher rabbit abundance than the top open plots at all times after release, although the highest differences was recorded during the first two weeks, which could be due by raptor predation in the short-term. Indeed, various authors (Rouco et al., 2008; Cabezas et al., 2011) have suggested that aerial predation could be focused upon the rabbit enclosures, in which the rabbits are more vulnerable to predation due to the high rabbit concentration within them. On the other hand, five enclosures in a small area could attract birds of prey much more than independent enclosures. We detected 10 carcasses with clear signs of predation by birds of prey (evidence of feathers, tufts of hair or remains of long bones) in the open top plots during the study period, which represented a 76.92 % of the total individuals found dead in top open plots (n =13), although raptors may have consumed sick, weak or dead animals as carrion. In addition, the highest differences in rabbit abundance between both kinds of plots occurred in the first two weeks after release (Figure 1), when the movements as a consequence of exploratory behavior (Letty et al., 2008) and the stress caused by capture, handling, transport and release in an unfamiliar environment (Letty et al., 2003; Cabezas et al., 2011) might have increased the rabbits vulnerability to death by birds of prey.

Our results further showed that the differences between open and closed plots remained constant after the third week (Figure 1), suggesting that raptor predation of rabbits did not cause an important effect after this time. Indeed, despite the initial predation rate, Rouco et al. (2008) concluded that the predation by raptors cannot prevent rabbits from achieving a high abundance in fenced plots during the reproductive season. However the landscape structure may determine the rate of predation and therefore the effect of raptor predation on restocking success may play a different role depending on habitat type (Ontiveros et al. 2005; Kamieniarz et al. 2013) and should be locally evaluated.

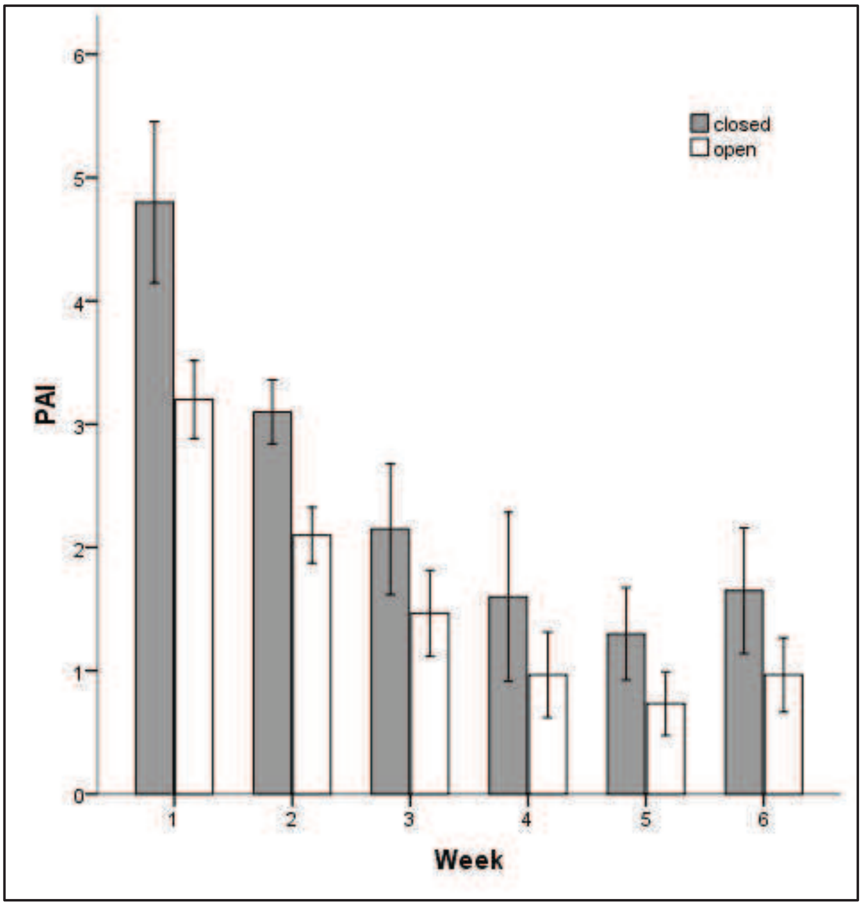

Figure 1: Effect of experimental treatment (open or closed plots) regarding to rabbit pellet abundance index (PAI) during the first six weeks after release. Bars represent mean values and 95 % intervals are shown

Nevertheless, both types of enclosure had an abrupt decrease in rabbit abundance during the first two weeks, and hence, causes of death other than predation (mainly stress) may also affect rabbit survival (Rouco et al. 2008; Cabezas et al. 2011). In our study the high density of released rabbit (50 individuals/ha) and a big bias in the sex ratio could have induced a huge social stress, thus increasing rabbit mortality. Therefore, although predation by raptors does not have a high effect on rabbit restocking success in the short-term, our results suggest that the use of nets to roof the fences plots could improve the rabbit survival in small enclosures (less than 0.5 ha). However, due to the high cost of a top fence, particularly in bigger plots, roofed enclosures may not be a cost-effective measure for raptors conservation and other measures as confinement of rabbits by warren fencing appear to be a more cost-effective measure to enhance rabbit survival. Moreover, an increase of refuge cover by distribution of pallets within the fenced plots could help prevent the aerial predation and, therefore, improve the short-term rabbit survival, although further research in long-term and large-scale in this topic is necessary to assess the trade off between cost and benefit of excluding aerial predation.

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Ethical standards: All processes of capture, transport and release were carried out by the same team from the Andalusia Environmental Agency in agreement with the standards and procedures lay down by the Spanish legislation (R.D. 1082/2009).

CHAPTER 3

CHAPTER 3

Factors affecting wild rabbit production in extensive breeding enclosures: how can we optimise efforts?

José Guerrero-Casado[*1], Leire Ruiz-Aizpurua[1], Antonio J. Carpio[1] & Francisco S. Tortosa[1]

Abstract

The decline in the wild rabbit population in the Iberian Peninsula has led hunters and authorities to rear rabbits in captivity systems for the purpose of their subsequent release. An alternative method to intensive rabbitry systems is extensive breeding enclosures, since they produce animals of a greater quality for hunting and conservation purposes. However, some of the factors that affect rabbit production in breeding enclosures are still unknown. We have therefore used partial least squares regression (PLSR) to analyse the effect of plot size, scrub cover, slope, initial rabbit abundance, the resources needed to dig warrens, predation and proximity to others enclosures on rabbit abundance. The results of our study show a positive effect of the number of other fenced plots within a radius of 3 km, a positive relationship with the availability of optimal resources to build warrens and a positive influence of intermediate values of scrub cover. According to our results, in order to maximize rabbit production in the enclosures it would be advisable to concentrate the restocking effort by ensuring that the restocking plots are close to each other, thus avoiding isolated enclosures. Furthermore, the selection of plots with an appropriate scrub cover and a great availability of elements that favour the construction of warrens such as large stones, sloping land or tall shrubs, may optimize results.

Keywords: captive breeding, *Oryctolagus cuniculus*, extensive system, wild rabbit.

Resumen

Factores que afectan a la producción de conejo de monte en cercados de cría extensivos: ¿cómo podemos optimizar los esfuerzos?- El declive de las poblaciones de conejo en la Península Ibérica ha aumentado la cría en cautividad de conejo de monte para su posterior liberación. Un método alternativo a la cría intensiva de conejo en cautividad son los cercados extensivos, ya que ellos producen animales con una gran calidad para su uso en las repoblaciones. Sin embargo, algunos de los factores que afectan la producción de conejos en estos cercados no han sido evaluados aún. Por lo tanto, nosotros hemos usados regresiones por mínimos cuadrados parciales (PLSR) para analizar el efecto del tamaño del cercado, cobertura de matorral, pendiente, recursos para excavar madrigueras, presión de depredación y la proximidad a otros cercados sobre la abundancia de conejo. Los resultados de nuestro estudio muestran un efecto positivo del número de otros cercados en un radio de 3 km, una relación positiva con la disponibilidad de recursos óptimos para excavar las madrigueras, y una influencia positiva de valores intermedios de cobertura de matorral. De acuerdo con estos resultados, para maximizar la producción de conejo en estos cercados es recomendable concentrar los esfuerzos construyendolos próximos unos a otros, evitando cercados aislados. Además la selección de zonas con una cobertura de monte adecuada y una gran disponibilidad de elementos que favorezcan la construcción de madrigueras como grandes rocas, taludes y matas de monte alto podría optimizar los resultados.

Palabras clave: cercados extensivos; conejo de monte; cría en cautividad; *Oryctolagus cuniculus*

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Introduction

The decline of the wild rabbit population is a major concern in Mediterranean ecosystems, in which rabbit is an important prey for more than 30 Iberian predators and one of the principal game species. Therefore, the interest in wild rabbit production for releasing purposes has increased over the last few decades (Sánchez-García et al., 2012). In fact, Sánchez-García et al., 2012 reported that the proportion of rabbits released in Spain that had been reared in captivity might exceed 50% of the total number of wild rabbits released.

The captive breeding of wild rabbits is normally carried out in intensive systems, but this process is difficult and not very productive owing to high levels of stress, behavioural problems (González-Redondo & Zamora-Lozano, 2008) and the low reproduction rate (González-Redondo, 2010). Semi-extensive breeding systems have attained a higher productivity (Arenas et al., 2006), but these smaller enclosures (500-800 m^2), do not reproduce natural conditions, and the animals reared in these enclosures might not therefore be appropriate for the purpose of release. In theory, the most appropriate system in which to produce wild rabbits for the purpose of release is extensive production in higher enclosures, since they simulate natural environmental conditions (food availability, soil type and aerial predator pressure), thus enabling the establishment of social interactions and the development of a dietary pattern and anti-predatory behaviour (Díez & Pérez-Garrido, 2003).

For these reasons, the establishment of extensive captive rabbit breeding enclosures has therefore become a widely used technique in conservation projects over the last few years (Ferreira & Delibes-Mateos, 2010). However, the importance of certain logistic issues such as enclosure sizes, the number of enclosures to be created or the distances between them, and their effect on further rabbit abundance, is not well known.

These factors are undoubtedly of great practical importance in optimizing rearing success owing to the fact that the high cost of fenced plots makes it difficult for private owners to afford them. Here we show results of a wild rabbit restocking project in which we analyzed the data from twenty three semi-extensive captivity breeding enclosures in order to evaluate the effect of the size of the plots, the distances between them, the presence of carnivores, the rabbits' facility to build their own warrens, the scrub cover and the slope on further rabbit abundance.

Materials and methods

Study Area

This study was carried out in central Sierra Morena, Córdoba, in Southern Spain (Figure 1). The study area included different ecosystems: Mediterranean scrubland, pine forest and oak savannah (*dehesa*). There are five species of terrestrial predator: the red fox (*Vulpes vulpes*), the egyptian mongoose (*Herpestes ichneumon*), the marten (*Martes foina*), the genet (*Genetta genetta*) and the wildcat (*Felis silvestris*); and six birds of prey: the Spanish imperial eagle (*Aquila adalberti*), the golden eagle (*Aquila chrysaetos*), the eagle owl (*Bubo bubo*), the bonellis eagle (*Aquila fasciata*), the booted eagle (*Hieraetus pennatus*) and the buzzard (*Buteo buteo*).

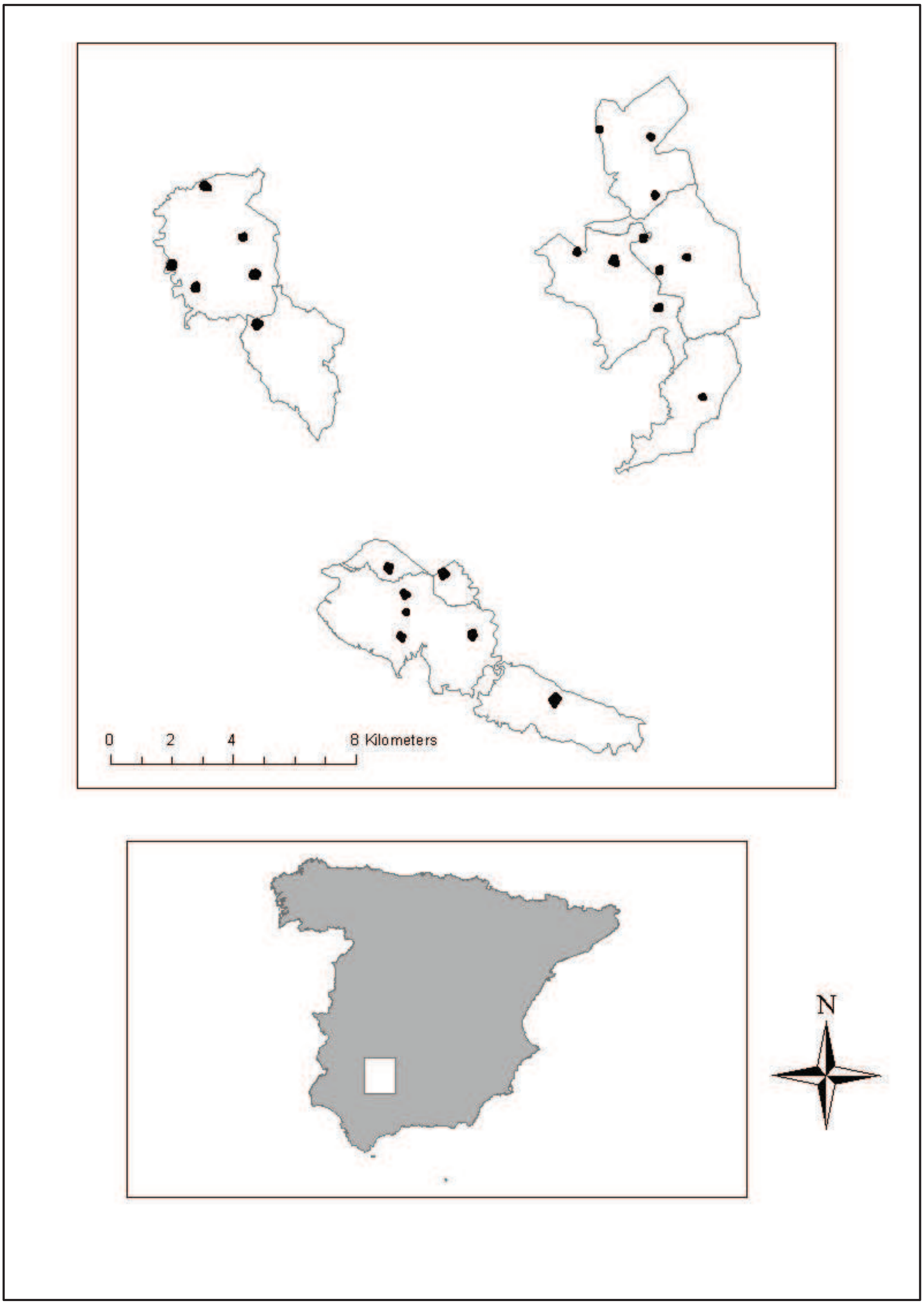

Figure 1: The white box indicates the location of study area in Spain. The enlargement shows the spatial distribution of the fenced enclosures (black circles), and the boundary of hunting states.

Rabbit release protocol and enclosures features

Twenty-three fences (range: 0.5-7.7 ha) were built throughout the year 2008 in the study area (Figure 1). All the plots were fenced 0.5 m below the ground and 1.7 m above the ground with two electric wires at a height of 30 and 150 cm above the ground level. Several artificial warrens made of pallets (3.5 ± 0.4, mean ±SE) were built in each plot. Water and food (grain mixture) were also supplied ad libitum throughout the year. The donor rabbit population was of a high density and was located in an agriculture area in the south of Córdoba province, and the release areas were located in the north of the same province (Figure 1). Both areas lie within the distribution limit of the genetic lineage traditionally associated with the sub-species *Oryctolagus cuniculus algirus* (Branco et al., 2000). The rabbits were translocated in October 2008, and the animals were mainly adults. Ferrets were used to capture the rabbits from their warrens in the morning, and they were immediately transported in commercial boxes to the release fences with no vaccines, no acclimation period or quarantines. The sex ratio was approximately 2:3 (males:females) in each fenced plot (0.62 ± 0.01, mean ± SE), the number of rabbits released ranged from 75 to 90 rabbits per hectare and the animals were released inside the artificial warrens. All processes of capture, transport and release were carried out by the same staff from the Andalusia Environmental Government.

Rabbit abundance

Rabbit abundance was estimated from November 2008 to July 2009 through the use of monthly pellet counts in fixed 0.5 m^2 circular sampling points (Fernández-de Simón et al., 2011) in a 20 m square grid located in the centre of the enclosure. The number of sampling points ranged from 15 in the enclosures less than 1 ha to 30 in the biggest one (more than 4 ha). Pellets were removed from the circular sampling plots after each count to insure that only fresh pellets of less than 1 month old were counted. This way, a pellet abundance index was created through the average density of pellets per day and surface for each month and enclosure (pellets/m^2/day). Since the objectives of the fenced areas were (1) to attain a high number of rabbits during the breeding season in order to permit them to later disperse into the surrounding area by opening the enclosures or by extraction, and (2) maintain a constant abundance to provide preys for endangered predators, the responses variable selected were the maximum abundance attained in each fenced plot (Model 1), and the mean rabbit abundance during the whole study period (Model 2).

Variables

Given that the rabbits released were slightly different in each enclosure, we included the initial rabbit density, as the number of rabbits released per hectare. In the field, the critical period for rabbit restocking is the first few weeks after their release (Calvete et al., 1997), so the abundance after this period of adaptation may be different in each fenced area and could affect further rabbit abundance. To account for this variation, the rabbit abundance one month after release was also included in the statistical analysis.

The number of artificial warrens per hectare was also included in the models. The size of each enclosure was obtained by geo-referencing the corners using GPS and ArcGIS software (ERSI, Inc, Redlands, CA, USA). Since the rabbit enclosures had different slopes, the average slope of each fenced plot was included in the analysis, which was calculated through Horn's method (Horn, 1981), by using the Digital Terrain Model of Andalusia (DTM, 10 x 10 m. resolution). In order to quantify the coverage of bushes, we performed 4 transects of a length of 50 m per hectare within the enclosures, in which vegetation was characterized at intervals of 50-cm. The percentage of cover occupied by the scrub stratum was calculated by applying the point-line intercept method (Canfield, 1941). However, we included the scrub cover in the models as a categorical variable (low: 0-25 %, medium: 25-50 %, high: > 50 %). In order to record the availability of optimal resources to build warrens, we defined a categorical variable with 4 levels according to the presence of appropriate structure and protection (rocks, tall scrub or sloping land) in the enclosures: 1- low (< 10 %), 2- medium (10-25 %), 3- abundant (25-50 %) and 4- very abundant (> 50 %).

Notwithstanding that the enclosures had two electric wires, they did not always prevent the entry of terrestrial predators. All the fenced areas were visited once per week during the study period, and during these visits, carnivore scats were annotated and removed. The model 1 shows the total number of scats found until the maximum rabbit abundance and the model 2 included the total sings during the whole study period. In all cases, the mammalian predators were removed from the rabbit enclosures by using live cage-traps.

An index of aerial predation was created too. This was done by dividing the set of the enclosures into three zones, in which a census of birds of prey was carried out at fixed points during the spring of 2009, with a total number of 21 hours of observation in each zone over three days (Redpath & Thirgood, 1997, Rouco, 2008). The total amount of flight time of the birds of prey was divided between the total number of observation hours, thus obtaining the average flight time for each zone (Rouco, 2008). This variable was then included in the models. Finally, in order to test the effect of the presence of other restocking fenced plots in the surrounding areas, we did different models which included the number of fenced areas in a radius of 1, 2, 3 and 4 km of each enclosure.

Statistical analysis

The statistical analysis was carried out by using partial least squares regression (PLSR). PLSR is a useful regression calibration technique when the number of predictor variables is similar to or higher than the number of observations, and/or the predictors are highly correlated (Carrascal et al., 2009), and it reduces the exploratory variables into a few components that have maximum covariance with the dependent variable. A PLSR should therefore be used to deal with the structure of our data with 23 cases and 10 exploratory variables. The number of significant components to be included in the model was selected by following the cross validation test described in (Umetrics, 2012), through the cross-validation index (Q^2), which was used to assess model significance ($Q^2 > 0.05$ for significant models). Moreover, the regression coefficient (R^2Y) and the predictor set variance (R^2X) used for the PLSR model were also used to interpret the PLS regression model. In order to determine the influence of individual variables as predictors of maximum annual abundance in the PLSR model, we used the variable importance in the projection (VIP) (Eriksson et al., 1999; Umetrics, 2012). Exploratory variables with a VIP value of over 1 were considered to be more relevant in explaining the variation observed in the variable response (Eriksson et al., 1999). In the first model, the response variable used was the maximum rabbit abundance reached in each breeding enclosure, and in the second model, the response variable was the mean rabbit abundance throughout the period of the study. The differences in rabbit abundance during the study period we tested by general linear models (GLM), with the month as categorical predictor and the enclosures as random factor. Post-hoc Tukey tests were conducted to illustrate differences among the monthly counts. All variables fitted a normal distribution ($P>0.05$; Shapiro-Wilk normality test). We used SIMCA-P software (version 13.0; Umetrics AB; Umeå, Sweden) to perform the PLS regression. Normality tests and GLM analysis were carried out with Statistica 7.0 software.

Results

The GLM revealed that rabbit abundance was different during the study period ($F_{8, 32} = 30.87$; $P = 0.001$). Indeed, the dynamics of the confined populations showed the typical oscillations of the species in a Mediterranean environment, with the onset of reproduction at the end of winter, reaching a maximum abundance at the end of spring and beginning of summer (June and July) (Figure 2). In the set of enclosures, the maximum abundance oscillated between 1.65 and 9.9 pellets/m^2/day, with an average (± SE) of 4.67 ± 0.46.

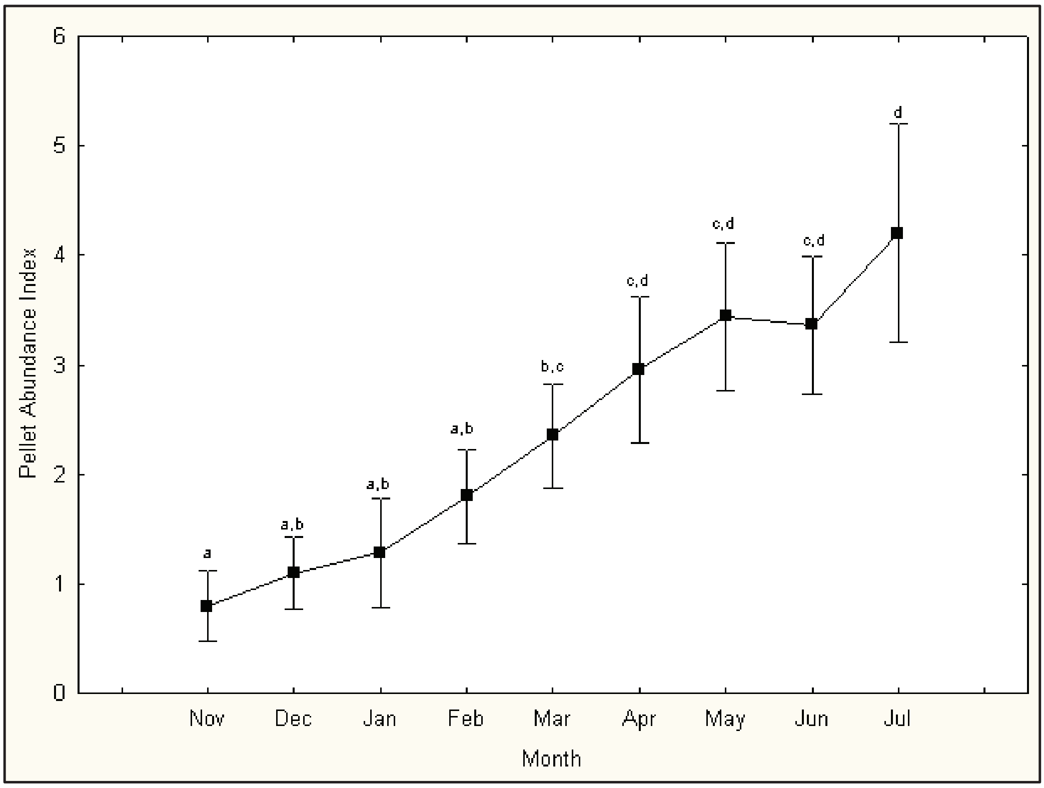

Figure 2: Average monthly rabbit abundance inside fenced plots expressed as a pellet abundance index (pellet/m2/day) during the study period. The errors bars represent a confidence interval of 95 %. Small case letters indicate significant differences (P < 0.05) between groups, as assessed using post-hoc Tukey tests.

Firstly, the model that best explained the maximum rabbit abundance (model 1) included the number of enclosures within a radius of 3 km ($R^2Y = 0.78$; $R^2X = 0.31$; Q2 = 0.42). In contrast, models including radii of 1, 2 and 4 km showed lower R2Y values ($R^2Y = 0.6$, $R^2Y = 0.63$ and $R^2Y = 0.67$ respectively). Similarly in model 2, the model with greatest value of R^2Y also included the number of enclosures in 3 km ($R^2Y = 0.61$; $R^2X = 0.27$; Q2 = 0.18), since the models that include the number of enclosures in 1, 2 and 4 km showed lower R^2Y values ($R^2Y = 0.51$, $R^2Y = 0.52$ and $R^2Y = 0.54$ respectively). Whatever the case, only three variables affected the rabbit abundance (VIP >1): the number of rabbit enclosures at a distance of 3 km, the availability of optimal resources to build warrens and the percentage of scrub cover (Table 1). The coefficients regression showed a positive effect of number of enclosures, a positive relationship with the availability of optimal resources to build warrens and a positive influence of medium values (25-50 %) of scrub cover (Table 1). Conversely, the models showed an adverse effect of lower values of warren resources and scrub cover on rabbit abundance.

Finally, we found 30 scats of terrestrial predators during the study period: 10 from common genet, 18 from stone marten and 2 from wild cat, in 12 of the 23 surveyed plots (54.5 %).

Tabla1: Influence (VIP) and coefficient of the exploratory variables used in the PLS regression models. R^2X = variance in the set of predictors used for the PLS model. R^2Y = explained variance by the PLS model. Q^2 = cross-validation index. Significant correlations coefficients are shown in bold type.

Variables	Model 1		Model 2	
	VIP	Coefficient	VIP	Coefficient
Number of enclosures in 3 km	**2,09**	0,47	**1,47**	0,23
Warren resources (very abundante)	**1,70**	0,31	**1,74**	0,18
Warren resources (low)	**1,28**	-0,32	**1,87**	-0,48
Scrub cover (medium)	**1,14**	0,22	**1,18**	0,08
Scrub cover (low)	**1,11**	-0,10	**1,08**	-0,07
Warren resources (medium)	**1,06**	0,10	0,92	0,01
Raptors time flight	0,71	0,10	0,76	0,13
Average slope	0,71	0,04	0,67	0,02
Initial rabbit density	0,65	-0,16	0,43	0,05
Artificial warren per hectare	0,65	0,15	0,87	0,21
Size of enclosures	0,45	0,11	0,37	0,05
Rabbit abundance on month after release	0,42	0,04	0,65	0,08
Warren resources (medium)	0,25	-0,03	0,45	0,12
Scrub cover (high)	0,16	-0,03	0,23	-0,05
Carnivore signs	0,16	-0,02	0,60	0,17
R^2Y	0,78		0,61	
R^2X	0,31		0,27	
Q2	0,42		0,18	

Discussion

Our results showed that the rabbit abundance has been proportionally higher in enclosures next to each other's, with great availability of optimal resources to build warrens and intermediate values of scrub cover. The increase in rabbit abundance during the study period bears witness to wild rabbit reproduction inside the enclosures. This might therefore be an appropriate tool with which to produce wild rabbits, since these semi-extensive systems avoid the usual problems related to the intensive rearing of wild rabbit, and produce animals with a greater ability to adapt to local conditions (Piorno, 2007a), which would then be highly adapted for their release into the wild. On the other hand, these enclosures also avoid genetic risks caused by hybridization with domestic rabbits that often occur on commercial wild rabbit farms (Piorno, 2007b). Finally, extensive systems also decrease human handling and improve the animals' welfare (Arenas et al., 2006).

Our results suggest that the spatial concentration of the enclosures favours wild rabbit production, since the number of enclosures in a radius of 3 km was positively correlated with the maximum and mean rabbit abundance (Figure 3). This could be attributed to the scattering of the predators in nearby enclosures, in which the set of enclosures might have enabled the rabbits to escape from the predation pit with greater ease, and this result motivate us to consider that an isolated enclosure is not viable, since it cannot support the impact of aerial predation. Furthermore, a radius of 3km forms a circumference of approximately 28 km2 around the enclosure, similar to the spatial scale (25 Km^2) often used at the home range level in studies on birds of prey (Martínez et al., 2003, López-López et al., 2006). The low rabbit density in the study area, the large quantity of rabbit predators and the elevated abundance attained in the enclosures, where the rabbits are highly vulnerable, might have attracted birds of prey (Rouco, 2008). Indeed, the aerial predation index showed a positive relationship with the rabbit abundance in both models, which may be owing to a higher raptor concentration in those spots with higher rabbit abundance.

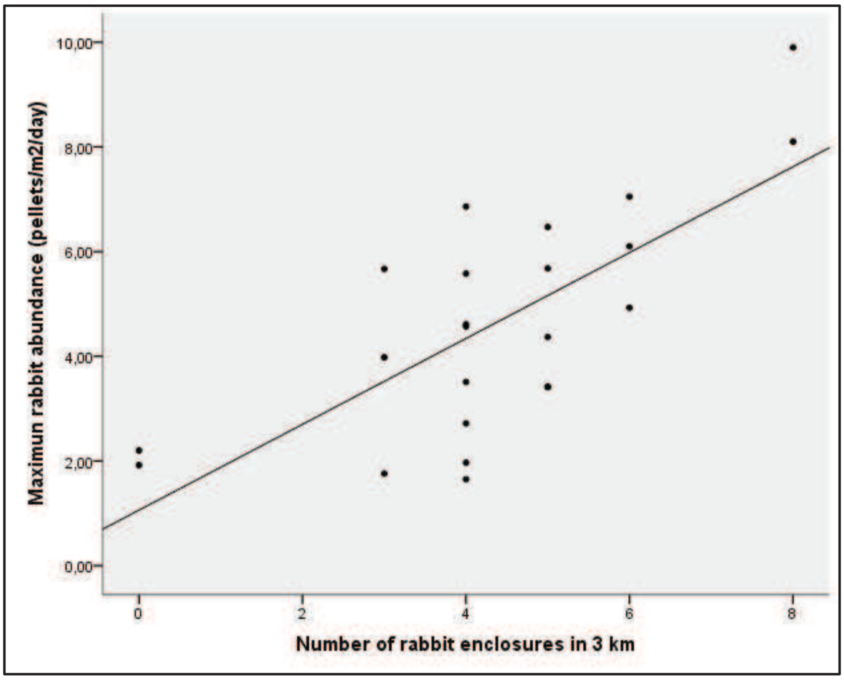

Figure 3: Correlation between the number of enclosures in a radius of three kilometres and the monthly maximum abundance reached in each fenced plots.

As expected, the availability of optimal places for the rabbits to dig warrens also had an important weight in both models. Several works have reported the rabbit's preference to build warrens under protective structures, such as trees, tall scrub and rocks (Palomares, 2003a, Barrio et al., 2009b). This may be owing to the fact that heavy rain can cause the death of juveniles as a result of flooding and/or tunnel-collapse, thus making unprotected warrens much more vulnerable to these phenomena (Palomares, 2003b). Likewise, protected warrens are less affected by predation (Villafuerte, 1994). In our experiment, the relatively low number of artificial warrens provided for the rabbits in the enclosures might be monopolized by the dominant rabbits (Mykytowycz & Gambale, 1965), and as a result of this, the subordinate animals must dig their own warrens, and then, a greater number of favourable places in which the rabbits can build their warrens would therefore allow secondary females to breed and thus contribute to a higher offspring production.

The percentage of scrub cover in each enclosure also had an important effect on rabbit production. Indeed, the PLS models showed a positive influence of intermediate values (25-50 %) and a negative correlation with low bush coverage (0-25 %). These results highlight the role of habitat features in wild rabbit abundance, since in the wild, rabbits attain high abundance in those places in which shelter (scrub) and food resources (pastures) are widely available. The highest values of scrub cover (more than 50 %) did not show a significant effect, since the range of rabbit abundance in these plots was very huge. Hence, the breeding enclosures should be built in places with an optimal availability of shelter, and enclosures with very low or very high bush cover should be avoided.

Our data showed that the electric fence was not completely effective in preventing the entry of these terrestrial predators, although the entry of some carnivores did not affect rabbit abundance. We deem that a small curved overhang on the top of fences (Moseby & Read, 2006) could prevent the entry of mammalian predators. Lastly, the slope, the size of the fenced plots, the initial rabbit density and the abundance one month after release did not affect further rabbit abundance, perhaps because the distances between plots and the presence of elements that favour the construction of warrens and shelter played a more relevant role in the model.

Conclusions

In agreement with the findings, we suggest that the new extensive rabbit captive enclosures should be created less than 3km apart from each other in order to minimize aerial predation, and that isolated enclosures should be avoided, thus minimizing the impact of predation. Our results also suggest that the availability of the optimal resources needed to dig warrens and the scrub coverage would appear to be others crucial factors, and then, the selection of plots with an appropriate structure and protection such as large stones or the presence of tall shrubs might therefore optimize the rearing results.

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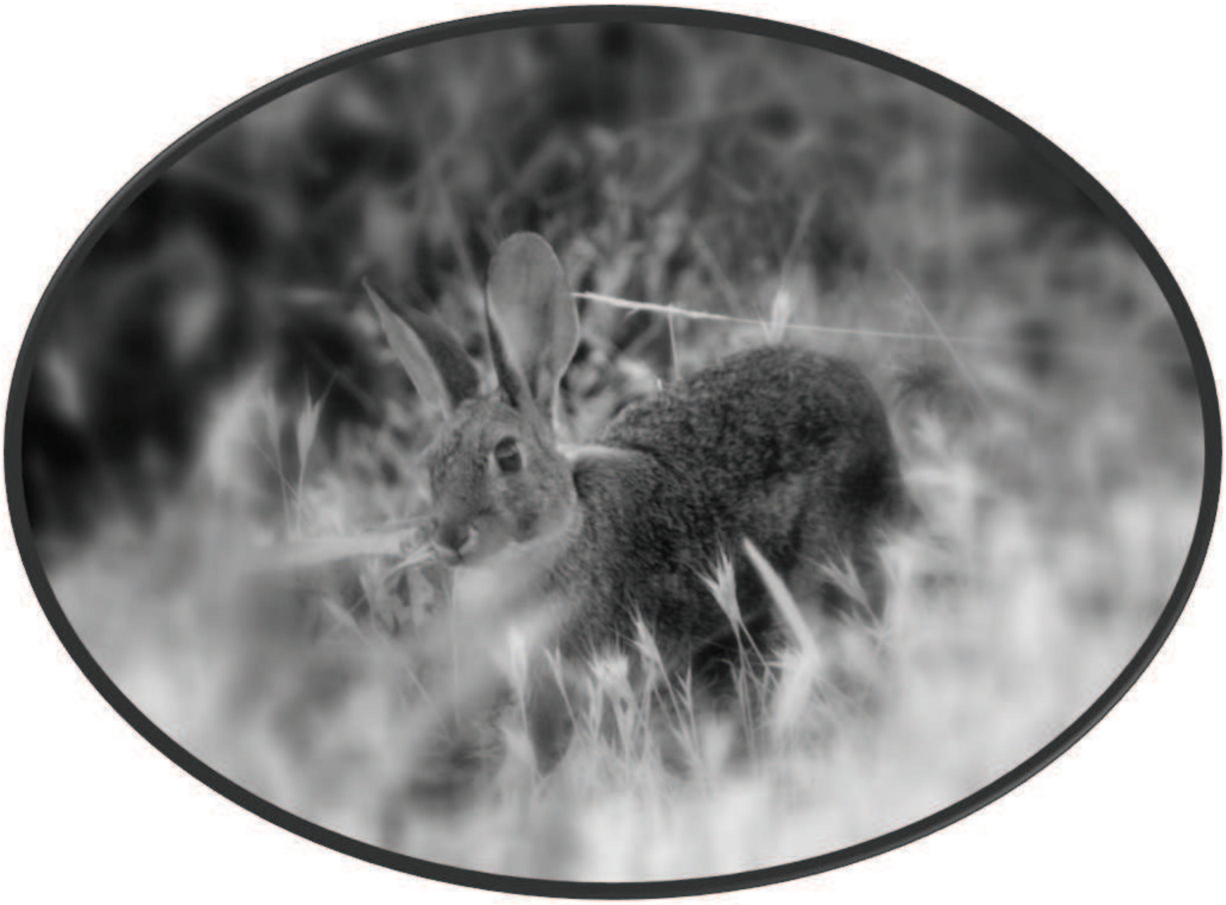
CHAPTER 4



CHAPTER 4

Restocking a keystone species in a biodiversity hotspot: recovering the European rabbit on a landscape scale.

José Guerrero-Casado*[1], Antonio J. Carpio[1], Leire Ruiz-Aizpurua[1] & Francisco S. Tortosa[1].

Abstract

Rabbit populations in Iberia remain at low densities in several areas in which their endangered predators still coexist, and the recovery of these populations is therefore urgent if the integrity of Iberian Mediterranean ecosystems is to be maintained. The enhancement of wild rabbit populations has been attempted through the use of *in situ* extensive rabbit captive breeding enclosures (restocking plots), which reduce mortality caused by terrestrial predators and dispersal movements and permit the breeding of young individuals which can then naturally disperse to settle in the surrounding areas. However, their effectiveness, the role of its size, the optimal habitat management that should be promoted around them and the habitat features remains uncertain. Here, we show results from a four years study of an ambitious rabbit restocking plan on a landscape scale. We measured rabbit abundance in a vast area in which thirty-two restocking plots were built to create an initial rabbit population for further dispersion, in addition to an intensive habitat management program. We also compared rabbit abundance between managed and unmanaged UTM cells of 2.5 x 2.5 km. Our results showed that rabbit abundance was 3 times higher in managed cells, but four years after restocking, rabbit abundances had only reached the threshold needed to support stable Iberian lynx presence (at least 10 latrines per Kilometre^{-1}) in 9 of the 23 managed cells. Rabbit abundance was strongly affected by habitat treatment and scrub coverage. The increase of shelter was useful in low cover areas but ineligible in places with high scrub cover, where the increase of refuge plus scrub clearing to create pastures improve rabbit abundance more effectively. In the light of our results, restocking plots should be built only in places with suitable habitat, whereas pastures should be created in dense scrublands and refuge in low cover areas.

Keywords: habitat management; *Oryctolagus cuniculus*; translocation; wildlife management; wild rabbit.

Resumen

Recuperación de una especie clave en un ecosistema de alta biodiversidad: repoblaciones de conejo europeo a gran escala.- Las poblaciones de conejo en la Península Ibérica se mantienen a bajas densidades en varias zonas donde sus depredados amenazados todavía existen, y por lo tanto la recuperación de estas poblaciones es urgente para mantener la integridad de los ecosistemas Mediterráneos. Para mejorar las poblaciones de conejo se han utilizado cercados de cría extensivos (cercados de repoblación), los cuales reducen la mortalidad causada por carnívoros terrestres así como los movimientos iniciales de dispersión, permitiendo la cría de individuos que pueden ser utilizados para colonizar las aéreas adyacentes a los cercados. Sin embargo su efectividad, el papel del tamaño, el manejo de hábitat que debe ser realizado alrededor de los mismos y las características del hábitat todavía no han sido evaluados. En este trabajo hemos mostrado los resultados de 4 años de estudio de un ambicioso proyecto de repoblación a escala de paisaje. Nosotros medimos la abundancia de conejo en una extensa área donde se construyeron 32 cercados de repoblación para crear poblaciones iniciales de conejo para su futura dispersión, conjunto con un intensivo programa de mejora de hábitat. Se comparó la abundancia de conejo entre celdas UTM de 2.5 x 2.5 kilómetros con y sin actuaciones. Los resultados muestran que la abundancia de conejo era 3 veces mayor en celdas con actuaciones, sin embargo 4 años después de las repoblaciones, la abundancia de conejo sólo había alcanzado el umbral para soportar la presencia estable de lince ibérico (al menos 10 letrinas por kilómetro) en 9 de las 23 celdas manejadas. La abundancia de conejo estuvo fuertemente influenciada por el tratamiento del hábitat realizado y la cobertura de matorral. El incremento de refugio fue útil en areas con baja cobertura pero insignificante en lugares con alta cobertura de matorral, donde el incremento de refugio junto con el desbroce del monte para crear pastizales mejora más efectivamente la abundancia de conejo. A la luz de nuestros resultados, los cercados de repoblación deberían construirse en lugares con un hábitat adecuado, mientras que la creación de pastizales en zonas de matorral denso y el aumento de refugio en zonas con escases de cobertura es necesario para aumentar la capacidad de carga.

Palabras clave: conejo de monte; manejo del hábitat; manejo de la fauna silvestre; trasnlocaciones

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Introduction

The Mediterranean Basin is a global hotspot of biodiversity owing to the exceptional concentrations of endemic species (Myers et al., 2000). In the Iberian Peninsula, the European rabbit (*Oryctolagus cuniculus*) plays a key role in the conservation of the Mediterranean basin hotspot, since the rabbit is an ecosystem engineer and an important prey for more than 30 predator species (Delibes-Mateos et al., 2008a). The rabbit is also the principal small game specie in France, Portugal and Spain (Delibes-Mateos et al., 2008a; Letty et al., 2008). However, local extinction or very low rabbit abundances is frequent in south-western Europe after the abrupt fall in rabbit populations during the 20th century as a result of habitat loss, over-harvesting and the outbreak of two viral diseases (myxomatosis and rabbit haemorrhagic disease). This sharp decline in rabbit populations may have had important cascading effects on the functioning of the Iberian-Mediterranean ecosystem, with serious ecological and economic consequences (Delibes-Mateos et al., 2008a), particularly for the Iberian lynx (*Lynx pardinus*) and the Spanish Imperial Eagle (*Aquila adalberiti*), two endangered rabbit-specialist predators (Ferrer & Negro, 2004).

Much emphasis has therefore been placed on recovering rabbit populations owing to the interest in the species for hunters and conservation (Angulo, 2003; Delibes-Mateos et al., 2008b; Letty et al., 2008). The strategies most frequently used are habitat management, a reduction of hunting pressure, predator control and vaccination (Angulo, 2003), although rabbit restocking has recently undergone a significant increase in Iberia and France (Letty et al., 2008). However, despite the fact that half a million rabbits are translocated in south-western Europe every year (Letty et al., 2008), very low success rates have been recorded in traditional rabbit restocking efforts (Calvete et al., 1997). This low restocking success signifies that wildlife managers have started to combine certain tools in order to improve rabbit survival, such as acclimatization in the release site or habitat management. More recently, the establishment of in situ extensive rabbit captive breeding enclosures (hereafter restocking plots) has also taken place and has become a widely employed technique in conservation projects (Ferreira & Delibes-Mateos, 2010; Guerrero-Casado et al., 2013b). The role of fences is to keep the rabbits confined in order to reduce their mortality as a result of terrestrial predators and their initial dispersal movements, and finally to obtain a high density inside the fenced plots and well-acclimated in situ animals in order to allow them to further colonise the surrounding areas (Rouco et al., 2008; Guerrero-Casado et al., 2013a).

Nevertheless, most rabbit translocation programs are not monitored (Cabezas & Moreno, 2007), and no studies have been conducted to address the final restocking success in the long-term and on a large-scale. Furthermore, despite the high cost of restocking plots, the effectiveness of further release in order to establish self-sustaining rabbit populations and the role of some important issues such as their size, habitat attributes or the optimal habitat management around them remain uncertain. In this four year study, we assessed rabbit abundance around 32 restocking plots spread over 14000 hectares in order to test the effectiveness of restocking 4 years after release. The aim of this paper is to analyze the effect of habitat features, the effectiveness of habitat management (creating shelter, artificial warrens, and pastures) and the importance of restocking plots size and distribution in facilitating the long-term and large-scale settlement of new free-ranging wild rabbit populations.

Material and Methods

Study area

The fieldwork took place in Sierra Morena mountain chains (Southern Spain, Figure 1), in which the current scarcity of rabbits is a major conservation concern, since the Iberian lynx and the Spanish Imperial Eagle still coexist in this area. The study area included tree species such as holm oak (*Quercus ilex*) and cork oak (*Quercus suber*), pine reforestations

(*Pinus spp.*), Mediterranean scrubland dominated by *Cystus* spp., *Pistacia* spp., and Rosmarinus spp and pasture areas occupied by oak savannah (*dehesa*).

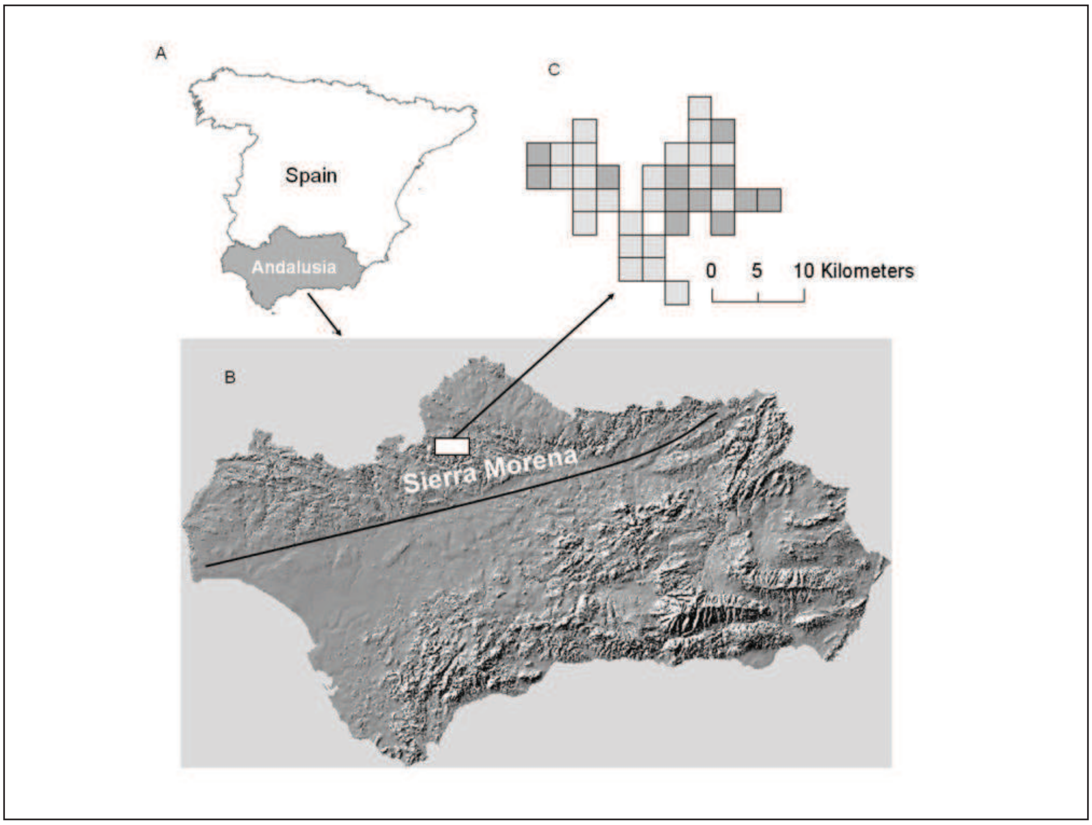

Figure 1: A) Localization of the study area in Spain. B) Sierra Morena mountains chain is shown in schematic form in Andalusia. C) The UTM 2.5 x 2.5 km UTM-cells with management actions and control are shown in light grey and dark grey respectively.

Rabbit release protocol and enclosures features

Thirty-two restocking plots (range 0.5 – 7.7 hectares) were built in the study area throughout the years 2008-2010 (Figure 1). Rabbits were released with no vaccines, and no acclimation period or quarantines during the years 2008 and 2009 in autumn or winter (for more details see Guerrero-Casado et al., 20113a). In the subsequent years (2009-2011) the fenced plots were opened by small gates on the fences at the end of the breeding season when rabbits had reached their highest abundances inside the restocking plots (Guerrero-Casado et al., 2013a) in order to permit their natural dispersal into adjacent areas. All processes of capture, transport and release were carried out by the same staff from the Andalusia Environmental Council.

Rabbit abundance

Around each restocking plots, wild rabbit abundance was estimated through the use of latrine counts by walking 4 transects of 500-m each (n = 128), signifying that there was a total transect distance of 2-km around every restocking plot. These surveys were performed both before and after these were opened (in the summers of 2008-2009 and 2012, respectively). A latrine was defined as any pellet accumulation containing at least 20 pellets over a surface of 20 x 30 cm (Virgós et al., 2003). Latrine counts have already been used as an index with which to estimate relative rabbit abundance in many scientific works (Virgós et al., 2003; Calvete et al., 2004a; Lozano et al., 2007; Cabezas-Diaz et al., 2011), since this rabbit abundance index can provide a useful estimation of rabbit abundance in large-scale studies (Calvete et al., 2006). Furthermore, latrine abundance and rabbit density estimated by direct observations are highly correlated in Sierra Morena (Gil-Sánchez et al., 2011). All these transects were GPS referenced, as was the location of every restocking plot. In each transect, the distances from the transect to the perimeter of the closest restocking plot were calculated every 100-m, and an average value was estimated in each one. This variable and how many restocking plots there were in a 3 km radio were obtained by using ArcGIS software (ERSI, Inc, Redlands, CA, USA).

In summer 2012, following the methodology employed by Gil-Sánchez et al., (2011), the study area was divided into 2.5 x 2.5 UTM grill cells (figure 1), in which rabbit abundance was sampled by latrine counts in managed (n = 23) and unmanaged cells (n = 12). The aforementioned authors reported that in Sierra Morena, stable lynx presence and reproduction were confirmed in grids with at least 10 rabbit latrines per Km^{-1}, and this value was therefore used to evaluate the effectiveness of the rabbit restocking plan.

Habitat features and Management

Scrub coverage (%) was estimated visually in circular plots of 25-m radius every 100-m in each walked transect, following similar protocols to general habitat-rabbit studies (Virgós et al., 2003). Prior to sampling, the same field workers performed trials to homogenise the percentage of the area covered by shrub vegetation. We used average values of scrub cover for each transect, which was reclassified in a categorical variable with 3 levels: low (0-30 %), medium (30-60 %) and high (> 60 %). Three different habitat enhancement strategies were then carried out in a 500-m radius of each enclosure: (1) no action; (2) an increase in the amount of refuge available by heaped wooden branches and artificial warrens created with wooden pallets and covered with stones, branches and earth; and (3) an increase in refuge plus the creation of pasture lands by clearing scrubland.

Statistical analysis

First, a generalized linear mixed model (GLMM) was performed to assess differences in rabbit abundance before and after opening, including the restocking plot as random variable (model 1). In this case, the number of latrines per Km-1 around each restocking plot was used as dependent variable and it was modeled by generalized mixed models, using a Poisson distribution with a log-link function as the error distribution. Second, a general linear mixed model (LMM) was performed to test differences in rabbit abundance between managed and unmanaged UTM cells (model 2), using the number of latrines per Km-1 in each cell as response variable, which fitted a normal distribution at

UTM-cell level. The coordinates X and Y of the center of UTM cells were considered to avoid spatial autocorrelation. Finally, in summer 2012, the effect of habitat management and restocking plots features on rabbit abundance around these was evaluated by generalized linear mixed models (model 3), in which the number of latrines in each transect was used as a dependent variable, which fitted a Poisson distribution with a log-link function. Since transects were grouped into each restocking plots, and data from transects were correlated within each restocking plots, it was included as a random variable. In this last model (model 3), the mean distance from each transect to the restocking plot, its surface (ha) and the number of restocking plots in a radius of 3km were included as continuous variables, whereas the percentage of scrub, the amount of years since each plot had been opened and the habitat management were treated as categorical variables with 3 levels. The interactions between habitat treatment and scrub coverage, and between habitat management and 'years opened' were also included. Fisher's least significant difference test (LSD test) for comparisons of the estimated means within mixed analysis was developed to check differences among the level of categorical variables and to illustrate the interactions. The assumptions of normality, homogeneity and independence of residuals were confirmed (Zuur et al., 2009), and we therefore log-transformed the size of enclosures, and the mean distance from the segment to the enclosure in order to fulfill normality. The entire statistical analysis was performed using InfoStat software.

Results

Prior to restocking, the rabbit abundance in the study area was very low and uniform, with an average value of 1.028 ± 0.314 latrines/km (mean ± S.E.), whereas after management actions the rabbit abundance was considerably higher (9.34 ± 1.91, mean ± S.E.; model 1, $X^2_{1, 29} = 246.04$, $P < 0.001$). Furthermore, in managed cells rabbit abundance (9.048 ± 1.98, mean ± S.E.) was statistically higher (model 2, $F_{1, 33} = 7.9$; P =

0.011) than in unmanaged areas (2.91 ± 1.32, mean ± S.E.). Our results further showed that 3 or 4 years after opening the restocking plots 9 of the 23 UTM cells in which wild rabbit conservation actions had taken place had attained the minimum rabbit density needed to support stable lynx presence (at least 10 latrines per Km^{-1}).

Regarding the factors affecting rabbit abundance around the restocking plots, the model 3 showed a significant effect of habitat management actions, scrub coverage, and of the years since the plots had been opened, while its area, the number of these in a radius of 3 km and the distance of transect to the restocking plot did not have a significant relationship with latrine abundance (Table 1). Nevertheless, the interaction between the habitat management strategy and scrub coverage also had a statistically significant effect, which indicates that the habitat management tools did not have the same effect on wild rabbit abundance depending of scrub coverage (Figure 2). There were no significant differences between unmanaged segments and an increase in refuge in high cover areas, whereas the increase in refuge did enhance rabbit abundance in low cover areas (Figure 2). The interaction between habitat treatment and the years since the plots had been opened also showed a significant differences, which are illustrated by Fisher test (Figure 3).

Table 1: Chi-square, p-values and coefficients of the variables included in the mixed model (model 3) to explain rabbit abundance. df show the degree of freedom of the numerator. Coefficients for the level of fixed factors were calculated using reference values of 1 in the variable 'years opened', 'low' in the variable 'scrub coverage', and 'shelter plus scrub clearing' in 'management'.

Variables	Chi-square	d.f.	P	Coeffcients ± E.S.
Management	154.55	2	<0.0001	Unmanaged = -1.97 ± 0.79 Shelter = 0.56 ± 0.8
Years opened	17.37	2	0.0002	2 = -0.15 ± 0.97
				3 = 0.78 ± 0.95
Scrub coverage	27.19	2	<0.0001	Medium = 1.49 ± 0.42
				High = 1.31 ± 0.45
Restocking plots in 3 km	0.01	1	0.9239	0.04 ± 0.11
Area	0.07	1	0.7888	-0.47 ± 0.85
Distance	0.97	1	0.3235	0.13 ± 0.14
Management*Years	21.39	4	0.0003	
Management* Scrub	22.16	4	0.0002	

Discussion

In southern Spain, the Spanish Imperial Eagle and the Iberian lynx, which are endangered rabbit predator species, are confined into those areas in which non-trophic detrimental factors have been relatively low and rabbit densities are sufficient (Ferrer & Negro, 2004; Real et al., 2009). Rabbit recovery in these spots is a critical concern if the local extinction of these specialist predator species is to be avoided (Ferrer & Negro, 2004; Real et al., 2009), and it is therefore imperative to establish guidelines with which to recover wild rabbit populations in these areas. The results presented here evidence the effectiveness of the restocking plots combined with habitat management on a landscape scale. We have even shown that rabbit abundance attained with these techniques could support stable lynx presence in some circumstances, and it might thus be considered an appropriate tool to promote the establishment of new lynx and imperial eagle territories or enhance rabbit abundance in the existing territories. Indeed, two new Spanish Imperial Eagle breeding pairs were located in the study area after management actions (author unpublished data).

In our study, the effect of the habitat enhancement actions depended of scrub cover. In high cover areas, the zones with an increase in refuge did not significantly differ from those which were unmanaged (Figure 2). Refuge was easily available in these zones, may explain why the increase in shelter with branches and artificial warrens did not enhance rabbit abundance, and the creation of grazing areas was also necessary to increase rabbit densities. In the study area, the loss of the traditional management of scrubland would appear to be one of the main factors explaining rabbit scarcity (Delibes-Mateos et al., 2010). Hence, the enclosures should be placed in habitats with great diversity and a complex structure, and the objective of habitat management should be to minimize predation rate by increasing habitat heterogeneity in areas adjacent to restocking plots. In fact, the highest rabbit abundance was attained in those areas in which the habitat complexity was enhanced by increase of refuge and scrub clearing (Figure 2).

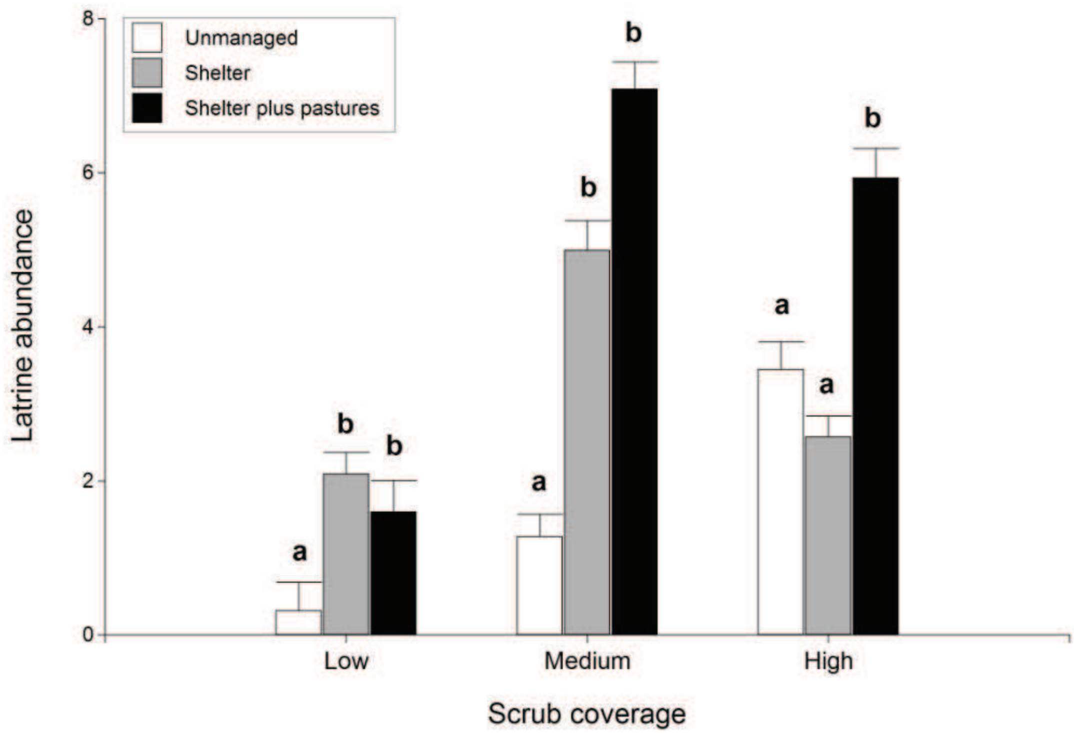

Figure 2: predicted mean values (± S.E.) of latrine counts with different habitat management actions in low, medium and high scrub cover. Small case letters indicate significant differences (P= 0.05) between groups of habitat treatment according to Fisher LSD tests.

The increase in refuge with heaped wooden branches and artificial warrens was effective in places with low and medium availability of shelter (Figure 2). Artificial warrens and branches provide protection against predators and against extreme climates, favour the establishment of social ties and provide an optimal place for breeding (Catalán et al., 2008; Rouco et al., 2011a). Since rabbit abundance in unmanaged areas was higher in high and medium cover areas, we deem that it is better to build restocking plots in those habitats than to perform an insensitive management in inappropriate areas. However, caution is needed when interpreting our results, and the management strategies should be carefully based on the landscape and vegetation features in each situation.

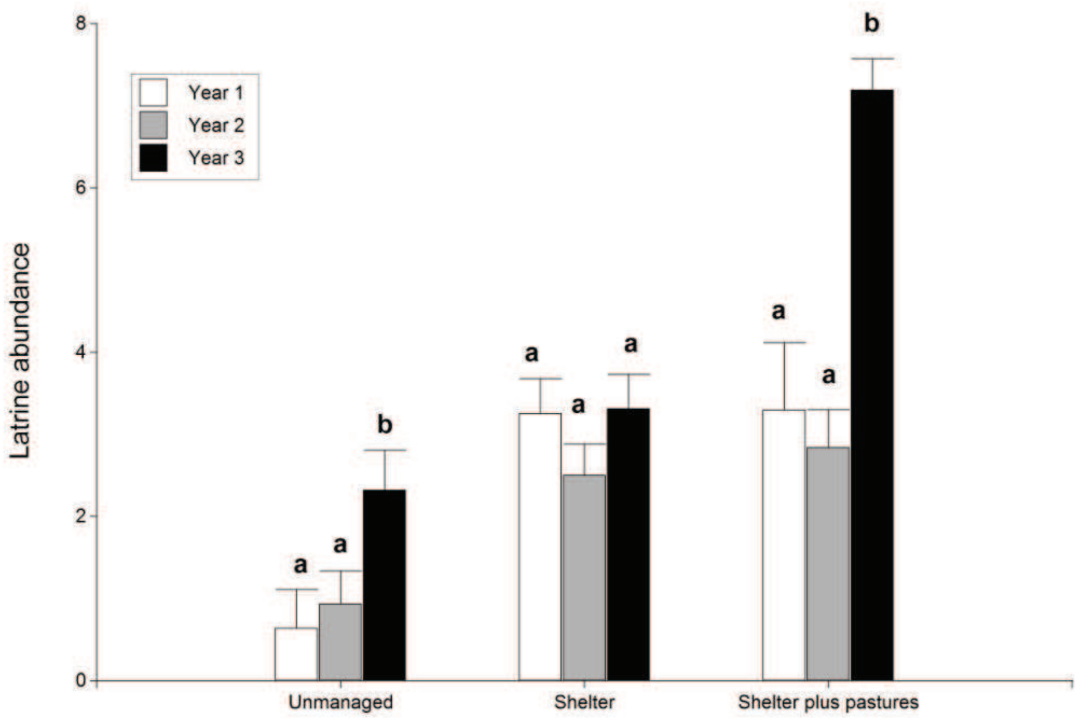

Figure 3: predicted mean values (± S.E.) of latrine counts with different habitat management actions in the years since the plots had been opened. Small case letters indicate significant differences (P= 0.05) between years according to Fisher LSD tests.

The distance from the transect to the restocking plots, their concentration and their surface did not have any significant effect on rabbit abundance. This may be owing to the fact that the habitat quality and the management actions play more relevant roles in the establishment of rabbit populations around the restocking plots. However, concentrating restocking effort by building restocking plots close to each other would perhaps favour rabbit establishment in adjacent areas under similar habitat conditions, since intra-specific interactions such as proximity to nearby warrens might be playing a primary role in these low-density populations (Barrio et al., 2009b). Small isolated and fragmented populations are much more vulnerable to stochastic phenomena (Wilcox & Murphy, 1985), and we therefore suggest that it would be advisable to concentrate the management actions in small areas rather than to establish dispersed actions in large areas (Carvalho & Gomes, 2003). Lastly, the years since the plots had been opened had also a significantly statistically effect, and the higher values were recorded in the restocking plots which had been opened 3 years before in those places with increase of refuge and creating of pastures (Figure 3). It suggests that the rabbit colonization is a long-time process, and it is necessary monitoring rabbit density in long-term to assess the effectiveness of restocking actions.

Conclusions

Our data suggest that restocking in enclosures for further release plus habitat management increase rabbit populations to support territories of top specialist predators, despite the flaws in the environment in these areas. In summary, restocking plots should be concentrated in those areas that are close to suitable habitats, and in the case of rabbit translocation in sub-optimal habitat, habitat enhancement should take place to promote mosaics of pastures and scrubs in order to raise the carrying capacity around the restocking plots, through the creation of feeding habitats in dense scrublands and the increase of shelter in low cover areas.

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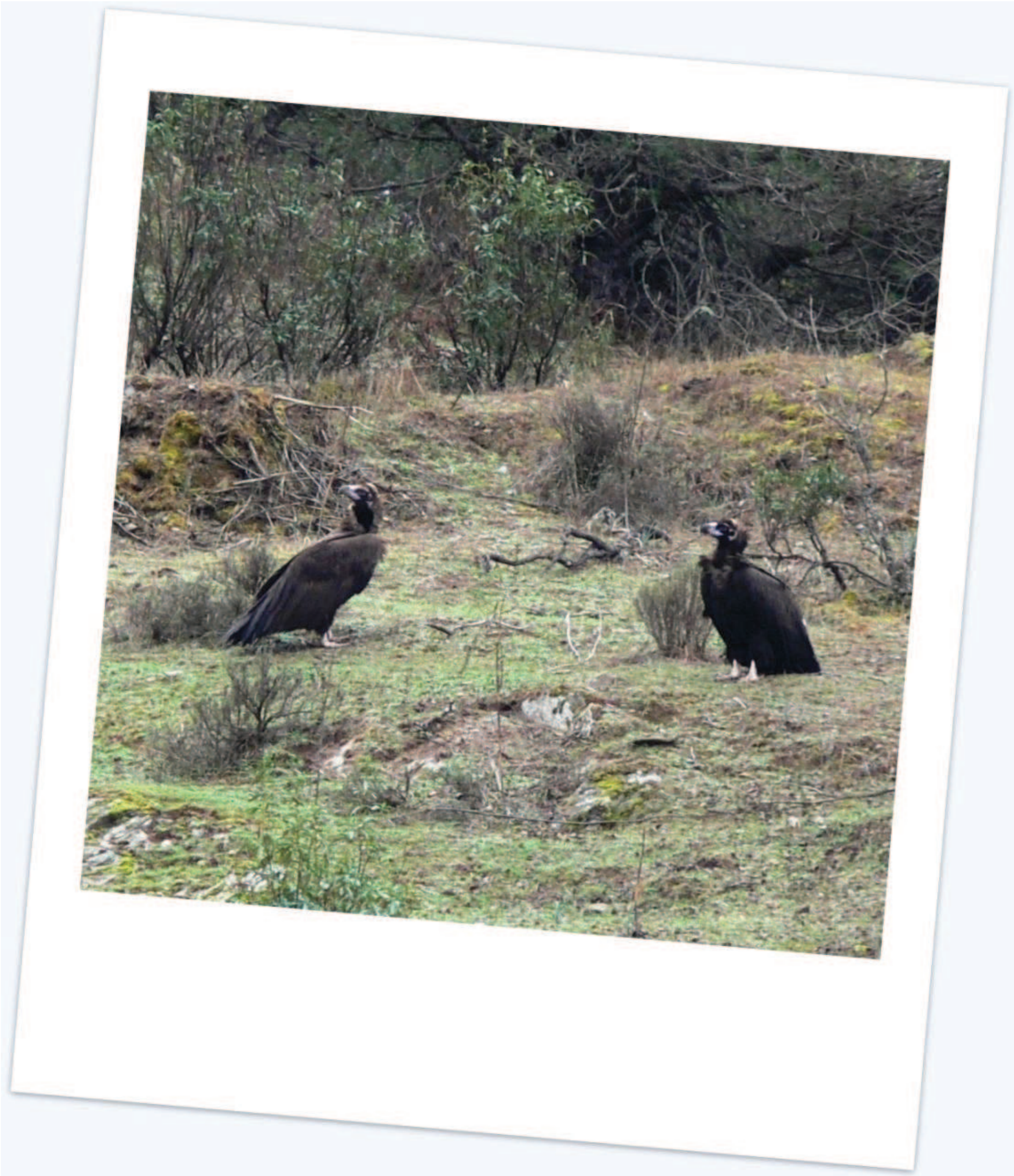
CHAPTER 5



CHAPTER 5

Modelling the nesting-habitat of the cinereous vulture (*Aegypius monachus*) on a fine scale for conservation purposes.

José Guerrero-Casado[1, 2]., Rafael Arenas[3], & Francisco S. Tortosa[1].

Abstract

The cinereous vulture´s nesting-habitat is highly affected by human disturbance and widespread habitat loss, and it is therefore imperative to identify suitable areas in which the harmful factors are not present. The aim of this work is to apply habitat modelling techniques through the use of a logistic regression approach and GIS tools in order to generate a predictive habitat suitability map for the cinereous vulture´s nesting-habitat on a fine scale (50-m resolution) for local conservation applications within its breeding area. This has been done by comparing the habitat features of 43 nest-locations and random points in order to identify the nest-habitat selection in the region of the Hornachuelos Natural Park (Spain). Compared with random points, nests were found to be located farther from roads, villages and patch edge, in large vegetation patches containing high cork oak cover on stepper slopes with lower solar radiation. The predictive map revealed that less than 8 % of the study area had higher probability value than 0.8. The low values of favourableness suggested that the conservation of the best suitable areas is a major concern. The fine scale employed, suggests that the predictive map may be valuable in designing conservation priority areas.

Keywords: conservation planning, GIS, habitat modeling, nest-site selection, predictive map, raptors.

Resumen

Modelación del hábitat del Buitre Negro (*Aegypius monachus*) a fina escala con fines de conservación. El hábitat de nidificación del Buitre Negro está altamente amenazado por las molestias causadas por el hombre y la continua pérdida de hábitat, y por lo tanto la identificación de las áreas más propicias para la cría es vital para asegurar la viabilidad de sus poblaciones. El objetivo de este trabajo es aplicar técnicas de modelación del hábitat usando regresiones logísticas y sistemas de información geográfica para generar un mapa predictivo de las zonas potencialmente favorables para la nidificación del buitre negro a fina escala (50 metros de resolución), el cual puede ser utilizado para acciones de conservación dentro de su área de cría. Esto fue realizado mediante la comparación de las características del hábitat de la ubicación de 43 nidos y puntos aleatorios en la colonia del Parque Natural de Hornachuelos. Comparados con los puntos aleatorios, los nidos están localizados alejados de carreteras, núcleos urbanos y del borde del parche de vegetación, en grandes parches de vegetación con una alta cobertura de alcornoques, pronunciadas pendientes y baja radiación solar. El mapa predictivo reveló que menos del 8 % de la zona de estudio tiene una alta probabilidad de albergar nuevos nidos, por lo la conservación de las áreas más adecuadas debería de ser una prioridad. La fina escala empleada sugiere que el mapa predictivo podría ser válido para diseñar las áreas prioritarias para su conservación.

Palabras clave: lugar de nidificación; mapa predictivo; modelación del hábitat; planes de conservación; SIG; rapaces.

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Introduction

Understanding the strength of the relationships between habitat and the distribution of raptors may be important in the management of protected areas and in contributing to the development of successful conservation programs (Limiñana et al., 2011, Di Vittorio et al., 2012). To address this, modelling of species' distributions has been increasingly used in order to identify suitable habitat and to predict potential distributions (Austin 2002, Rushton et al., 2004). Predictive habitat distribution modelling based on statistical techniques and GIS tools have important potential applications, such as forecasting species occurrence in poorly documented areas, the identification of adequate target areas for species reintroduction or conservation programmes or predicting the climatic change impact (Rodríguez et al., 2007, Muñoz et al., 2013). However, there are only a few published examples of high-resolution predictive habitat distribution models (e.g. < 100-m precision) built to predict suitable nesting-areas for raptors within their breeding range.

Like many birds, raptors are usually highly selective with regard to their habitats, particularly as regards the availability of suitable nesting areas (Limiñana et al., 2011). This is the case of the Cinereous Vulture (*Aegypius monachus*), a highly selective tree-nesting raptor. This raptor is considered to be an umbrella flagship species (Carrete & Donázar 2005) and is classified as Near Threatened in the IUCN Red List, and Vulnerable in the Spanish Red List. In the Iberian Peninsula, Cinereous Vulture populations have undergone a sharp decline, particularly throughout the first half of the 20th century, as a consequence of habitat loss and alteration, a decrease in food availability, poisoning and human disturbance (Donázar et al., 2002, Madroño et al., 2004).

Previous studies have shown that nest site selection and occupancy by Cinereous Vultures are most likely to be affected by either loss of nests and nesting habitat as result of human activities or disturbance during the pre-laying and laying period, particularly if these circumstances occur consistently over time (Donázar et al., 2002, Gavashelishvili et al., 2006). Consequently, the Cinereous Vulture selects its nesting-habitat in undisturbed areas, in mature vegetation patches with steep slopes (Poirazidis et al., 2004, Morán-López et al., 2006a, Moreno-Opo et al. 2012). The optimal habitat for Cinereous Vulture has been dramatically reduced, making its current distribution in Europe small in historical terms (Gavashelishvili et al., 2012), and it is thus urgent to identify and protect those suitable areas. The aim of this work is therefore to apply habitat modelling techniques with the use of a logistic regression approach and GIS tools to generate an explicative and predictive habitat suitability model for the Cinereous Vulture´s nesting-habitat at a fine spatial resolution (50-m resolution) within its breeding range.

Study area

The study was conducted in Córdoba province, in the Sierra Morena mountains (southern Spain, Fig. 1) in and around the Hornachuelos' Natural Park, where the Cinereous Vulture breeds currently and historically (Dobado & Arenas 2012). The Hornachuelos mountains were designated as a Natural Park in 1989, and cover 60 000 ha. The climate is typically Mediterranean, with moderately cool rainy winters and hot dry summers. The altitude ranges from 200 to 800 m. The dominant vegetation includes tree species such as holm oak (*Quercus ilex*) and cork oak (*Quercus suber*), reforestations of stone pine (*Pinus pinea*) and cluster pine (*Pinus pinaster*), Mediterranean scrubland dominated by *Cystus spp., Erica spp., Pistacia spp., Phyllirea spp. and Rosmarinus spp* and pastures areas occupied by oak savannah (*dehesa*). Some nests belonging to the colony are located outside the Natural Park´s limits, and a 5-Km conservation buffer (the maximum distance between the two nearest nests) has therefore been designated around them, since it is known that Cinereous Vulture usually establishes the new nests in adjacent areas to pre-existing ones. This buffer zone is included in the Guadiato-Bembézar "Site of Community Importance" for the Natura 2000 Network.

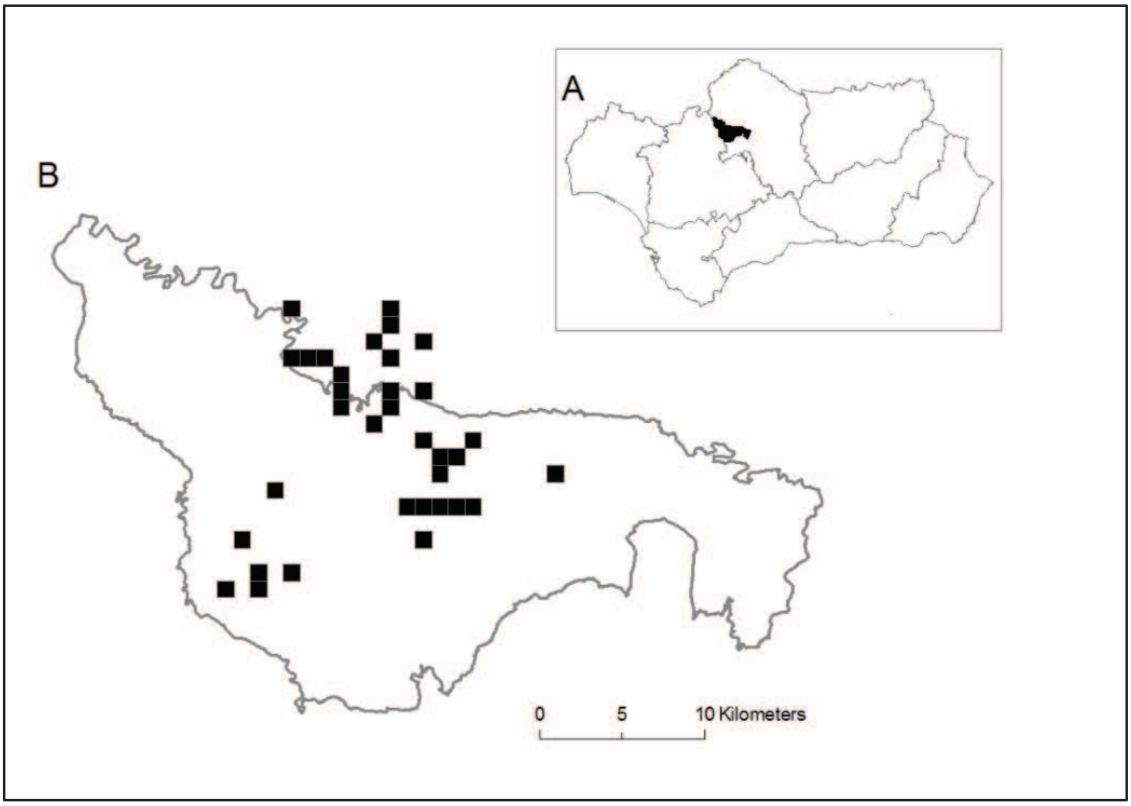


Figure 1: (A) The shaded area shows the location of Hornachuelos Natural Park in the region of Andalusia (southern Spain). (B) The black squares indicate the location of Cinereous Vulture nests in the study area, and the grey line is the limit of the Natural Park.

Nest survey

In 2011, the study area was searched for nest sites, and a minimum of three visits was made to every nest during the breeding season. A nest was considered to be occupied if we observed, a minimum of: typical pair behaviour, courtship, brood rearing activity or young (Carrete et al., 2001, López-López et al., 2006). The geographical coordinates of nests were recorded with a global positioning system (GPS). As habitat selection studies require a comparison of the selected sites (presences) with a randomly chosen control points of non-used sites (absences) (Manly et al., 1993), a geographic information system (GIS) was used to generate the same number of random points as nests, so that the number of sampled nests and the random plots was balanced (Moreno-Opo et al., 2012). Autocorrelation problems were reduced by constraining the random points to be at least 59-m apart (the minimum of the nearest neighbour distance of the black vulture in the study area).

Studied variables

Those variables that affect nest site selection by Cinereous Vultures in Spain were selected in accordance with previous studies (Morán-López et al. 2006a, Moreno-Opo et al., 2012). A total of 11 variables provided by GIS analyses were taken into account: altitude (m), slope (%), mean incident solar radiation from February to June (Wh/m^2), distance to nearest road (m), distance to nearest track (m), distance to the nearest village (m), the size of the homogenous land cover patch in which the nest is located (ha), the distance from the nest/random point to the border of the vegetation patch (m) and the canopy cover covered by *Quercus suber* (%), *Q. rotundifolia* (%) and *Pinus spp* (%). Topographic variables (altitude slope and solar radiation) were obtained from a Digital Elevation Model (DEM) with an accuracy of 10-m pixels of horizontal and vertical resolution, whereas vegetation variables were obtained from the third National Forestry Inventory (1997-2007) and the Corine land cover (2006). Climatic factors were not considered owing to the relatively small study area, which implies similar values of temperature, rainfall and humidity throughout the study area (Moreno-Opo et al., 2012).

Statistical analysis and predictive map

Univariate and multivariate statistical techniques were used to determine which factors affect the Cinereous Vulture´s nest-site selection. First, Mann–Whitney U-tests were used to provide a simple description of the main differences in the mean values of the variables between nests and random sites. Second, a binomial logistic regression (with a logit link function), a particular case of Generalised Linear Model (GLM), was used to identify which variables were the best predictors (Pearce & Ferrier 2000). Two different models were built: considering the distance to the nearest-neighbour (model 1), because this variable has biological meaning and captures the spatial configuration (Barrio et al., 2009b); and without including this spatial variable (model 2). In both cases, a forward-backward stepwise procedure was applied using the Akaike's Information Criterion with a small-sample bias adjustment (AICc) to select the most parsimonious models. The accuracy of the predictive distribution models were assessed by calculating the area under the receiver operating characteristic (ROC) curve (known as AUC). AUC values of > 0.7 indicate acceptable predictive power. Collinearity among continuous explanatory variables was low (Pearson correlation < 0.6; variance inflate factor (VIF) < 10). The distance to the nearest-nest was ln-transformed in order to eliminate non-linearity among the independent variables and the logit of the dependent variable. Finally, Moran's *I* coefficient was calculated to examined spatial autocorrelation in residuals of the models in order to confirm the assumption of independent errors (Dormann et al., 2007).

Results

In total, 43 nest-site locations were recorded in 2011, and the mean distance to the nearest neighbour was 1031 m (± 927 S.D; range 59-5353 m). Nests were located farther from roads, villages and patch edges, in larger homogenous patches with high proportion of cork oak cover and on steeper slopes with lower solar radiation (Table 1). Both most parsimonious models showed a positive effect of slope, cork oak cover, and to the distance of vegetation patch border, and the model 2 also showed a significant positive effect with the distance from the villages (Table 2). As we predicted, the model 1 demonstrated an effect of the nearest nest (Table 2). Model 1 and model 2 had AUC values of 0.93 and 0.87 respectively, indicating a good discrimination capacity. The Moran's *I* coefficient of the model with the distance to the nearest nest was particularly low, thus indicating no significant spatial autocorrelation among residuals (Moran's I = -0.007, P = 0.98), whereas the residuals of the model without this spatial variable showed spatial autocorrelation (Moran's I = -0.44, P < 0.001).

Table 1. Means (± standard error) of variables measured for the nests and the random points.

Variables	Nest site	Random	U-test	P
Distance to roads (m)	4661 (± 305)	3425 (± 386)	806	<0.001
Distance to tracks (m)	124 (± 16.8)	85 (± 10.5)	1115	0.076
Distance to villages (m)	12793 (± 425)	10520 (± 476)	913	0.002
Altitude (m.a.s.l.)	339 (± 10)	359 (± 13)	1306	0.566
Slope (%)	37.4 (± 1.8)	21.5 (± 1.9)	570	<0.001
Distance to neighbour (m)	1031 (± 141)	4893 (± 486)	345	<0.001
Patch size (ha)	31907 (± 2196)	19367 (± 1817)	1011	0.010
Distance to edge patch (m)	340 (± 45)	186 (± 22.8)	919	0.003
Solar Radiation (W/m^2)	536068 (± 1064)	571933 (± 5419)	1011	0.015
Quercus suber (%)	14.7 (± 1.12)	7.43 (± 1.03)	14776	<0.001
Quercus rotundifolia (%)	14.6 (± 1.67)	17.09 (± 1.73)	1291.5	0.505
Pinus spp (%)	9.5 (± 2.57)	6.12 (± 1.86)	1214	0.152

Table 2. Logistic regression models of cinereous vulture habitat requirements in Hornacuelos Natural Park. ΔAIC indicates the improvement in model fit of the final model compared with the next best model.

	Model 1ΔAICc = 4.08		
Parameter	Coefficient	Wald	Sig
Intercept	5.86 ± 2.58	5.15	0.023
Ln nearest nest	-1.37 ± 0.34	16.26	<0.001
Slope	0.088 ± 0.023	14.58	<0.001
Distance to patch edge	0.003 ± 0.002	4.48	0.034
Cork oak cover	0.032 ± 0.028	1.35	0.035

	Model 2 ΔAICc = 5.01		
Parameter	Coefficient	Wald	Sig
Intercept	-5.98 ± 1.31	20.7	<0.001
Slope	0.075 ± 0.019	16.21	<0.001
Distance to patch edge	0.004 ± 0.001	9.61	0.002
Cork oak cover	0.058 ± 0.022	7.08	0.008
Distance to villages	0.001 ± 0.001	20.74	0.01

Discussion

Given that the Cinereous Vulture has a long reproductive season, this species is strongly affected by anthropogenic disturbance (Madroño et al., 2004, Morán-López et al., 2006b, Margalida et al., 2011). Our results were in agreement with those of previous studies in that they showed that the Cinereous Vulture selected steep slope areas far away from roads, villages and from the edges of larger vegetation patches, suggesting that this species selected areas less disturbed by humans.

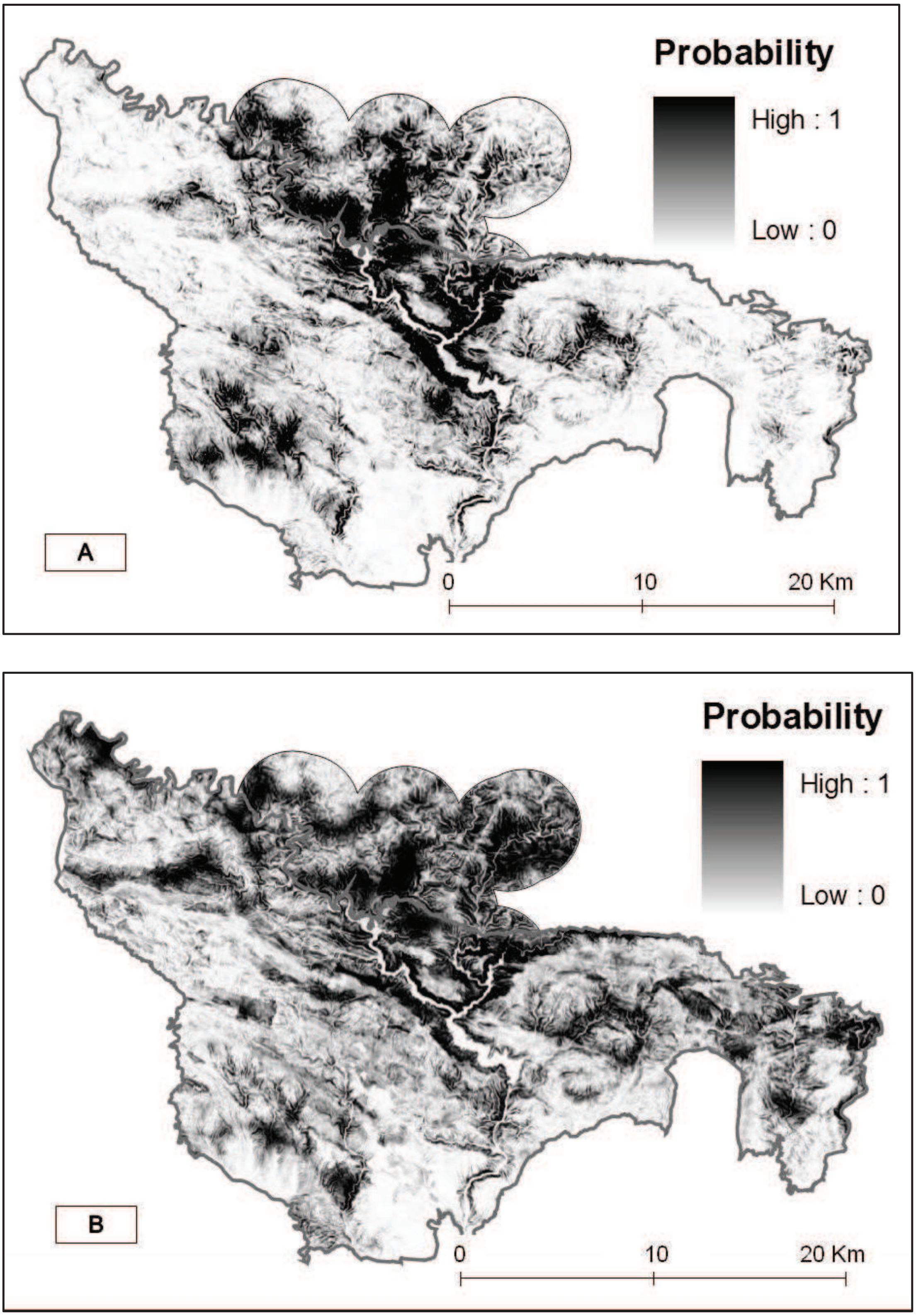


Figure 2: Probability of presence of Cinereous Vultures derived from logistic regressions at a 50 x 50 m resolution, considering the distance to nearest-nest (A) or without include it (B).

Moreover, because the maximum temperature in the study area can reach 40 ºC, the thermoregulation of the young may constitute a limiting factor, and therefore these birds tend to select areas with lower solar radiation, highlighting the importance of avoiding warmer places under these harsh conditions (Morán-López et al., 2006b). Lastly, some previous studies have pointed up the positive effect of high cork oak coverage, since the Cinereous Vulture selects old cork oaks as a priority nesting trees, because they support better the weight of nests and are easier to land on and take off (Dónazar et al. 2002, Moreno-Opo et al., 2012).

Since this study was carried out inside the limits of a current and historical breeding area for the Cinereous Vulture, the effects of anthropogenic disturbance were likely to be uniformly weak (Table 1) in contrast to other areas where they are an important predictor (Morán-López et al. 2006a), thus the results should be interpreted in this context. Therefore, although distance from tracks and roads to the nest was not a very important negative predictor, road access is restricted in many areas of the study area, and the roads which cross the study area are secondary roads with little car transit. In the same way, since the study area has low human population density, the distance from villages between occupied and unoccupied areas is also less marked (Table 1).

With regard to habitat nest-selection for colonial or semi-colonial raptors species, conspecific attraction may play an important role in breeding spatial distribution (Sergio & Penteriani 2005, Limiñana et al. 2011). Indeed, the first model confirmed that the proximity to the nearest nest was an important predictor in determining nest-location, and therefore most of the predicted suitable areas were located surrounding pre-existing vulture nests (Fig. 2a). Moreover, this model had a higher AUC value, lower AICc value and no spatial autocorrelation among residuals, thus suggesting that the aggregated spatial pattern of the nests should be considered in any predictive model for Cinereous Vulture. Model 2 can also be considered for predicting the spread of the colony in the long-term, because this model allows the identification of places far away from the current existing nests (Fig. 2b). However, in the light of the aforementioned reasons, we believe that the model that included the distance to the nearest nest is more appropriate, particularly in order to predictive the immediate colonization of new territories. Therefore, for this semi-colonial species, increasing the availability of preferred habitats in the periphery of the existing nests might be more efficient in ensuring the occupancy of given areas when designing areas for conservation priority.

Predictive species distribution models have been used in a wide range of applications including regional biodiversity assessments, conservation biology, wildlife management and conservation planning (Rodríguez et al. 2007). However, the lack of fine grain predictive maps may result in their low effectiveness for designing further protected areas (López-López et al. 2007, Fernández & Gurrutxaga 2010). In the light of these considerations, our predictive maps may be valuable for the local management of the Cinereous Vulture. For instance, here we have shown that many favourable areas are situated outside to the protected limits of the Natural Park, but are inside the Guadiato-Bembézar "Site of Community Importance" for the Natura 2000 Network (Fig. 2). In these conservation priority areas for the Cinereous Vulture, the implementation of spatial and temporal buffer zones around potentially sensitive areas (breeding sites) where disturbances are limited or prohibited (Margalida et al. 2011), and the preservation of large vegetation patches with high coverage of cork oak should favour the establishment of new breeding pairs. Lastly, the probability maps may also be very helpful in identifying sites for the construction of artificial nests, although microhabitat variables such as tree species and tree height would be necessary to select the exact sites for these.

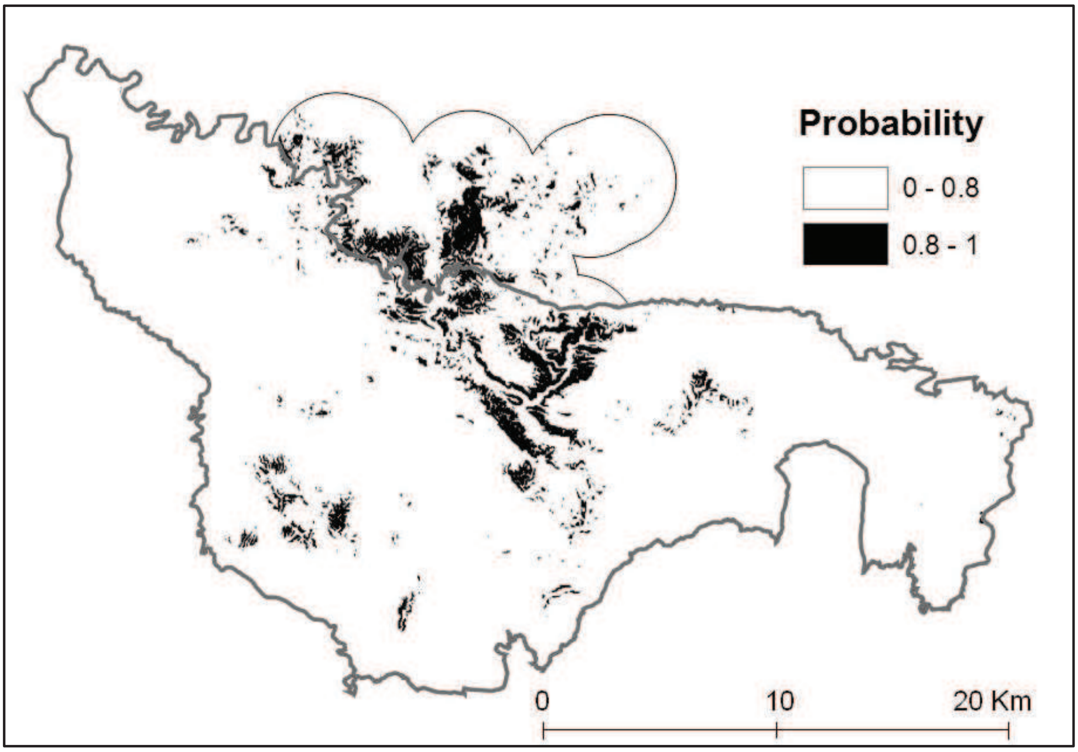


Figure 3. Potential distribution map taking 0.8 as the threshold above which presence is more likely than absence.

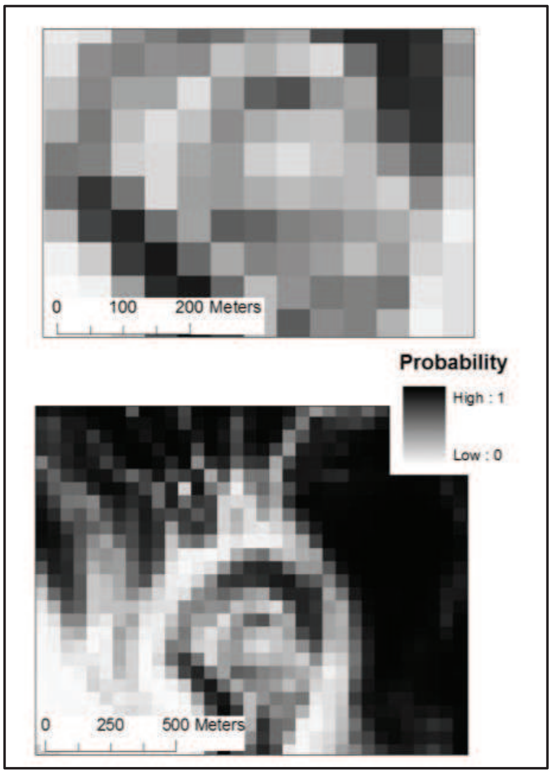


Figure 4: Figure that illustrates the accurate scale of the model

The Cinereous Vulture can be considered as an habitat indicator species owing to its nest-site selection in areas with high conservation status that are important to many other species (Moreno-Opo et al. 2012). The predictive map that included the distance to the nearest neighbour also revealed that less than 8 % of the study area had a higher probability value of occupancy than 0.8 (Fig. 2a), which can be considered a reasonable threshold value, because the odds are higher than 4:1 (Real et al. 2006). These low values of suitable habitat inside the breeding range imply that the conservation of these best suitable areas is a major concern in order to minimize the negative impact of habitat loss. The scale used in our experiment (50-m resolution) permits the delimitation of protection areas in which the occurrence of target species is more probable, thus allowing the application of fine scale management decisions.

Acknowledgments: *A. monachus* nesting population in the Hornachuelos' colony was surveyed with the aid of staff from the Andalusia Environmental Agency. We should first like to express our gratitude to the staff from Andalusia Environmental Government for collecting the data, particularly to Pablo M. Dobado. We would also like to thank I.C. Barrio and A. J. Carpio and for their useful comments and suggestions, and S. Crespo for her support. This work is funded by the Andalusia Environmental Government through a project for the conservation of the Cinereous Vulture in Cordoba province.

GENERAL DISCUSSION

GENERAL DISCUSSION

Further perspectives in rabbit restocking research

According to Griffith et al., (1989), the success of translocations is more frequently achieved when the animals involved are native herbivorous game species, introduced into a high quality area of their core historical range with few or no competitors, and are early breeders with large clutches. Under these guidelines, the European rabbit could be considered as a good candidate for success in restocking programs (Cotilla & Villafuerte 2007). However, rabbit translocations are frequently unsuccessful in their objective of establishing a self-sustaining population (Calvete et al., 1997; Letty et al., 2002b), and its final success depends on several intrinsic and environmental factors, in addition to the application of different management strategies.

Bearing in mind that the rabbit is a keystone and pest species, the scientific literature on rabbit management are more scarce (Ferreira 2012), and thereby management decisions are frequently made on the basis of insufficient scientific information. In the case of rabbit restocking, the scientific information reviewed herein has proved to have important knowledge gaps. Additionally, some papers have revealed that some known factors recommended by researchers are not always taken into account (e.g. Calvete & Estrada 2004; Cotilla & Villafuerte 2007; Delibes-Mateos et al., 2008c), probably because hunters attempt to achieve a significant increase in rabbit density in a relatively short-time (Calvete et al., 1997). Seddon et al., (2007) pointed out that wildlife managers manipulate systems to achieve management objectives rather than to discover more about how systems work what may produce a lack of controls, replicates, adequate monitoring, or even the guidance of explicit objectives. In this scenario, wildlife managers and hunters do not have a clear protocol to apply in rabbit translocations, thus increasing the biological cost of these activities. Any further efforts should therefore be targeted at bridging these knowledge gaps and attempting to implement the scientific recommendations with the aim of establishing accurate management guidelines for subsequent rabbit restocking.

In the late 90s, Calvete et al., (1997) made evident the low effectiveness of traditional wild rabbit restocking. Since that date, several works have tested different ways and methods with which to improve success. Nevertheless, these works have traditionally been focused on those factors that determine whether or not reintroductions are successful, and little attention has been paid to the consequences of restocking operations at the meta-population and ecosystem levels (Amstrong & Seddon 2008). These authors propose ten key questions focusing at the population, metapopulation and ecosystem level, which can be used to encourage a more integrated approach to reintroduction biology. With regard to the ecosystem level, the last two questions place stress on whether the ecosystem will be affected by the target species and its parasites and if the reintroductions will affect the ultimate species composition. Both questions could be relevant in the case of the wild rabbit owing to the role of rabbit as a keystone species and ecosystem engineer, and hence, the further rabbit restocking programs should consider this new framework, and researchers should answer the key questions regarding the influence of rabbit restocking at meta-population and ecosystem levels.

This thesis improves scientific awareness of the topic, bridging some previously identified knowledge gaps. The main output of this thesis is the assessment of the restocking plots plus habitat management as conservation tools with which to establish wild populations at landscape scale.

Considering the impact of aerial predation

The establishment of semi-extensive rabbit captive breeding enclosures is an increasingly used technique to avoid the initial dispersal movements and the high mortality resulting from carnivores, favouring the establishment of the animals on the release site. Although most of the studies assume that carnivores are the main rabbit predators during the first days after release, there are no studies that test the effect of avian predation exclusion on restocking success. In Chapter 2 we evidenced the positive effect of excluding raptors predation through the use of roofed enclosures. However, rabbit survival differences between open and closed plots remained constant after the second week, suggesting that raptor predation of rabbits occurred intensively during the first few days after release suggesting that in our case two weeks was the period that the rabbits needed to adapt to the new environment (Letty et al., 2008).

In agreement with this result, Rouco et al., (2008) found that raptors prey more often on fenced plots than on unfenced rabbit restocking plots, and Cabezas et al., (2011) likewise found that the risk of being killed by raptors was higher in carnivorous exclusion plots than in others plots without fences with additional shelter and food. All the same, despite the initial high predation rate, raptors may not fatally affect the rabbit population in the long-term. In fact, Rouco (2008) concluded that the predation by raptors cannot prevent rabbits from achieving a high abundance in fenced plots during the reproductive season, and the extraction of rabbits from the enclosures may become necessary. However, this thesis has evidenced that aerial predation should also be considered on these plots in the long-term since: a) the proximity among enclosures was correlated with higher rabbit abundance, probably by the scattering of aerial predation; b) the flight time of raptors was higher in those enclosures with higher rabbit abundance; and c) the enclosures with scarce refuge reached lower rabbit abundance.

Although it is necessary to stress this long-lasting effect with greater accuracy, the exclusion of aerial predators by roofing the smaller enclosures could be applied in further extensive breeding enclosures. Indeed, semi-extensive wild rabbit farms often are equipped with a fence on the roof to prevent birds of prey from gaining access (e.g. Arenas 2002; Piorno 2007a). However, roofing bigger enclosures might not be a cost-effective tool, and hence others measures such as selecting plots with appropriate cover (see Chapter 3), applying soft-release protocols through the use of warren fencing (Letty et al., 2000) or the increase in refuge near artificial warrens using pallets (Ferreira 2011) or heaps of wood branches (Rouco et al., 2008) would appear to be most appropriate tools from the point of view of cost-effectiveness.

The extensive enclosure as in situ farms to produce wild rabbits

The role of restocking plots is to avoid the initial predation or dispersion to produce wild rabbit for further spread in the surrounding areas, by opening the enclosures or extract them. The production of wild rabbits in these enclosures has several advantages in comparison to the release of wild rabbit from intensive either farms or the wild:

1)- It avoids the usual problems that wild rabbits undergo in intensive rearing systems: stress levels, maladapted behaviour, low reproduction rate (González-Redondo & Zamora-Lozano, 2008; González-Redondo, 2010) and genetic problems resulting from hybridisation with domestic animals (Piorno 2007a).

2)- These enclosures simulate the natural environmental conditions in terms of landscape, food type, soil type and aerial predation pressure, thus enabling the development of a dietary pattern and antipredatory behaviour (Díez & Pérez-Garrido, 2003).

3)- They favour the establishment of social ties of released stock.

4)- They reduce human handling and improve the animals' welfare (Arenas et al., 2006).

5)- They require less maintenance than intensive farms.

6)- The annual production of animals for releasing purposes avoids the continuous extraction of the source populations in the wild, thus minimising the risk of over-harvesting them. However, the high rabbit abundances in some agricultural areas may provide a high number of wild rabbits to be restocked.

Consequently the rabbits from the enclosures would be pre-adapted to the local conditions, and it is expected that they can survive better than those animals reared in intensive systems or translocated from other areas. In this thesis, the original stock came from an intensive agricultural area 100-km apart from the release site (see Barrio 2010), with little similarity between capture and release areas in terms of landscape, food resources, parasites, soil type or predation pressure. This means that it was expected that the rabbits would not adapt to the new environment, and hence difficulties in adaptation may have arisen (Letty et al., 2008). Rearing rabbits in these in situ enclosures could consequently be a more efficient way of improving their survival and the establishment of the founder population in the new environment

Improving wild rabbit production in extensive breeding systems

Our results enhance the awareness of producing wild rabbits in extensive systems with restocking purposes. The data presented here suggest that rabbit production can be significantly enhanced by selecting plots with appropriate cover availability and structures in which to build warrens. Cover is essential for hide from predators (Lombardi et al., 2007), it decreases the need for group vigilance and individual distances to forage (Villafuerte & Moreno 1997), provides important food resources during the summer period (Beja et al., 2007), increases breeding opportunities with positive outcomes on productivity (Lombardi et al. 2003), and it may be essential for the younger and more subordinate animals when they are expulsed from the burrows (Cowan 1987; Kolb 1994). What is more, in some areas with very thick ground cover it seems that rabbits prefer to use cover as refuge more rather than burrows, probably because this provides greater security from predators (Kolb 1991).

However, pasture cover is also a good predictor of high rabbit abundance, and a trade-off therefore exists between shelter and food availability. Results in Chapter 3 showed that selected plots with intermediate values of scrub cover (25-50 %) significantly increase rabbit density. As some of the enclosures are devoted to providing a food supply for endangered raptors, another trade-off exists between the enhancement of wild rabbit production in selected areas with good scrub cover and the facilitation for their capture by the birds of prey by building the enclosures in low cover areas. In this case, selecting plots with scrub coverage values of about 25 % may compensate both requirements.

Rabbits usually dependen on warrens for breeding and for protection from predators and climatic extremes, and it is therefore expected that the facility to build burrows enhances the reproduction. In these plots, the maximum abundance was strongly correlated with both the number of warrens and the percentage of protected warrens (Figure 1), suggesting that a greater number of protected places in which to dig warrens may allow secondary females to breed and thus contribute to the attainment of higher offspring production. It is known that rabbits prefer to build warrens under some structure of protection, such as stones, tall scrub, or tree roots (Palomares 2003a; Gea-Izquierdo et al., 2005) preferably on well drained sloping lands (Barrio et al., 2012), which could be related both to warren stability and to impeding the access of digging predators (Palomares 2003a).

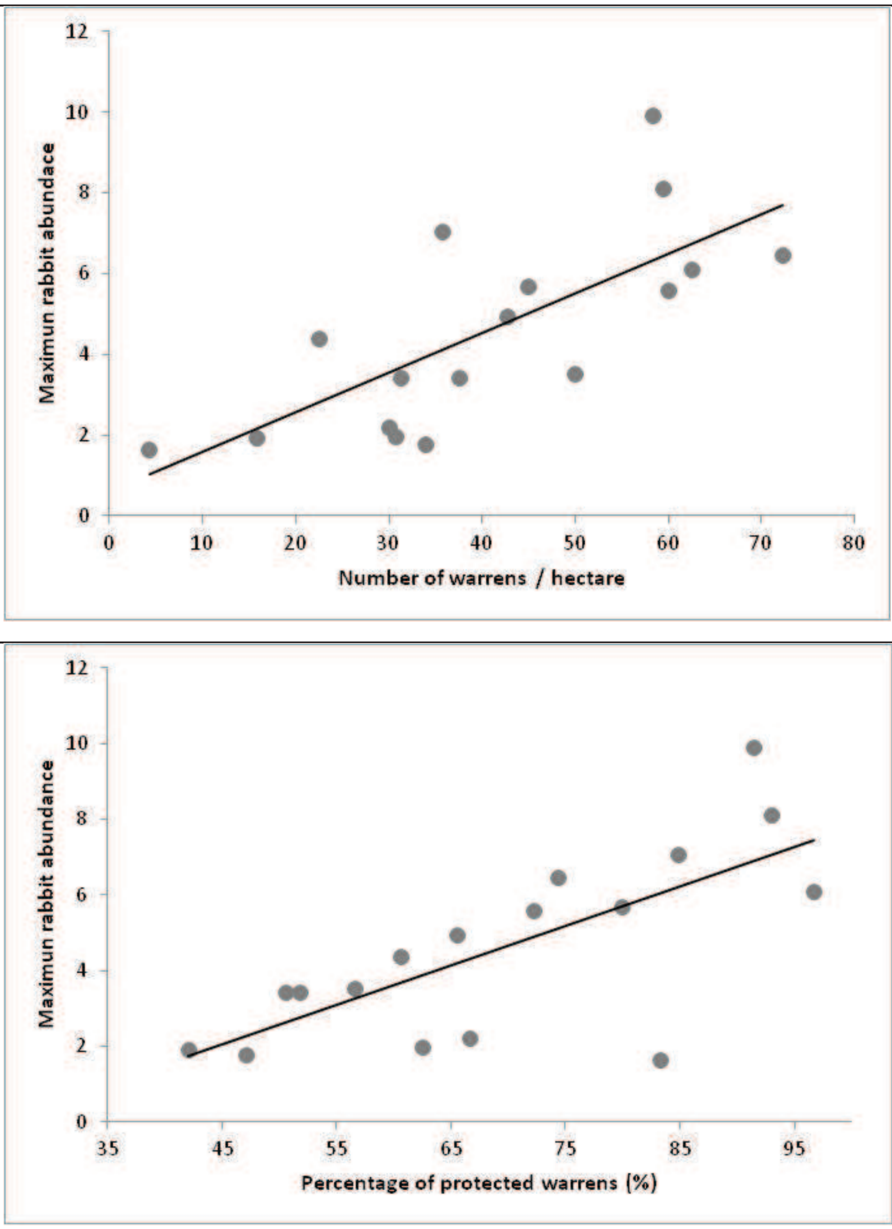


Figure1: Correlation between the maximum rabbit abundance and (A) the number of warrens per hectare^{-1} (R^2 = 0.53) and (B) the percentage of protected warrens (R^2 = 0.53).

Rabbit population growth inside these breeding systems could be limited by intrinsic mechanisms, such as physiological stress and social interactions, since individuals compete for social rank, food resources and breeding sites (Myers & Poole, 1961). Ruiz-Aizpurua (2013) has recently found a lower growing rate (poplation increase in relation to density) in enclosures with higher densities. This density-dependence could be explained by a) a decrease in fecundity as a consequence of a higher proportion of young females with a lower reproductive performance resulting from their poorer physical condition and lower social rank (Rödel et al., 2004b), and b) an increase in mortality at higher densities produced by a decrease in food resources and a certain amount of antagonist interactions (Myers & Poole, 1961; Rödel et al., 2004a). In any case, to prevent these density-dependent effects, Ruiz-Aizpurua (2013) concluded that the extraction of the surplus stock could improve productivity during the breeding season. Although food was supplied ad libitum in the form of commercial pellets and grain mixture, the rabbits consumed the whole pasture resources in plots with higher densities, probably because they prefer to feed on high quality natural pastures (Gea-Izquierdo et al., 2005). Because of this, the status of pastureland could be an indicator of density-dependent phenomena, and the extraction of animals should be carried out in order to avoid food limitation.

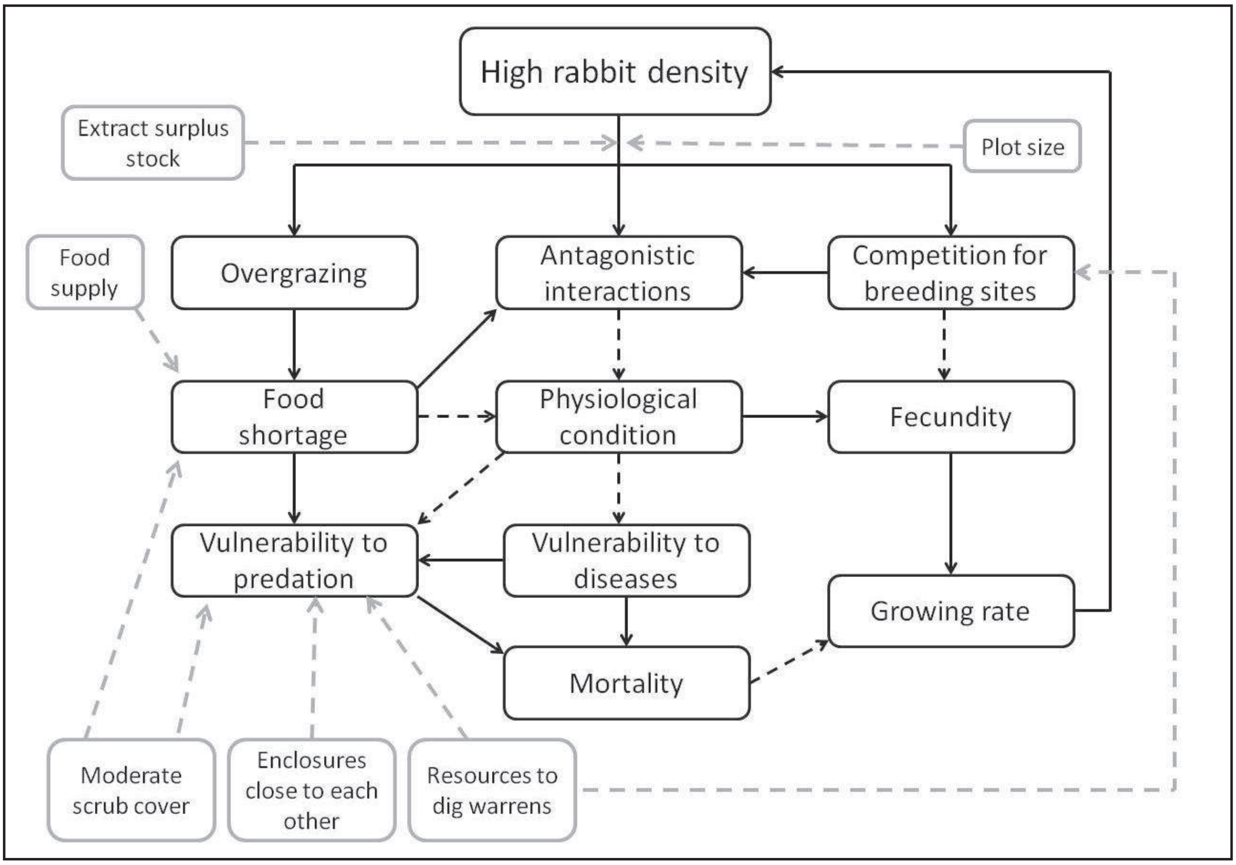


Figure 2: factors affecting wild rabbit production in extensive breeding enclosures (dark squares) and the management actions that can be used to palliate these factors (grey). The continuous and discontinues arrows represent direct or inverse relationships respectively.

Although the total number of terrestrial carnivore signs found into the fenced plots was not related to the maximum abundance of rabbits attained, the large number of mammalian carnivores scats found inside the plots evidenced the ineffectiveness of electric fences as regards preventing their access. It is therefore advisable to apply the recommendations made by Moseby & Read (2006), who suggested that a 60-cm wide external netting overhang curved in an arc with two electric wires at the base of the overhang (to provide a shock to animals exploring it) is more effective with carnivores. Future research should be performed to test the impact of terrestrial carnivores, particularly as regards the impact of marten and genet; the most frequent carnivorous activity occurs inside the enclosures, and these works should be focused on evaluating the cost-effectiveness of their exclusion.

The extensive breeding enclosures as dispersal centres

In populations with very low rabbit density, habitat management may not be effective enough to reverse its negative trends (Ferreira et al., 2013), and it is therefore necessary to create a founder population, which can be obtained by installing extensive breeding enclosures. The role of fences is that of keeping the rabbits confined in order to maintain a population density which is sufficiently stable and high to make it possible to utilise the rabbits from fenced plots as colonisers of surrounding areas (Rouco et al., 2008). It is known that bigger populations are less sensitive to predation (Trout & Tittensor, 1989), diseases (Calvete 2006; Cotilla et al., 2010), stochastic phenomena (Wilcox & Murphy 1985) and the Allee effect (Stephens & Sutherland 1999), and the enclosures can be therefore considered as an appropriate measure to found an initial population at very low local rabbit abundances. All these arguments support the hypothesis that concentrating management efforts in close areas with bigger enclosures rather than disperse the actions in large areas could reach bigger and more stable populations in long-term thus improving the overall results.

In the light of these considerations, the bigger plots (> 2 ha) would be a better solution since they can reach higher abundances. Moreover, they simulated the natural home range of the species and provide more dispersal opportunities inside the plots, thus decreasing the denso-dependent effects (see above). Surprisingly, the size of the enclosures did not have a significant impact on rabbit abundance inside the enclosures during the first year after release, probably because the distance among them played a more important role. It is therefore necessary a complete assessment of the role of plot size in the long-term (3 - 4 years) to evaluate the effectiveness of this high cost structures.

To date, there are no data concerning the chronology of breeding after release, including the possible waiting period before the onset of breeding in the release area (Letty et al., 2008). In this project, the fact that the animals were released in autumn, with a continuous increase in abundance until the following summer, bears witness to wild rabbit reproduction inside the enclosures thus suggesting a good adaptation. The plots were opened at the end of the breeding season, and the animals could therefore be adapted to the dispersal area, and they may have a better ability to colonise the surrounding areas. Furthermore, translocating animals in autumn or winter before the breeding season optimises the population growth (Cotilla & Villafuerte 2007), prevents the disruption of reproduction activity (Moreno et al., 2004) and may ensure a high antibody concentration against viral diseases (see Chapter 1). In the light of these considerations, the release in the enclosures in autumn or winter, and the further opening at the end of the reproductive season could enhance population establishment. However, if abundances within the fenced plots were too high, then the plots might be opened to mitigate the negative denso-dependent effects (Ruiz-Auizpurua 2013).

Recovering European rabbit populations in Sierra Morena

Reversing rabbit decline is one of the greatest challenges for nature conservation in Spain and Portugal (Delibes-Mateos et al., 2008a), but effective techniques for the widespread recovery of wild populations have not yet been devised (Ward 2005). This thesis shows that restocking in enclosures combined with an intensive habitat improvement around them can be considered as an effective tool with which to promote wild rabbit populations on a large-scale, both temporally and spatially. Indeed, some managed UTM cells reached the threshold value needed to support stable lynx presence in Sierra Morena (10 latrines km^{-1}). Furthermore, if the formula developed by Gil-Sánchez et al., (2011) were used to calculated the rabbit density from the number of latrines in Sierra Morena, the average value in treated areas would be 0.8 rabbits ha^{-1}, whereas in unmanaged UTM cells the recorded rabbit density would be 0.06 rabbits ha^{-1}. This value is near the threshold value of 1 rabbits ha^{-1} necessary for the residence of Iberian lynx (Palomares et al., 2001) and to be considered as high habitat quality for the Spanish Imperial eagle (González et al., 2008), and it should be sufficient to obtain good hunting yields (>0.2 captured rabbits ha^{-1} in Andalusia according to Vargas et al., 2007).

Nevertheless, rabbit density was strongly influenced by habitat treatment and scrub coverage, and its abundance could be enhanced in accordance with the results presented herein. Land-use changes have decreased the habitat heterogeneity in Sierra Morena (Delibes-Mateos et al., 2010), and there are thus currently 3 main habitat types, in which different habitat treatments could be carried out:

Dense scrubland patches: the loss of sparse scrubland and the increase of large areas with dense scrubland seems to be detrimental for the wild rabbit in Sierra Morena, since there is a widely shelter availability, but scarce pasture quality and quantity. In this habitat, the scrub clearance is a powerful tool by which to promote mosaics of pastures and scrubs by creating feeding areas in the dense scrubland (Moreno & Villafuerte 1995), thus increasing the habitat heterogeneity and complexity.

Pastureland (*dehesa*): in the study area, the wild rabbit abundance has been traditionally associated with the dehesa landscape, because this habitat had an important scrub layer (Delibes-Mateos et al., 2010). However, this scrub layer has disappeared as a consequence of the intensification of herbaceous crops and livestock (Fernández-Alés et al., 1992), and this kind of habitat has therefore become unsuitable for wild rabbits as a result of low shelter availability. However, the habitat quality can be improved by increasing refuge with brush piles and artificial warrens. Besides, it is essential to recover the scrub layer in this habitat through reforestation and decreasing the grazing pressure caused by livestock or wild ungulates (Pulido et al., 2001).

Pine forest: in the middle of the 20th century, Mediterranean scrubland and oak forest were replaced with dense pine forest plantations, which are an inappropriate habitat for the wild rabbit (Virgós et al., 2003) since the high tree prevents the growth of pastureland and scrubland, and shelter and food resources are therefore scarce. In this project, some forestry actions were also carried out, but the small sample size prevented us from evaluating their effectiveness. Although Arenas et al., (2011) reported an increase in rabbit abundance after pruning dense pine mass plus providing shelter, it is more advisable to release in a high quality habitat than to invest heavily in these inappropriate areas. Anyway, it seems imperative to perform habitat improvement techniques in dense pine mass in order to restore wild rabbit populations in a landscape context.

In all the cases, the habitat management should be targeted towards providing shelter, food, and water, and towards promoting opportunities for dispersal. In this context, the installation of artificial warrens can provide better shelter for breeding, particularly in areas in which soft soils for digging warrens are absent (Fernández-Olalla et al., 2010), while other less costly measures such as planting crops and supplying water (Ferreira et al., 2013) may facilitate rabbit establishment around the enclosures. In conclusion, since environments suitable for rabbits have become impoverished in Sierra Morena (Delibes-Mateos et al., 2010), rabbit translocation programmes should therefore be carried out in conjunction with habitat improvement actions in the target area to ensure that the investment is worthwhile.

To reach a more sustained and widespread rabbit recovery in Sierra Morena it would be crucial to link the smaller isolated populations into more continuous larger connected populations (Ward 2005) in order to avoid the aforementioned problems of fragmentation. This indicates that enclosures can also be considered as a powerful tool by which to connect fragmented rabbit populations and enhance the likelihood of re-colonisation of areas in which the species has become quasi-extinct (Virgós et al., 2003). This could also be achieved through habitat restoration by favouring small-scattered patches of natural tall shrubs and the establishment of new ones in low cover areas (Carvalho & Gomes 2003).

European Rabbit and Cinereous Vulture conservation

Different responses of predators to the rabbit population collapse have been recorded: a reduction in red fox litter size (Villafuerte et al., 1996); a reduction in the number of territories occupied by eagle owls (Martínez & Zuberogoita 2001); a decrease in golden eagles reproduction rates (Fernández 1993); an increase in the home range size of adult lynx female (Ferreras et al., 2011); or a reduction in rabbit consumption together with an increase in trophic diversity by mammalian carnivores (Ferreras et al., 2011) and the cinereous vulture (Costillo et al., 2007). In order to mitigate these drawbacks, a huge effort is been undertaken to enhance the availability of prey (e.g. Simón et al., 2012). Nevertheless, the outcome of the increase in rabbit abundance by management actions on theirs predator has been rarely assessed.

The number of cinereous vulture breeding pairs recorded in and around the Hornachuelos Natural Park increased from 34 breeding pairs in 2007 to 49 pairs in 2012. This population increase is probably owing to the combined effect of the different conservation strategies: food supply in "vulture restaurants", increase of rabbit abundance, the control and persecution of illegal poisoning and the re-construction of pre-existing nests (BirdLife International 2012, Dobado & Arenas 2012). Anyway, the emplacement of new vulture's nests in areas closed to the enclosures may have modified the spatial configuration of the colony, with a concentration of nest in those areas. In this regard, the ellipse of standard deviation and the centroide can be useful tools to check patterns in spatial distribution (Levine 1996). As shown in Figure 3, a slight expansion to the managed areas occurred, what may be due to the attraction effect of the rabbit enclosures. Although the high density of big game species in the proximity of the colony may provide enough food resources (Costillo et al., 2007), the higher rabbit availability may supply food out of the hunting season (from March to October), when the mortality of wild ungulates is lower and the rabbit mortality by viral diseases is higher (Calvete et al., 2002). However further research is necessary to stress the real effect of rabbit abundance increase on the breeding success and dietary response of black vulture.

The proximity of the food resources has been identified as an important factor for the central-place foragers such as the cinereous vulture which are obliged to return every day to their nest or roost-site at the colony (Orians & Pearson, 1979; Carrete & Dónazar 2005). Therefore, although the large home range of black vultures allow nesting and foraging to be not so close, the installation of rabbit enclosures and rabbit friendly-habitat restoration around the colony would reduce the cost and risks associated with long travel distances from breeding cores to feeding areas (Carrete & Dónazar 2005), providing overall positive effects.

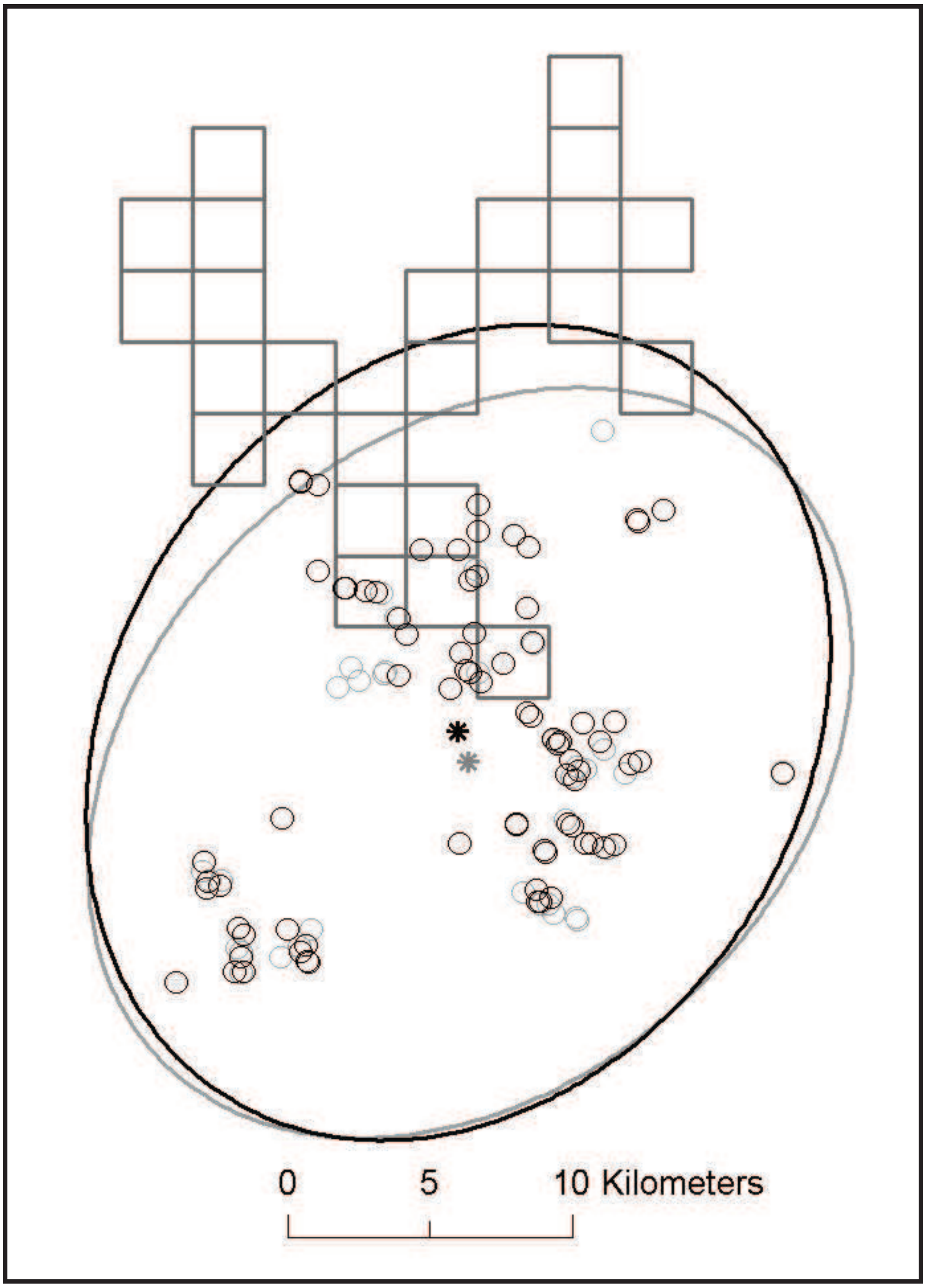
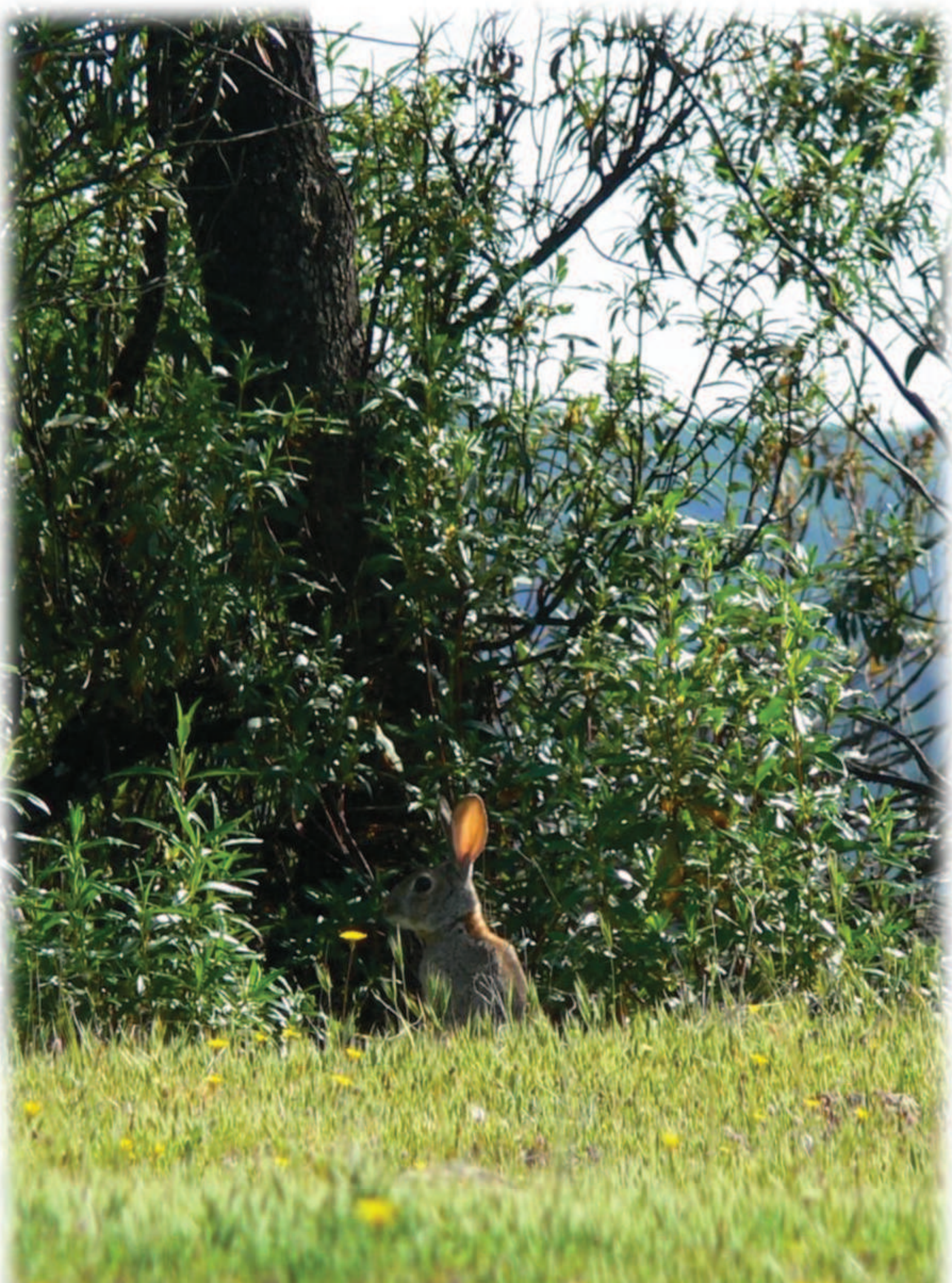


Figure 3: Ellipse of standard deviation showing the spatial pattern of the cinereous vulture nests before (grey) and after rabbit restocking (dark). The grey and dark asterisks represent the centroid of the colony before (2004-2007) and after rabbit restocking activities (2008-2011) respectively. The UTM-cells with management actions are shown.

It is also noteworthy that the cinereous vulture selects dense patches of mature vegetation as nesting habitat, which do not confer optimum habitat conditions for rabbits (Delibes-Mateos et al., 2008d), since rabbits attain a higher abundance in mixed areas of scrub and pastures or crops. Therefore, the cinereous vulture conservation programs should consider both its breeding habitats and the preference habitat for its principal prey to ensure a better conservation benefit. From this point of view, the strict protection of mature vegetation in their breeding habitats is essential to mitigate habitat loss, reduce human disturbance and enhance breeding success (Dónazar et al., 2002; Morán-López et al., 2006a), whereas the existence of an adequate habitat for wild rabbits in the foraging area may enhance food availability.

CONCLUSIONS



CONCLUSIONS

1- Eight of the 17 selected items of the IUCN guidelines for re-introduction were identified as partly studied or unknown, including important items such as the management and release of captive-reared wild rabbits, the development of transport and monitoring programmes, the application of vaccine programmes, and post-release long-term studies, and any further efforts should be focused on bridging these knowledge gaps.

2- The exclusion of aerial predation increases rabbit abundance during the first weeks after release, suggesting that roofing the smaller enclosures could be as an appropriate tool during this critical period.

3- The proximity among enclosures favoured the population increase inside the enclosures, probably as a result of the scattering of aerial predation. It would therefore be advisable to concentrate the restocking effort by ensuring that the restocking plots are close to each other, thus avoiding isolated enclosures.

4- Rabbit production in the extensive enclosures was greater in plots with intermediate values of scrub coverage, since there is a high availability of shelter (scrub) and food (pastures) in these plots.

5- Building enclosures in areas with high availability in materials in which to dig protected warrens, such as stones, tall scrub or trees, may also improve rabbit production.

6- Rabbit density in the whole study area was significantly higher after the management actions and higher than in areas with no conservations measures, reaching the threshold value to support stable lynx presence in 40 % of the managed UTM cells. Therefore, restocking plots plus habitat management around them can be considered as an effective measure with which to recover wild rabbit populations in long-term and on a landscape scale.

7- Rabbit abundance around the enclosures is influenced by scrub cover, while a lower value has been recorded in areas with a scarcity of scrubland, and restocking plots should therefore be concentrated in those areas that are close to suitable habitats.

8- Habitat management boosts rabbit abundance around the enclosures. Habitat enhancement should take place to promote mosaics of pastures and scrubs in order to raise the carrying capacity, through the creation of feeding habitats (pastures) in dense scrublands via scrub clearance and the increase of refuge in low cover areas.

9- The higher abundances were recorded around restocking plots which had been opened 3 years before, and the effectiveness of the various habitat treatments was more evident than the first two years. This suggests that rabbit colonisation is a medium-term process, and it is therefore necessary to monitor rabbit density during several years in order to assess the effectiveness of restocking actions.

10-In Hornachuelos Natural Park, the cinereous vulture selected as nesting-habitat areas that are far away from roads, village and the edges of patch vegetation, in large pathces with a high cover of cork oak and steep slopes and lower solar radiation. The proximity to the nearest nest had an important weight in the nest-location, and it is thus necessary to consider the conspecific attraction when designing areas for conservation priority.

11- The predictive map showed that less than 8 % of the Natural Park surface is favourable for cinereous vulture nesting-habitat, the conservation of these best suitable areas being a major concern.

CONCLUSIONES

1- Ocho de los 17 puntos selecciones de las directrices de la UICN para las reintroducciones fueron identificados como parcialmente estudiados o desconocidos, incluyendo importantes aspectos como el manejo y la suelta de conejos criados en cautividad, el desarrollo de planes de transporte y programas de monitoreo, la aplicación de vacunas, y estudios a largo plazo tras la suelta. Los futuros esfuerzos en investigación deberían estar enfocados a suprimir estas lagunas de conocimiento.

2- La exclusión de la depredación aérea incrementa la abundancia de conejo durante los primeros días tras la suelta, por lo que techar los cercados más pequeños podría mejorar la supervivencia durante este período crítico.

3- La proximidad entre los cercados, menor a 3 km, favoreció el aumento de la población de conejo en el interior, probablemente debido a la dilución de la depredación aérea. Por lo tanto es recomendable agrupar la construcción de cercados y evitar los cercados aislados.

4- La producción de conejo silvestre en los cercados extensivos fue mejor en zonas con valores intermedios de matorral, ya que estos cercados presentan una alta disponibilidad de refugio (matorral) y comida (pastizales).

5- Construir los cercados en áreas con una gran disponibilidad de elementos de protección tales como rocas, matorral alto o pie de árboles para construir madrigueras puede mejorar la producción de conejo.

6- Tras la apertura de los cercados, la densidad de conejo fue significativamente mayor tras las actuaciones así como respecto a las zonas sin actuaciones, alcanzándose el umbral mínimo para soportar la presencia estable de lince ibérico en el 40% de las cuadrículas UTM manejadas. Por lo tanto, las repoblaciones de conejo en los cercados junto con la mejora de hábitat alrededor de los mismos puede ser considerada una medida efectiva para recuperar las poblaciones de conejo a escala de paisaje.

7- Alrededor de los cercados, la abundancia de conejo estuvo influenciada por la cobertura de matorral, registrándose los valores más bajos en lugares con escasez de refugio, por lo que los cercados deberían situarse en zonas próximas a hábitas favorables.

8- El manejo del hábitat aumentó la abundancia de conejo alrededor de los cercados. Esta mejora de hábitat debería de promover la creación de mosaicos de pastos y matorral con el fin de aumentar la capacidad de carga, mediante el desbroce en parches de matorral denso para la creación de pastizales y la construcción de refugios en áreas con escasa cobertura.

9- La abundancia de conejo fue mayor alrededor de aquellos cercados que habían sido abiertos 3 años antes, y la efectividad de los diferentes tratamientos del hábitat fue más evidente que en los dos primeros años. Esto sugiere que la colonización por parte de los conejos es un proceso a medio plazo, y que es necesario el seguimiento durante varios años para evaluar la efectividad de las repoblaciones.

10- En el Parque Natural de la Sierra de Hornachuelos, el buitre negro seleccionó como área de nidificación zonas alejadas de carreteras, pueblos, y del borde del parche de vegetación, prefiriendo grandes parches con alta cobertura de alcornoque, pendientes pronunciadas y baja radiación solar. La proximidad al vecino más cercano también tuvo un peso importante en la localización del nido, y por lo tanto es necesario considerar este factor a la hora de designar las aéreas prioritarias para la conservación de su área de nidificación.

11- El mapa predictivo mostró que menos del 8% de la superficie del Parque Natural es favorable para el buitre negro, por lo que la estricta conservación de estas áreas debería de ser prioritario.

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SHORT COMMUNICATION

The short-term effect of total predation exclusion on wild rabbit abundance in restocking plots

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Abstract About half a million rabbits are translocated in southwestern Europe every year for conservation and hunting purposes. However, the success of traditional rabbit restocking is generally extremely low, and this has been attributed to short-term predation by mammalian carnivores. Hence, recent recovery programs have tackled the problem of terrestrial predators with the use of exclusion fences, but no additional measures have been employed to avoid aerial predation. In this study, we have therefore conducted a field experiment to test the short-term effect of total predation exclusion in rabbit restocking enclosures, comparing rabbit abundance in plots which are only accessible to raptors (top-open plots) and plots which are accessible to neither carnivores nor raptors (top-closed plots). The results showed that the top-closed plots had higher rabbit abundance in the short term, and the highest difference in rabbit abundance between the two kinds of fences was attained in the first 2 weeks. We therefore conclude that the top-closed plots were an effective tool to increase rabbit abundance during the first weeks after release through the exclusion of raptor predation.

Keywords *Oryctolagus cuniculus* · Predator exclusion · Rabbit conservation · Raptors · Restocking

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Introduction

After their decline, around half a million European wild rabbits (*Oryctolagus cuniculus*), within its native range, are translocated each year in order to boost rabbit population (Letty et al. 2008). This is done because the scarcity of rabbits in Iberia and France constitutes a serious problem due to their economical and biological value (Delibes-Mateos et al. 2008), since they are considered to be the primary small-game species in sport hunting (Calvete and Estrada 2004) and are additionally a keystone species in Mediterranean ecosystems (Delibes-Mateos et al. 2007). Nevertheless, several scientific studies (Calvete et al. 1997; Letty et al. 2002) show a very low success rate in rabbit restocking. In lagomorphs, some works have highlighted that the crux of the problem is the high short-term mortality (Calvete et al. 1997) as a consequence of predation by terrestrial carnivores, environmental novelty, and stress (Calvete et al. 1997; Moreno et al. 2004; Letty et al. 2008; Misiorowska and Wasilewski 2012). Because of this, to prevent the impact of predation and rabbit dispersal after release, the construction of predator exclusion fences, within which the rabbits are released, is one of the most frequent actions in recent rabbit recovery programs (Ferreira and Delibes-Mateos 2010; Ward 2005).

Although some authors have suggested that the impact of raptors on rabbit enclosures could be very high and may therefore decrease restocking efficiency (Rouco et al. 2008; Cabezas et al. 2011), wildlife managers hardly ever carry out measures to tackle the problem of avian predation, other than providing more shelter. Thus, in this study, we aim to test the short-term effect of total predation exclusion in rabbit restocking enclosures. This is done by comparing the most common plots which are only accessible to birds of prey with those which are accessible to neither mammalian predators nor raptors. To date, this is the first study that reported on the effect of total predator exclusion in a rabbit translocation program.

Material and methods

Field work was carried out in central Sierra Morena, in southern Spain, where six of the main raptors that consume rabbit (Delibes-Mateos et al. 2007) were present in the study area: the Spanish imperial eagle (*Aquila adalberti*), the golden eagle (*Aquila chrysaetos*), the eagle owl (*Bubo bubo*), the Bonelli's eagle (*Aquila fasciata*), the booted eagle (*Hieraaetus pennatus*), and the buzzard (*Buteo buteo*). Five fences were built close to each other in an area of 3 ha, three of which had no top net and were therefore only accessible to raptors (top-open plots), and two enclosures with no access to either raptors or terrestrial carnivores (top-closed plots), which were also equipped with a fence on the roof to prevent birds of prey from gaining access. All the fenced plots were of 0.5 ha, had the same number of artificial warrens (five per plot), and had similar vegetation, structure, and cover. All the plots were fenced 0.5 m below the ground and 2 m above the ground with two electric wires and a floppy overhang to exclude terrestrial carnivores (Moseby and Read 2006). Additionally, water and food were supplied ad libitum during the study period. In each plot, 25 adult rabbits were released inside the artificial warrens in February 2010, in the same sex ratio: four females and one male in each warren (total: 20 females and 5 males per plot). All the rabbits were captured with the use of ferrets in a high rabbit density area in the south of the Córdoba province, and they were immediately transported to the release fences (in the north of the Córdoba province) with no vaccines, no confinement period, or quarantines.

Rabbit abundance was estimated through the use of pellet counts at fixed sampling sites in 0.5-m^2 circular sampling (Fernández-de-Simón et al. 2011). Within each plot, 20 fixed points (4×5 grids) were set 20 m from each other, where pellets were removed in all visits to ensure that only fresh pellets of less than 1-week-old were counted. To standardize all pellets counts, a pellet abundance index (PAI) was estimated in each sampling site by dividing the number of pellets at each counting station by the number of days since the last count (Rouco et al. 2011). The counts were repeated on a weekly basis for 6 weeks after release. To evaluate the effect of top fences, we used generalized linear mixed models (GLMM), where pellet abundance index in the counting points was the dependent variable, with Poisson error distribution and logit link function. "Treatment" (closed and open plots) and "week" (each weekly pellet count) were regarded as fixed factors; and "plot" (each individual plot) and "sampling site" (each counting point) were included as random factors. Finally, to compare rabbit abundance between weeks of sampling and treatment, we used Bonferroni's test for pairwise multiple comparisons within the mixed-model analysis. We used SPSS 20.0 software (IBM corp. Chicago, USA) to perform the mixed models.

Table 1 Results of the generalized linear mixed models (GLMM) of the effect of treatment (closed and open top plots) and week on the rabbit abundance

Variables	*F*	$d.f._n$, $d.f._d$	*P*
Intercept	35.82	11, 288	<0.001
Treatment	16.86	1, 288	<0.001
Week	75.59	5, 288	<0.001
Treatment × week	0.26	5, 288	0.932

$d.f._n$ degrees of freedom of numerator, $d.f._d$ degrees of freedom of denominator

Result and discussion

We found a significant effect of treatment (Table 1): the "week" factor had a statistically significant effect (Table 1), but the interaction between "treatment" and "week" was not significant (treatment × week, Table 1). Bonferroni pairwise test showed that rabbit abundance was higher in closed-top plots than open-top plots ($P<0.05$) at all times after release (Fig. 1). Rabbit abundance was different from the first to the third week in both plots (Bonferroni, $P<0.05$), this being more stable since the fourth week, when the rabbit abundance remained constant (Bonferroni, $P>0.05$).

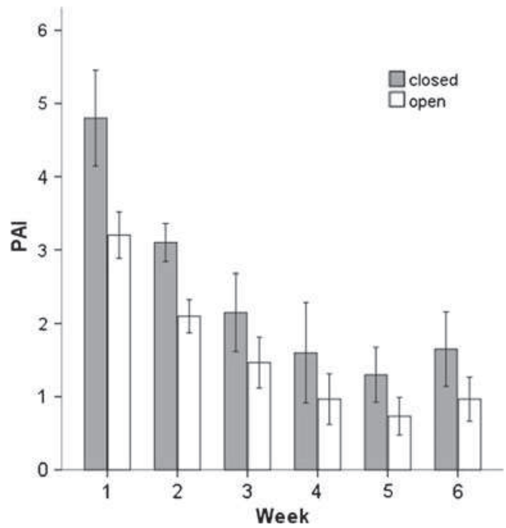

Fig. 1 Effect of experimental treatment (open or closed plots) regarding rabbit pellet abundance index (PAI) during the first 6 weeks after release. *Bars* represent mean values and 95 % intervals are shown

Our results show that the top-closed plots had higher rabbit abundance than the top-open plots at all times after release, although the highest difference was recorded during the first 2 weeks, which could be due to raptor predation in the short term. Indeed, various authors (Rouco et al. 2008; Cabezas et al. 2011) have suggested that aerial predation could be focused upon the rabbit enclosures, in which the rabbits are more vulnerable to predation due to the high rabbit concentration within them. On the other hand, five enclosures in a small area could attract birds of prey much more than independent enclosures. We detected ten carcasses with clear signs of predation by birds of prey (evidence of feathers, tufts of hair, or remains of long bones) in the open-top plots during the study period, which represented a 76.92 % of the total individuals found dead in the top-open plots (n=13) although raptors may have consumed sick, weak, or dead animals as carrion. In addition, the highest difference in rabbit abundance between both kinds of plots occurred in the first 2 weeks after release (Fig. 1), when the movements as a consequence of exploratory behavior (Letty et al. 2008) and the stress caused by capture, handling, transport, and release in an unfamiliar environment (Letty et al. 2003; Cabezas et al. 2011) might have increased the rabbits' vulnerability to death by birds of prey.

Our results further showed that the difference between open and closed plots remained constant after the third week (Fig. 1), suggesting that raptor predation of rabbits did not cause an important effect after this time. Indeed, despite the initial predation rate, Rouco et al. (2008) concluded that the predation by raptors cannot prevent rabbits from achieving a high abundance in fenced plots during the reproductive season. However, the landscape structure may determine the rate of predation, and therefore, the effect of raptor predation on restocking success may play a different role depending on habitat type (Ontiveros et al. 2005; Kamieniarz et al. 2013) and should be evaluated locally.

Nevertheless, both types of enclosure had an abrupt decrease in rabbit abundance during the first 2 weeks, and hence, causes of death other than predation (mainly stress) also affected rabbit survival (Rouco et al. 2008; Cabezas et al. 2011). In our study, the high density of released rabbit (50 individuals/ha) and a big bias in the sex ratio could have induced a huge social stress, thus increasing rabbit mortality. Therefore, although predation by raptors does not have a high effect on rabbit restocking success in the short term, our results suggest that the use of nets to roof the fences plots could improve rabbit survival in small enclosures (less than 0.5 ha). However, due to the high cost of a top fence, particularly in bigger plots, roofed enclosures may not be a cost-effective measure for raptors conservation and other measures, as confinement of rabbits by warren fencing appear to be a more cost-effective measure to enhance rabbit survival. Moreover, an increase of refuge cover by distribution of pallets within the fenced plots could help prevent the aerial predation and therefore improve the short-term rabbit survival, although further research in long-term and large-scale in this topic is necessary to assess the trade-off between the cost and benefit of excluding aerial predation.

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FACTORS AFFECTING WILD RABBIT PRODUCTION IN EXTENSIVE BREEDING ENCLOSURES: HOW CAN WE OPTIMISE EFFORTS?

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Abstract: The declining rabbit population in the Iberian Peninsula has led hunters and authorities to rear rabbits in captivity systems for their subsequent release. One alternative method to intensive rabbitry systems is the use of extensive breeding enclosures, since they produce animals of greater quality for hunting and conservation purposes. However, some of the factors that affect rabbit production in breeding enclosures are still unknown. The present study used partial least squares regression (PLSR) to analyse the effects of plot size, scrub cover, slope, initial rabbit abundance, the resources needed to dig warrens, predation and proximity to other enclosures on rabbit abundance. The results of our study show a positive effect of the number of other fenced plots within a radius of 3 km, a positive relationship with the availability of optimal resources for building warrens and a positive influence of intermediate values of scrub cover. According to our results, to maximise rabbit production in the enclosures it would be advisable to concentrate the restocking effort by ensuring that the restocking plots are close to each other, thus avoiding isolated enclosures. Furthermore, the selection of plots with an appropriate scrub cover and high availability of elements that favour the construction of warrens, such as large stones, sloping land or tall shrubs, may optimise results.

Key Words: captive breeding, *Oryctolagus cuniculus*, extensive system, wild rabbit.

INTRODUCTION

The decline in the wild rabbit population is a major concern in Mediterranean ecosystems, where rabbit is one of the principal game species and an important prey for over 30 Iberian predators. The interest in wild rabbit production for releasing purposes has therefore increased over the last few decades (Sánchez-García *et al*., 2012). In fact, Sánchez-García *et al.,* 2012 reported that the proportion of rabbits released in Spain that had been reared in captivity might exceed 50% of the total number of wild rabbits released.

Captive breeding of wild rabbits is normally carried out in intensive systems, but this process is difficult and not very productive owing to high stress levels, behavioural problems (González-Redondo and Zamora-Lozano, 2008) and the low reproduction rate (González-Redondo, 2010). Semi-extensive breeding systems have attained higher productivity (Arenas *et al*., 2006), but these smaller enclosures (500-800 m^2) fail to reproduce natural conditions and the animals reared in these enclosures might not therefore be appropriate for the purpose of release. In theory, the most appropriate system in which to produce wild rabbits for the purpose of release is extensive production in higher enclosures, since they simulate natural environmental conditions (food availability, soil type and aerial predator pressure), thus enabling the establishment of social interactions and the development of a dietary pattern and anti-predatory behaviour (Díez and Pérez-Garrido, 2003).

For these reasons, setting up extensive captive rabbit breeding enclosures has become a widely used technique in conservation projects over the last few years (Ferreira and Delibes-Mateos, 2010). However, the importance of certain logistic issues such as enclosure sizes, the number of enclosures to be created or the distances between them and their effect on further rabbit abundance, is not well known. These factors are undoubtedly of great practical importance in optimising rearing success, owing to the fact that the high cost of fenced plots makes it difficult for private owners to afford them. Here we show the results of a wild rabbit restocking project in which we analysed the

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data from 23 semi-extensive captivity breeding enclosures to evaluate the effect of the size of the plots, the distances between them, the presence of carnivores, the rabbits' ability to build their own warrens, the scrub cover and the slope on subsequent rabbit abundance.

MATERIAL AND METHODS

Study Area

This study was carried out in central Sierra Morena, Córdoba, in Southern Spain (Figure 1). The study area included different ecosystems: Mediterranean scrubland, pine forest and oak savannah (dehesa). There are 5 species of terrestrial predator: the red fox (*Vulpes vulpes*), the Egyptian mongoose (*Herpestes ichneumon*), the marten (*Martes foina*), the genet (*Genetta genetta*) and the wildcat (*Felis silvestris*); and 6 birds of prey: the Spanish imperial eagle (*Aquila adalberti*), the golden eagle (*Aquila hrysaetos*), the eagle owl (*Bubo bubo*), Bonelli's eagle (*Aquila fasciata*), the booted eagle (*Hieraetus pennatus*) and the buzzard (*Buteo buteo*).

Rabbit release protocol and enclosures features

Twenty-three fences (range: 0.5-7.7 ha) were built throughout the year 2008 in the study area (Figure 1). All plots were fenced 0.5 m below ground and 1.7 m above ground, with 2 electric wires at a height of 30 and 150 cm above ground level. Several artificial warrens made of pallets (3.5±0.4, mean±standard error) were built in each plot. Water and food (grain mixture) were also supplied *ad libitum* throughout the year. The donor rabbit population was high density and located in an agricultural area in the south of Córdoba province, and the release areas were located in the north of the same province (Figure 1). Both areas lie within the distribution limit of the genetic lineage traditionally associated with the sub-species *Oryctolagus cuniculus algirus* (Branco *et al.,* 2000). The rabbits were translocated in October 2008 and the animals were mainly adults. Ferrets were used to capture the rabbits from their warrens in the morning; they were then immediately transported in commercial boxes to the release fences with no vaccines, acclimation period or quarantine. The gender ratio was approximately 2:3 (males:females) in each fenced plot (0.62±0.01); the number of rabbits released ranged from 75 to 90 rabbits per hectare and the animals were released inside the artificial warrens. All capture, transport and release processes were carried out by the same staff from the Andalusian Government Environmental Service.

Rabbit abundance

Rabbit abundance was estimated from November 2008 to July 2009 through the use of monthly pellet counts in fixed 0.5 m^2 circular sampling points (Fernández de Simón *et al.*, 2011) in a 20 m^2 grid located in the centre of the

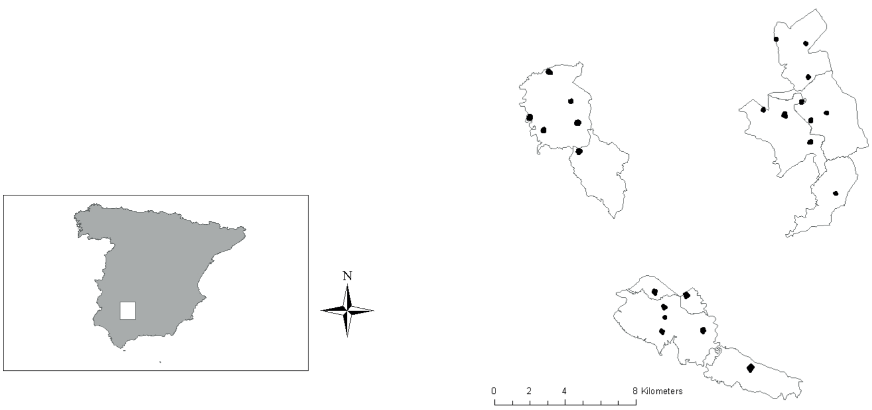

Figure 1: The white box indicates the location of study area in Spain. The enlargement shows the spatial distribution of the fenced enclosures (black circles) and the boundary of hunting states.

enclosure. The number of sampling points ranged from 15 in the enclosures of less than 1 ha to 30 in the largest one (more than 4 ha). Pellets were removed from the circular sampling plots after each count to ensure that only fresh pellets less than 1 mo old were counted. This way, a pellet abundance index was created through the average density of pellets per day and surface for each month and enclosure (pellets/m^2 per day). Since the objectives of the fenced areas were (1) to attain a high number of rabbits during the breeding season in order to permit them to later disperse into the surrounding area by opening the enclosures or by extraction, and (2) maintain a constant abundance to provide prey for endangered predators, the response variables selected were the maximum abundance achieved in each fenced plot (Model 1) and the mean rabbit abundance during the whole study period (Model 2).

Variables

Given that the rabbits released were slightly different in each enclosure, we included the initial rabbit density as the number of rabbits released per hectare. In the field, the critical period for rabbit restocking is the first few weeks after their release (Calvete *et al.,* 1997), so the abundance after this period of adaptation may be different in each fenced area and could affect further rabbit abundance. To account for this variation, the rabbit abundance 1 mo after release was also included in the statistical analysis.

The number of artificial warrens per hectare was also included in the models. The size of each enclosure was obtained by geo-referencing the corners using GPS and ArcGIS software (ERSI, Inc, Redlands, CA, USA). Since the rabbit enclosures had different slopes, the average slope of each fenced plot was included in the analysis, which was calculated through Horn's method (Horn, 1981), using the Digital Terrain Model of Andalusia (DTM, 10×10 m. resolution). To quantify the coverage of bushes, we performed 4 transects 50 m in length per hectare within the enclosures, where vegetation was characterised at intervals of 50 cm. The percentage of cover occupied by the scrub stratum was calculated by applying the point-line intercept method (Canfield, 1941). However, we included the scrub cover in the models as a categorical variable (1: 0-25%, 2: 25-50%, 3: >50%). To record the availability of optimum resources for warren building, we defined a categorical variable with 4 levels according to the presence of appropriate structure and protection (rocks, tall scrub or sloping land) in the enclosures: 1, low (<10%); 2, medium (10-25%); 3, abundant (25-50%) and 4, very abundant (>50 %).

Although the enclosures had 2 electric wires, they did not always prevent the entry of terrestrial predators. All the fenced areas were visited once per week during the study period and during these visits carnivore scats were annotated and removed. Model 1 shows the total number of scats found until maximum rabbit abundance and Model 2 included the total signs during the whole study period. In all cases, the mammalian predators were removed from the rabbit enclosures using live cage-traps.

An aerial predation index was also created. This was done by dividing the set of enclosures into 3 zones, in which a census of birds of prey was carried out at fixed points in the spring of 2009, with a total number of 21 h of observation in each zone over 3 d (Redpath and Thirgood, 1997; Rouco, 2008). The total amount of flight time of the birds of prey was divided between the total number of observation hours, thus obtaining the average flight time for each zone (Rouco, 2008). This variable was then included in the models. Finally, to test the effect of the presence of other restocking fenced plots in the surrounding areas, we applied different models that included the number of fenced areas within a 1, 2, 3 and 4 km radius of each enclosure.

Statistical analysis

Statistical analysis was carried out using partial least squares regression (PLSR). PLSR is a useful regression calibration technique when the number of predictor variables is similar to or higher than the number of observations and/or the predictors are highly correlated (Carrascal *et al.,* 2009), and it reduces the exploratory variables into a few components that have maximum covariance with the dependent variable. A PLSR should therefore be used to deal with the structure of our data with 23 cases and 10 exploratory variables. The number of significant components to be included in the model was selected following the cross validation test described in (Umetrics, 2012), through the cross-validation index (Q2), which was used to assess model significance (Q2>0.05 for significant model). Moreover, the regression coefficient (R^2Y) and the predictor set variance (R^2X) used for the PLSR model were also used to interpret the PLS regression model. To determine the influence of individual variables as predictors of maximum annual abundance in the PLSR model, we used the variable importance in the projection (VIP; Eriksson *et al.*, 1999; Umetrics, 2012). Exploratory variables with a VIP value of over 1 were considered to be more relevant in explaining the variation observed in the variable response (Eriksson *et al.*, 1999). In the 1st model, the response variable used was the maximum rabbit abundance reached in each breeding enclosure, and in the 2nd model, the response variable was the mean rabbit abundance throughout the period of study. Differences in rabbit abundance during the study period were tested by general linear models (GLM), with the month as categorical predictor and the enclosures as random factor. Post-hoc Tukey tests were conducted to illustrate differences among the monthly counts. All variables fitted a normal distribution (P>0.05; Shapiro-Wilk normality test). We used SIMCA-P software (version 13.0; Umetrics AB; Umeå, Sweden) to perform the PLS regression. Normality tests and GLM analysis were carried out with Statistica 7.0 software.

RESULTS

The GLM revealed that rabbit abundance was different during the study period ($F_{8, 32}$=30.87; P=0.001). Indeed, the dynamics of the confined populations showed the typical oscillations of the species in a Mediterranean environment, with the onset of reproduction at the end of winter, reaching a maximum abundance at the end of spring and beginning of summer (June and July) (Figure 2). In the set of enclosures, the maximum abundance oscillated between 1.65 and 9.9 pellets/m^2 per day, with an average of 4.67±0.46.

Firstly, the model that best explained the maximum rabbit abundance (Model 1) included the number of enclosures within a radius of 3 km (R^2Y=0.78; R^2X=0.31; Q2=0.42). In contrast, models including radii of 1, 2 and 4 km showed lower R^2Y values (R^2Y=0.6, R^2Y=0.63 and R^2Y=0.67 respectively). Similarly in Model 2, the model with greatest value of R^2Y also included the number of enclosures in 3 km (R^2Y=0.61; R^2X=0.27; Q2=0.18), since the models that include the number of enclosures in 1, 2 and 4 km showed lower R^2Y values (R^2Y=0.51, R^2Y=0.52 and R^2Y=0.54 respectively). Whatever the case, only 3 variables affected the rabbit abundance (VIP>1): the number of rabbit enclosures at a distance of 3 km, the availability of optimum resources for warren building and the percentage of scrub cover (Table 1). The regression coefficients showed a positive effect of number of enclosures, a positive relationship with the availability of optimum resources for warren building and a positive influence of medium values (25-50%) of scrub cover (Table 1). Conversely, the models showed an adverse effect of lower values of warren resources and scrub cover on rabbit abundance.

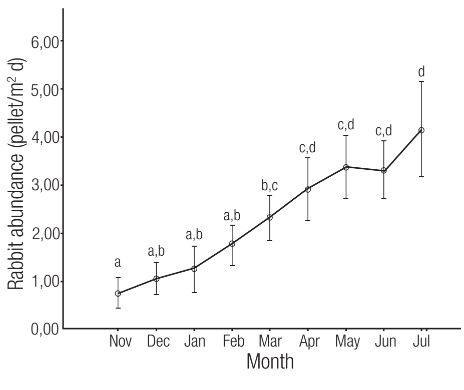

Figure 2: Average monthly rabbit abundance inside fenced plots expressed as a pellet abundance index (pellet/m^2 d) during the study period. Error bars represent a confidence interval of 95 %. Small case letters indicate significant differences (P < 0.05) between groups, as assessed using post-hoc Tukey tests.

Finally, we found 30 scats of terrestrial predators during the study period: 10 from common genet, 18 from stone marten and 2 from wild cat, in 12 of the 23 surveyed plots (54.5%).

DISCUSSION

Our result showed that the rabbit abundance was proportionally higher in enclosures next to each other, with great availability of optimum resources for warren building and intermediate scrub cover values. The increase in rabbit abundance during the study period bears witness to wild rabbit reproduction inside the enclosures. This might therefore be an appropriate tool with which to produce wild rabbits, since these semi-extensive systems avoid the usual problems related with the intensive rearing of wild rabbit and produce animals with a greater ability to adapt to local conditions (Piorno, 2007b), which would then be highly adapted for their release into the wild. On the other hand, these enclosures also avoid genetic risks caused by

Table 1: Influence (VIP) and coefficient of the exploratory variables used in the PLS regression models.

Variables	Model 1		Model 2	
	VIP	Coefficient	VIP	Coefficient
Number of enclosures in 3 km	2.09[a]	0.47	1.47[a]	0.23
Warren resources (4)	1.70[a]	0.31	1.74[a]	0.18
Warren resources (1)	1.28[a]	–0.32	1.87[a]	–0.48
Scrub cover (2)	1.14[a]	0.22	1.18[a]	0.08
Scrub cover (1)	1.11[a]	–0.10	1.08[a]	–0.07
Warren resources (2)	1.06[a]	0.10	0.92	0.01
Raptors time flight	0.71	0.10	0.76	0.13
Average slope	0.71	0.04	0.67	0.02
Initial rabbit density	0.65	–0.16	0.43	0.05
Artificial warren per hectare	0.65	0.15	0.87	0.21
Size of enclosures	0.45	0.11	0.37	0.05
Rabbit abundance on month after release	0.42	0.04	0.65	0.08
Warren resources (3)	0.25	–0.03	0.45	0.12
Scrub cover (3)	0.16	–0.03	0.23	–0.05
Carnivore signs	0.16	–0.02	0.60	0.17
R^2Y	0.78		0.61	
R^2X	0.31		0.27	
Q2	0.42		0.18	

VIP: variable importance in the projection. R^2Y: explained variance by the PLS model. R^2X: variance in the set of predictors used for the PLS model. Q2: cross-validation index. [a] Significant correlation coefficients ($P<0.05$).

the hybridisation with domestic rabbits that often occurs on commercial wild rabbit farms (Piorno, 2007a). Finally, extensive systems also reduce human handling and improve the animals' welfare (Arenas *et al.*, 2006).

Our results suggest that the spatial concentration of the enclosures favours wild rabbit production, since the number of enclosures in a radius of 3 km was positively correlated with the maximum and mean rabbit abundance (Figure 3). This could be attributed to the scattering of the predators in nearby enclosures, in which the set of enclosures might have enabled the rabbits to escape from the predation pit with greater ease, and this result prompted us to consider that an isolated enclosure is not viable, since it cannot support the impact of aerial predation. Furthermore, a radius of 3 km forms a circumference of approximately 28 km^2 around the enclosure, similar to the spatial scale (25 km^2) often used at the home range level in studies on birds of prey (Martínez *et al.*, 2003; López-López *et al.*, 2006). The low rabbit density in the study area, the large number of rabbit predators and the elevated abundance achieved in the enclosures, where the rabbits are highly vulnerable, might have attracted birds of prey (Rouco, 2008). Indeed, the aerial predation index showed a positive relationship with rabbit abundance in both models, which may be due to a higher raptor concentration in those spots with higher rabbit abundance.

As expected, the availability of optimal sites for the rabbits to dig warrens also had an important weight in both models. Several works have reported the rabbit's preference for building warrens under protective structures, such as trees, tall scrub and rocks (Palomares, 2003b; Barrio *et al.*, 2009). This may be because heavy rain can cause the death of juveniles as a result of flooding and/or tunnel-collapse, thus making unprotected warrens much more vulnerable to these phenomena (Palomares, 2003a). Likewise, protected warrens are less affected by predation (Villafuerte,

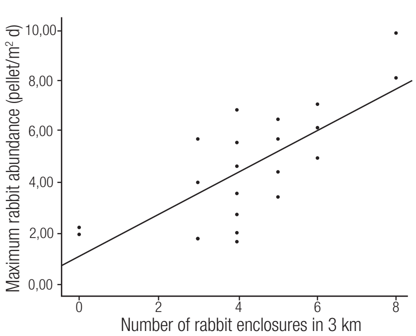

Figure 3: Correlation between the number of enclosures in a radius of 3 km and the monthly maximum abundance reached in each fenced plot.

1994). In our experiment, the relatively low number of artificial warrens provided for rabbits in the enclosures might be monopolised by the dominant rabbits (Mykytowycz and Gambale, 1965). As a result, the subordinate animals would have to dig their own warrens, so a greater number of favourable places where the rabbits can build their warrens would therefore allow secondary females to breed and thus contribute to higher offspring production.

The percentage of scrub cover in each enclosure also had an important effect on rabbit production. Indeed, the PLS models showed a positive influence of intermediate values (25-50%) and a negative correlation with low bush coverage (0-25%). These results highlight the role of habitat features in wild rabbit abundance, as rabbits in the wild reach high abundance in those places where shelter (scrub) and food resources (pastures) are widely available. The highest scrub cover values (more than 50%) showed no significant effect, as the range of rabbit abundance in these plots was very broad. Hence, the breeding enclosures should be built in places with optimum shelter availability, while enclosures with very low or very high bush cover should be avoided.

Our data showed that the electric fence was not completely effective in preventing the entry of terrestrial predators, although the entry of some carnivores did not affect rabbit abundance. We consider that a small curved overhang on the top of fences (Moseby and Read, 2006) could prevent the entry of mammalian predators. Finally, the slope, the size of the fenced plots, the initial rabbit density and the abundance 1 mo after release did not affect further rabbit abundance, perhaps because the distances between plots and the presence of elements that favour the construction of warrens and shelter played a more relevant role in the model.

CONCLUSION

In agreement with the findings, we suggest that new semi-extensive rabbit captive enclosures should be created less than 3 km apart from each other in order to minimise aerial predation, and that isolated enclosures should be avoided, thus minimising the impact of predation. Our results also suggest that the availability of optimum resources for digging warrens and the scrub coverage would appear to be other crucial factors, so the selection of plots with an appropriate structure and protection such as large stones or the presence of tall shrubs might therefore optimise the rearing results.

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Restocking a keystone species in a biodiversity hotspot: Recovering the European rabbit on a landscape scale

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ABSTRACT

Rabbit populations in Iberia remain at low densities in several areas in which their endangered predators still coexist, and the recovery of these populations is therefore urgent if the integrity of Iberian Mediterranean ecosystems is to be maintained. The enhancement of wild rabbit populations has been attempted through the use of *in situ* extensive rabbit captive breeding enclosures (restocking plots), which reduce mortality caused by terrestrial predators and dispersal movements and permit the breeding of young individuals which can then naturally disperse to settle in the surrounding areas. However, their effectiveness, the role of its size, the optimal habitat management that should be promoted around them and the habitat features remains uncertain. Here, we show results from a four year study of an ambitious rabbit restocking plan on a landscape scale. We measured rabbit abundance in a vast area in which thirty-two restocking plots were built to create an initial rabbit population for further dispersion, in addition to an intensive habitat management program. We also compared rabbit abundance between managed and unmanaged UTM cells of 2.5 km × 2.5 km. Our results showed that rabbit abundance was three times higher in managed cells, but four years after restocking, rabbit abundances had only reached the threshold needed to support stable Iberian lynx presence (at least 10 latrines per km^{-1}) in 9 of the 23 managed cells. Rabbit abundance was strongly affected by habitat treatment and scrub coverage. The increase of shelter was useful in low cover areas but ineligible in places with high scrub cover, where the increase of refuge plus scrub clearing to create pastures improve rabbit abundance more effectively. In the light of our results, restocking plots should be built only in places with suitable habitat, whereas pastures should be created in dense scrublands and refuge in low cover areas.

Introduction

The Mediterranean Basin is a global hotspot of biodiversity owing to the exceptional concentrations of endemic species (Myers et al. 2000). In the Iberian Peninsula, the European rabbit (*Oryctolagus cuniculus*) plays a key role in the conservation of the Mediterranean basin hotspot, since the rabbit is an ecosystem engineer and an important prey for more than 30 predator species (Delibes-Mateos et al. 2008a). The rabbit is also the principal small game species in France, Portugal and Spain (Delibes-Mateos et al. 2008a; Letty et al. 2008). However, local extinction or very low rabbit abundances is frequent in south-western Europe after the abrupt fall in rabbit populations during the 20th century as a result of habitat loss, over-harvesting and the outbreak of two viral diseases (myxomatosis and rabbit hemorrhagic disease). This sharp decline in rabbit populations may have had important cascading effects on the functioning of the Iberian-Mediterranean ecosystem, with serious ecological and economic consequences (Delibes-Mateos et al. 2008a), particularly for the Iberian lynx (*Lynx pardinus*) and the Spanish Imperial Eagle (*Aquila adalberti*), two endangered rabbit-specialist predators (Ferrer & Negro 2004).

Much emphasis has therefore been placed on recovering rabbit populations owing to the interest in the species for hunters and conservation (Angulo 2003; Delibes-Mateos et al. 2008b; Letty et al. 2008). The strategies most frequently used are habitat management, a reduction of hunting pressure, predator control and vaccination (Angulo 2003), although rabbit restocking has recently undergone a significant increase in Iberia and France (Letty et al. 2008). However, despite the fact that half a million rabbits are translocated in south-western Europe every year (Letty et al. 2008), very low success rates have been recorded in traditional rabbit restocking efforts (Calvete et al. 1997). This low restocking success signifies that wildlife managers have started to combine certain tools in order to improve rabbit survival, such as acclimatisation in the release site or habitat management. More recently, the establishment of *in situ* extensive rabbit captive breeding enclosures (hereafter restocking plots) has also taken place and has become a widely employed technique in conservation projects (Ferreira &

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Delibes-Mateos 2010; Guerrero-Casado et al. 2013b). The role of fences is to keep the rabbits confined in order to reduce their mortality as a result of terrestrial predators and their initial dispersal movements, and finally to obtain a high density inside the fenced plots and well-acclimated *in situ* animals in order to allow them to further colonise the surrounding areas (Guerrero-Casado et al. 2013a; Rouco et al. 2008).

Nevertheless, most rabbit translocation programs are not monitored (Cabezas & Moreno 2007), and no studies have been conducted to address the final restocking success in the long-term and on a large-scale. Furthermore, despite the high cost of restocking plots, the effectiveness of further release in order to establish self-sustaining rabbit populations and the role of some important issues such as their size, habitat attributes or the optimal habitat management around them remain uncertain. In this four year study, we assessed rabbit abundance around 32 restocking plots spread over 14,000 ha in order to test the effectiveness of restocking four years after release. The aim of this paper is to analyse the effect of habitat features, the effectiveness of habitat management (creating shelter, artificial warrens, and pastures) and the importance of restocking plot size and distribution in facilitating the long-term and large-scale settlement of new free-ranging wild rabbit populations.

Materials and methods

Study area

The fieldwork took place in Sierra Morena mountain chains (southern Spain, Fig. 1), in which the current scarcity of rabbits is a major conservation concern, since the Iberian lynx and the Spanish Imperial Eagle still coexist in this area. The study area included tree species such as holm oak (*Quercus ilex*) and cork oak (*Quercus suber*), pine reforestations (*Pinus* spp.), Mediterranean scrubland dominated by *Cystus* spp., *Pistacia* spp., and *Rosmarinus* spp. and pasture areas occupied by oak savannah (*dehesa*).

Rabbit release protocol and enclosures features

Thirty-two restocking plots (range 0.5–7.7 ha) were built in the study area throughout the years 2008–2010 (Fig. 1). Rabbits were released with no vaccines, and no acclimation period or quarantines during the years 2008 and 2009 in autumn or winter (for more details see Guerrero-Casado et al. 2013a). In the subsequent years (2009–2011) the fenced plots were opened by small gates on the fences at the end of the breeding season when rabbits had reached their highest abundances inside the restocking plots (Guerrero-Casado et al. 2013a) in order to permit their natural dispersal into adjacent areas. All processes of capture, transport and release were carried out by the same staff from the Environmental Council.

Rabbit abundance

Around each restocking plots, wild rabbit abundance was estimated through the use of latrine counts by walking four transects of 500-m each ($n = 128$), signifying that there was a total transect distance of 2-km around every restocking plot. These surveys were performed both before and after these were opened (in the summers of 2008–2009 and 2012, respectively). A latrine was defined as any pellet accumulation containing at least 20 pellets over a surface of 20 cm × 30 cm (Virgós et al. 2003). Latrine counts have already been used as an index with which to estimate relative rabbit abundance in many scientific works (Cabezas-Diaz et al. 2011; Calvete et al. 2004; Lozano et al. 2007; Virgós et al. 2003), since this rabbit abundance index can provide a useful estimation of rabbit abundance in large-scale studies (Calvete et al. 2006). Furthermore, latrine abundance and rabbit density estimated by direct observations are highly correlated in Sierra Morena (Gil-Sánchez et al. 2011). All these transects were GPS referenced, as was the location of every restocking plot. In each transect, the distances from the transect to the perimeter of the closest restocking plot were calculated every 100m, and an average value was estimated in each one. This variable and how many restocking plots there were in a 3 km radius was obtained by using ArcGIS software (ERSI, Inc., Redlands, CA, USA).

In summer 2012, following the methodology employed by Gil-Sánchez et al. (2011), the study area was divided into 2.5 × 2.5 UTM grill cells (Fig. 1), in which rabbit abundance was sampled by latrine counts in managed ($n = 23$) and unmanaged cells ($n = 12$). The aforementioned authors reported that in Sierra Morena, stable lynx presence and reproduction were confirmed in grids with at least 10 rabbit latrines per km^{-1}, and this value was therefore used to evaluate the effectiveness of the rabbit restocking plan.

Habitat features and management

Scrub coverage (%) was estimated by eye in circular plots of 25m radius every 100m in each walked transect, following similar protocols to general habitat-rabbit studies (Virgós et al. 2003). Prior to sampling, the same field workers performed trials to homogenise the percentage of the area covered by shrub vegetation. We used average values of scrub cover for each transect, which was reclassified in a categorical variable with three levels: low (0–30%); medium (30–60%); and, high (>60%). Three different habitat enhancement strategies were then carried out in a 500m radius of each enclosure: (1) no action; (2) an increase in the amount of refuge available by heaped wooden branches and artificial warrens created with wooden pallets and covered with stones, branches and earth; and (3) an increase in refuge plus the creation of pasture lands by clearing scrubland.

Statistical analysis

First, a generalised linear mixed model (GLMM) was performed to assess differences in rabbit abundance before and after opening, including the restocking plot as random variable (model 1). In this case, the number of latrines per km^{-1} around each restocking plot was used as dependent variable and it was modeled by generalised mixed models, using a Poisson distribution with a log-link function as the error distribution. Second, a general lineal mixed model (LMM) was performed to test differences in rabbit abundance between managed and unmanaged UTM cells (model 2), using the number of latrines per km^{-1} in each cell as response variable, which fitted a normal distribution at UTM-cell level. The coordinates *X* and *Y* of the centre of UTM cells were considered to avoid spatial autocorrelation. Finally, in summer 2012, the effect of habitat management and restocking plots features on rabbit abundance around these was evaluated by generalised linear mixed models (model 3), in which the number of latrines in each transect was used as a dependent variable, which fitted a Poisson distribution with a log-link function. Since transects were grouped into each restocking plots, and data from transects were correlated within each restocking plots, it was included as a random variable. In this last model (model 3), the mean distance from each transect to the restocking plot, its surface (ha) and the number of restocking plots in a radius of 3 km were included as continuous variables, whereas the percentage of scrub, the amount of years since each plot had been opened and the habitat management were treated as categorical variables with three levels. The interactions between habitat treatment and scrub coverage, and between habitat management and 'years opened' were also included. Fisher's least significant difference test (LSD test) for comparisons of the

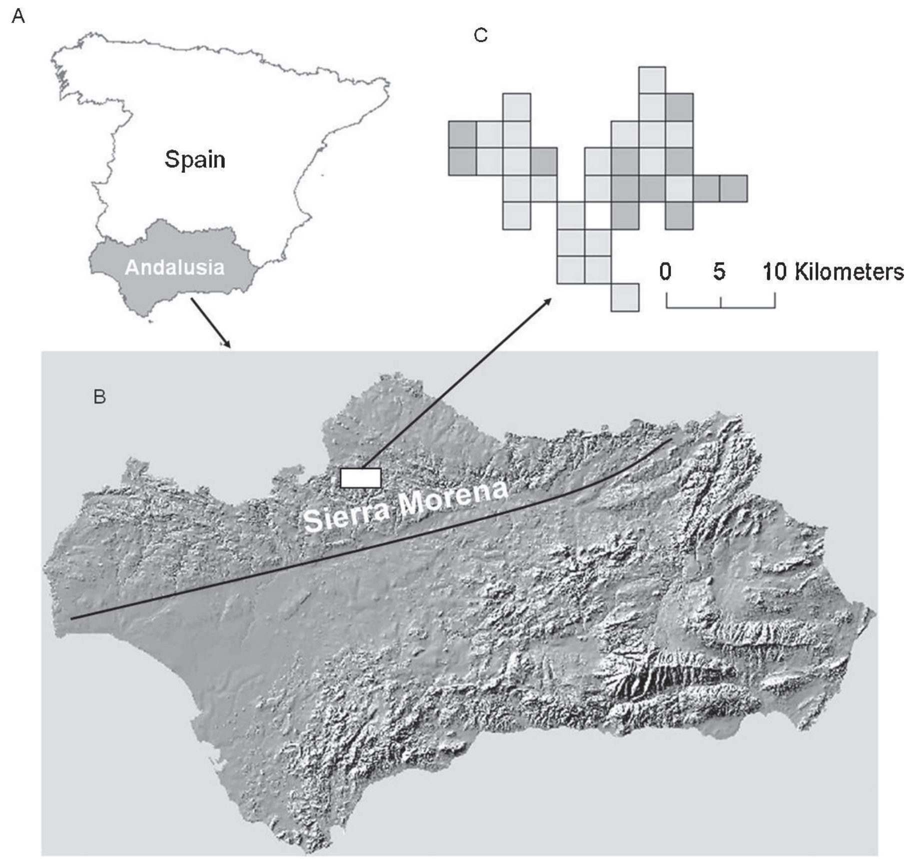

Fig. 1. (A) Localisation of Andalusia in Spain. (B) Sierra Morena mountains chain is shown in schematic form in Andalusia and the white square represents the study area. (C) The 2.5 km × 2.5 km UTM-cells with management actions and control are shown in light grey and dark grey, respectively.

estimated means within mixed analysis was developed to check differences among the level of categorical variables and to illustrate the interactions. The assumptions of normality, homogeneity and independence of residuals were confirmed (Zuur et al. 2009), and we therefore log-transformed the size of enclosures, and the mean distance from the segment to the enclosure in order to fulfill normality. The entire statistical analysis was performed using InfoStat software.

Results

Prior to restocking, the rabbit abundance in the study area was very low and uniform, with an average value of 1.028 ± 0.314 latrines/km (mean ± S.E.), whereas after management actions the rabbit abundance was considerably higher (9.34 ± 1.91, mean ± S.E.; model 1, $X^2_{1,29} = 246.04$, $P < 0.001$). Furthermore, in managed cells rabbit abundance (9.048 ± 1.98, mean ± S.E.) was statistically higher (model 2, $F_{1,33} = 7.9$; $P = 0.011$) than in unmanaged areas (2.91 ± 1.32, mean ± S.E.). Furthermore, our results showed that three or four years after opening the restocking plots 9 of the 23 UTM cells in which wild rabbit conservation actions had taken place had attained the minimum rabbit density needed to support stable lynx presence (at least 10 latrines per km^{-1}).

Regarding the factors affecting rabbit abundance around the restocking plots, model 3 showed a significant effect of habitat management actions, scrub coverage, and the years since the plots had been opened, while its area, the number of these in a radius of 3 km and the distance of transect to the restocking plot did not have a significant relationship with latrine abundance (Table 1). Nevertheless, the interaction between the habitat management strategy and scrub coverage also had a statistically significant effect, which indicates that the habitat management tools did not have the same effect on wild rabbit abundance depending on scrub coverage (Fig. 2). There were no significant differences between unmanaged segments and an increase in refuge in high cover areas, whereas the increase in refuge did enhance rabbit abundance in low cover areas (Fig. 2). The interaction between habitat treatment and the years since the plots had been opened also showed significant differences, which are illustrated by Fisher test (Fig. 3).

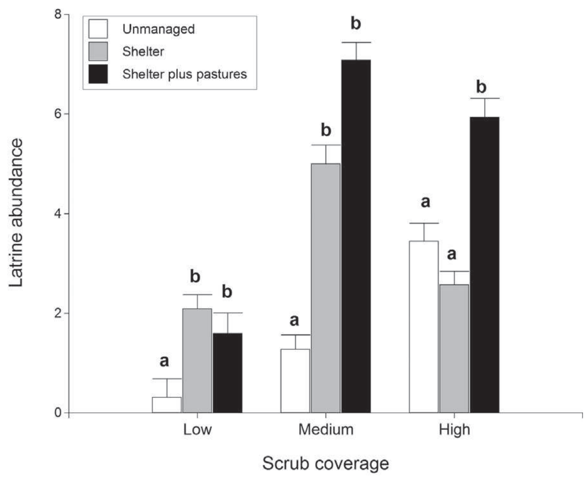

Fig. 2. Predicted mean values (±S.E.) of latrine counts with different habitat management actions in low, medium and high scrub cover. Small case letters indicate significant differences ($P = 0.05$) between groups of habitat treatment according to Fisher LSD tests.

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Table 1
Chi-square, *P*-values and coefficients of the variables included in the mixed model (model 3) to explain rabbit abundance. df show the degree of freedom of the numerator. Coefficients for the level of fixed factors were calculated using reference values of 1 in the variable 'years opened', 'low' in the variable 'scrub coverage', and 'shelter plus scrub clearing' in 'management'.

Variables	Chi-square	df	*P*	Coefficient ± E.S.
Management	154.55	2	<0.0001	Unmanaged = −1.97 ± 0.79 Shelter = 0.56 ± 0.8
Years opened	17.37	2	0.0002	2 = −0.15 ± 0.97 3 = 0.78 ± 0.95
Scrub coverage	27.19	2	<0.0001	Medium = 1.49 ± 0.42 High = 1.31 ± 0.45
Restocking plots in 3 km	0.01	1	0.9239	0.04 ± 0.11
Area	0.07	1	0.7888	−0.47 ± 0.85
Distance	0.97	1	0.3235	0.13 ± 0.14
Management * Years	21.39	4	0.0003	
Management * Scrub	22.16	4	0.0002	

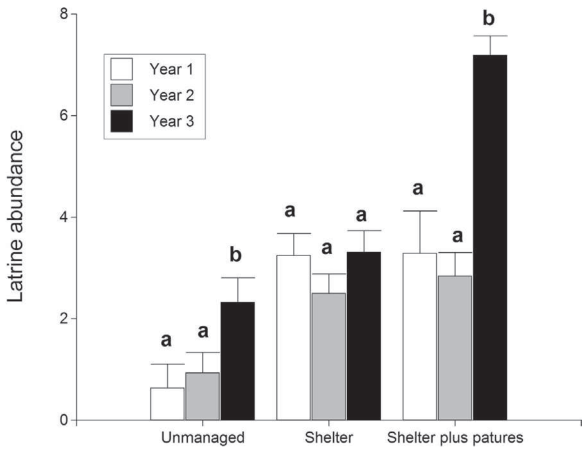

Fig. 3. Predicted mean values (±S.E.) of latrine counts with different habitat management actions in the years since the plots had been opened. Small case letters indicate significant differences ($P = 0.05$) between years according to Fisher LSD tests.

Discussion

In southern Spain, the Spanish Imperial Eagle and the Iberian lynx, which are endangered rabbit predator species, are confined into those areas in which non-trophic detrimental factors have been relatively low and rabbit densities are sufficient (Ferrer & Negro 2004; Real et al. 2009). Rabbit recovery in these spots is a critical concern if the local extinction of these specialist predator species is to be avoided (Ferrer & Negro 2004; Real et al. 2009), and it is therefore imperative to establish guidelines for the recovery of wild rabbit populations in these areas. The results presented here provide evidence of the effectiveness of the restocking plots combined with habitat management on a landscape scale. We have shown that rabbit abundance attained with these techniques could support stable lynx presence in some circumstances, and it might thus be considered an appropriate tool to promote the establishment of new lynx and imperial eagle territories or enhance rabbit abundance in the existing territories. Indeed, two new Spanish Imperial Eagle breeding pairs were located in the study area after management actions (author unpublished data).

In our study, the effect of the habitat enhancement actions depended of scrub cover. In high cover areas, the zones with an increase in refuge did not significantly differ from those which were unmanaged (Fig. 2). Refuge was easily available in these zones, and may explain why the increase in shelter with branches and artificial warrens did not enhance rabbit abundance, and the creation of grazing areas was also necessary to increase rabbit densities. In the study area, the loss of the traditional management of scrubland would appear to be one of the main factors explaining rabbit scarcity (Delibes-Mateos et al. 2010). Hence, the enclosures should be placed in habitats with great diversity and a complex structure, and the objective of habitat management should be to minimise predation rate by increasing habitat heterogeneity in areas adjacent to restocking plots. In fact, the highest rabbit abundance was attained in those areas in which the habitat complexity was enhanced by increase of refuge and scrub clearing (Fig. 2).

The increase in refuge with heaped wooden branches and artificial warrens was effective in places with low and medium availability of shelter (Fig. 2). Artificial warrens and branches provide protection against predators and against extreme climates, favour the establishment of social ties and provide an optimal place for breeding (Catalán et al. 2008; Rouco et al. 2011). Since rabbit abundance in unmanaged areas was higher in high and medium cover areas, we deem that it is better to build restocking plots in those habitats than to perform insensitive management in inappropriate areas. However, caution is needed when interpreting our results, and the management strategies should be carefully based on the landscape and vegetation features in each situation.

The distance from the segment to the restocking plots, their concentration and their surface did not have any significant effect on rabbit abundance. This may owe to the fact that the habitat quality and the management actions play more relevant roles in the establishment of rabbit populations around the restocking plots. However, concentrating restocking effort by building restocking plots close to each other would perhaps favour rabbit establishment in adjacent areas under similar habitat conditions, since intra-specific interactions such as proximity to nearby warrens might be playing a primary role in these low-density populations (Barrio et al. 2009). Small isolated and fragmented populations are much more vulnerable to stochastic phenomena (Wilcox & Murphy 1985), and we therefore suggest that it would be advisable to concentrate the management actions in small areas rather than to establish dispersed actions in large areas (Carvalho & Gomes 2003). Lastly, the years since the plots had been opened also had a significant statistical effect, and higher values were recorded in the restocking plots which had been opened three years before in places with an increase in refuge and creation of pastures (Fig. 3). It suggests that rabbit colonisation is a long-time process, and it is necessary to monitor rabbit density in the long-term to assess the effectiveness of restocking actions.

Our data suggest that restocking in enclosures for further release plus habitat management increase rabbit populations to support territories of top specialist predators, despite the flaws in the environment of these areas. In summary, restocking plots should be concentrated in those areas that are close to suitable habitats, and in the case of rabbit translocation in sub-optimal habitat, habitat enhancement should take place to promote mosaics of pastures and scrubs in order to raise the carrying capacity around the restocking plots, through the creation of feeding habitats in dense scrublands and the increase of shelter in low cover areas.

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Modelling the nesting-habitat of the Cinereous Vulture Aegypius monachus on a fine scale for conservation purposes

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Modelling the nesting-habitat of the Cinereous Vulture *Aegypius monachus* on a fine scale for conservation purposes

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Capsule Nests of Cinereous Vultures were found to be located farther from roads, villages and the edge of large vegetation patches. They preferred large vegetation patches containing extensive Cork Oak cover on steeper slopes and with lower solar radiation. Less than 8% of the study area was predicted to be suitable for nesting.
Aims To generate a predictive habitat suitability map for the Cinereous Vulture's nesting-habitat on a fine scale for conservation applications within its breeding range.
Methods Habitat features of 43 nest-locations and random points were compared in order to identify nest-habitat selected in the region of the Hornachuelos Natural Park (Spain). A logistic regression approach was used to create habitat models.
Results Compared with random points, nests were found to be located farther from roads, villages and patch edges, and in large vegetation patches containing extensive Cork Oak cover on steeper slopes with lower solar radiation. The predictive map revealed that less than 8% of the study area had a greater probability of occupancy than 0.8.
Conclusions Most habitats in the study area are unsuitable for nesting suggesting that conservation of the best suitable areas is important. The fine-scale predictive map approach may be valuable in designating conservation priority areas.

Understanding the strength of the relationships between habitat and the distribution of raptors may be important in the management of protected areas and in contributing to the development of successful conservation programmes (Limiñana *et al.* 2011, Di Vittorio *et al.* 2012). To address this, modelling of species' distributions has been increasingly used in order to identify suitable habitat and to predict potential distributions (Austin 2002, Rushton *et al.* 2004). Predictive habitat distribution modelling based on statistical techniques and geographic information system (GIS) tools have important potential applications, such as forecasting species occurrence in poorly documented areas, the identification of adequate target areas for species reintroduction or conservation programmes or predicting the climatic change impact (Rodríguez *et al.* 2007, Muñoz *et al.* 2013). However, there are only a few published examples of high-resolution predictive habitat distribution models (e.g. < 100-m precision) built to predict suitable nesting areas for raptors within their breeding range.

Like many birds, raptors are usually highly selective with regard to their habitats, particularly as regards the availability of suitable nesting areas (Limiñana *et al.* 2011). This is the case of the Cinereous Vulture (*Aegypius monachus*), a highly selective tree-nesting raptor. This raptor is considered to be an umbrella flagship species (Carrete & Donázar 2005) and is classified as *Near Threatened* in the IUCN Red List, and *Vulnerable* in the Spanish Red List. In the Iberian Peninsula, Cinereous Vulture populations have undergone a sharp decline, particularly throughout the first half of the 20th century, as a consequence of

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habitat loss and alteration, a decrease in food availability, poisoning and human disturbance (Donázar *et al.* 2002, Madroño *et al.* 2004).

Previous studies have shown that nest-site selection and occupancy by Cinereous Vultures are most likely to be affected by either loss of nests and nesting-habitat as result of human activities or disturbance during the pre-laying and laying period, particularly if these circumstances occur consistently over time (Donázar *et al.* 2002, Gavashelishvili *et al.* 2006). Consequently, the Cinereous Vulture selects its nesting-habitat in undisturbed areas, in mature vegetation patches with steep slopes (Poirazidis *et al.* 2004, Morán-López *et al.* 2006a, Moreno-Opo *et al.* 2012). The optimal habitat for Cinereous Vulture has been dramatically reduced, making its current distribution in Europe small in historical terms (Gavashelishvili *et al.* 2012), and it is thus urgent to identify and protect those suitable areas. The aim of this work is therefore to apply habitat modelling techniques with the use of a logistic regression approach and GIS tools to generate an explicative and predictive habitat suitability model for the Cinereous Vulture's nesting-habitat at a fine spatial resolution (50-m resolution) within its breeding range.

METHODS

Study area

The study was conducted in Córdoba Province, in the Sierra Morena mountains (southern Spain, Fig. 1) in and around the Hornachuelos Natural Park, where the Cinereous Vulture breeds currently and historically (Dobado & Arenas 2012). The Hornachuelos mountains were designated as a Natural Park in 1989 and cover 60 000 ha. The climate is typically Mediterranean, with moderately cool rainy winters and hot dry summers. The altitude ranges from 200 to 800 m. The dominant vegetation includes tree species such as Holm Oak (*Quercus ilex*) and Cork Oak (*Quercus suber*), reforestations of Stone Pine (*Pinus pinea*) and Cluster Pine (*Pinus pinaster*), Mediterranean scrubland dominated by *Cistus* spp., *Erica* spp., *Pistacia* spp., *Phyllirea* spp. and *Rosmarinus* spp and pastures areas occupied by oak savannah (*dehesa*). Some nests belonging to the colony are located outside the Natural Park's limits, and a 5-km conservation buffer (the maximum distance between the two nearest nests) has, therefore, been designated around them, since it is known that Cinereous Vulture usually establishes the new nests in adjacent areas to pre-existing ones. This buffer zone is included in the Guadiato-Bembézar 'Site of Community Importance' for the Natura 2000 network.

Nest survey

In 2011, the study area was searched for nest sites, and a minimum of three visits was made to every nest during the breeding season. A nest was considered to be occupied if we observed a minimum of typical pair behaviour, courtship, brood rearing activity or young (Carrete *et al.* 2001, López-López *et al.* 2006). The geographical coordinates of nests were recorded with a global positioning system. As habitat-selection studies require a comparison of the selected sites (presences) with a randomly chosen control points of non-used sites (absences) (Manly *et al.* 1993), a GIS was used to generate the same number of random points as nests, so that the number of sampled nests and the random plots was balanced (Moreno-Opo *et al.* 2012). Autocorrelation problems were reduced by constraining the random points to be at least 59-m apart (the minimum of the nearest neighbour distance of the Cinereous Vulture in the study area).

Studied variables

Those variables that affect nest-site selection by Cinereous Vultures in Spain were selected in accordance with previous studies (Morán-López *et al.* 2006a, Moreno-Opo *et al.* 2012). A total of 11 variables provided by GIS analyses were taken into account: altitude (m), slope (%), mean incident solar radiation from February to June (Wh/m^2), distance to nearest road (m), distance to nearest track (m), distance to the nearest village (m), the size of the homogenous land cover patch in which the nest was located (ha), the distance from the nest/random point to the border of the vegetation patch (m) and the canopy cover covered by *Q. suber* (%), *Quercus rotundifolia* (%) and *Pinus* spp. (%). Topographic variables (altitude slope and solar radiation) were obtained from a digital elevation model with an accuracy of 10-m pixels of horizontal and vertical resolution, whereas vegetation variables were obtained from the third National Forestry Inventory (1997–2007) and the Corine land cover (2006). Climatic factors were not considered owing to the relatively small study area, which implies similar values of

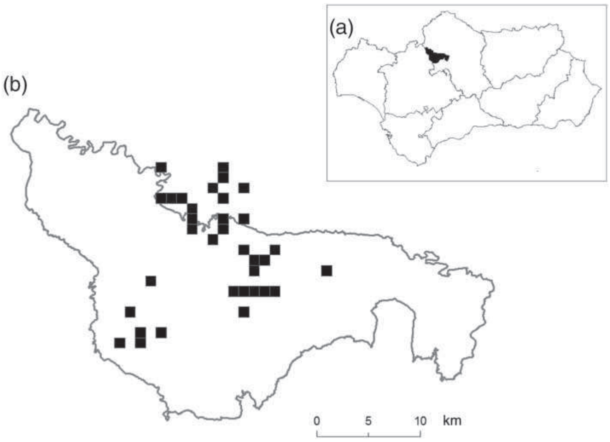

Figure 1. (a) The shaded area shows the location of Hornachuelos Natural Park in the region of Andalusia (southern Spain). (b) The black squares indicate the location of Cinereous Vulture nests in the study area, and the grey line is the limit of the Natural Park.

temperature, rainfall and humidity throughout the study area (Moreno-Opo *et al.* 2012).

Statistical analysis and predictive map

Univariate and multivariate statistical techniques were used to determine which factors affect the Cinereous Vulture's nest-site selection. First, Mann–Whitney *U*-tests were used to provide a simple description of the main differences in the mean values of the variables between nests and random sites. Secondly, a binomial logistic regression (with a logit link function), a particular case of generalised linear model, was used to identify which variables were the best predictors (Pearce & Ferrier 2000). Two different models were built: considering the distance to the nearest neighbour (model 1), because this variable has biological meaning and captures the spatial configuration (Barrio *et al.* 2009); and without including this spatial variable (model 2). In both cases, a forward–backward stepwise procedure was applied using the Akaike Information Criterion with a small-sample bias adjustment (AICc) to select the most parsimonious models. The accuracy of the predictive distribution models were assessed by calculating the area under the receiver operating characteristic curve (known as AUC). AUC values of > 0.7 indicate acceptable predictive power. Collinearity among continuous explanatory variables was low (Pearson correlation < 0.6; variance inflate factor < 10). The distance to the nearest nest was ln-transformed in order to eliminate nonlinearity among the independent variables and the logit of the dependent variable. Finally, Moran's *I* coefficient was calculated to examine spatial autocorrelation in residuals of the models in order to confirm the assumption of independent errors (Dormann *et al.* 2007).

The final models obtained by model selection were implemented in GIS using the inverse of logit transformation (Real *et al.* 2006). The predictive maps were built with a resolution of 50-m per pixel, a scale considered to represent the minimum suitable surface unit needed by the Cinereous Vulture for breeding (Poirazidis *et al.* 2004). GIS analyses were performed using ArcGIS 9.3.1 (ESRI Inc, Redlands, CA, USA) and all the statistical analysis was performed using SPSS 11 software (IBM corp. Chicago, USA).

RESULTS

In total, 43 nest-site locations were recorded in 2011, and the mean distance to the nearest neighbour was 1031 m (± 927 sd; range 59–5353 m). Nests were located farther from roads, villages and patch edges, in larger homogenous patches with high proportion of Cork Oak cover and on steeper slopes with lower solar radiation (Table 1). Both most parsimonious models

Table 1. Means (± se) of variables measured for the nests (n = 43) and the random points (n = 43).

Variables	Nest site	Random	*U*-test	*P*
Distance to roads (m)	4661 (±305)	3425 (±386)	806	<0.001
Distance to tracks (m)	124 (±16.8)	85 (±10.5)	1115	0.076
Distance to villages (m)	12 793 (±425)	10 520 (±476)	913	0.002
Altitude (m.a.s.l.)	339 (±10)	359 (±13)	1306	0.566
Slope (%)	37.4 (±1.8)	21.5 (±1.9)	570	<0.001
Distance to neighbour (m)	1031 (±141)	4893 (±486)	345	<0.001
Patch size (ha)	31 907 (±2196)	19 367 (±1817)	1011	0.010
Distance to edge patch (m)	340 (±45)	186 (±22.8)	919	0.003
Solar radiation (W/m^2)	536 068 (±1064)	571 933 (±5419)	1011	0.015
Q. suber (%)	14.7 (±1.12)	7.4 (±1.03)	14 776	<0.001
Q. rotundifolia (%)	14.6 (±1.67)	17.1 (±1.73)	1291.5	0.505
Pinus spp. (%)	9.5 (±2.57)	6.1 (±1.86)	1214	0.152

Table 2. Logistic regression models of Cinereous Vulture habitat requirements in Hornachuelos Natural Park.

	Model 1 ΔAICc = 4.08				Model 2 ΔAICc = 5.01			
Parameter	Coefficient	Wald	df	*P*	Coefficient	Wald	df	*P*
Intercept	5.86 ± 2.58	5.15	4	0.023	−5.98 ± 1.31	20.7	4	<0.001
Ln nearest nest	−1.37 ± 0.34	16.26	4	<0.001	–	–	–	–
Slope	0.088 ± 0.023	14.58	4	<0.001	0.075 ± 0.019	16.21	4	<0.001
Distance to patch edge	0.003 ± 0.002	4.48	4	0.034	0.004 ± 0.001	9.61	4	0.002
Cork Oak cover	0.032 ± 0.028	1.35	4	0.035	0.058 ± 0.022	7.08	4	0.008
Distance to villages	–	–	–	–	0.001 ± 0.001	20.74	4	0.01

ΔAIC indicates the improvement in model fit of the final model compared with the next best model.

showed a positive effect of slope and Cork Oak cover to the distance of vegetation patch border, and model 2 also showed a significant positive effect with the distance from the villages (Table 2). As we predicted, model 1 demonstrated an effect of the nearest nest (Table 2). Model 1 and model 2 had AUC values of 0.93 and 0.87, respectively, indicating a good discrimination capacity. Moran's *I* coefficient of the model with the distance to the nearest nest was particularly low, thus indicating no significant spatial autocorrelation among residuals (Moran's $I = -0.007$, $P = 0.98$), whereas the residuals of the model without this spatial variable showed spatial autocorrelation (Moran's $I = -0.44$, $P < 0.001$).

DISCUSSION

Given that the Cinereous Vulture has a long reproductive season, this species is strongly affected by anthropogenic disturbance (Madroño *et al.* 2004, Morán-López *et al.* 2006b, Margalida *et al.* 2011). Our results were in agreement with those of previous studies in that they showed that the Cinereous Vulture selected steep slope areas far away from roads, villages and from the edges of larger vegetation patches, suggesting that this species selected areas less disturbed by humans. Moreover, because the maximum temperature in the study area can reach 40°C, the thermoregulation of the young may constitute a limiting factor, and therefore, these birds tend to select areas with lower solar radiation, highlighting the importance of avoiding warmer places under these harsh conditions (Morán-López *et al.* 2006b). Lastly, some previous studies have highlighted the positive effect of extensive Cork Oak coverage, because the Cinereous Vulture selects old Cork Oaks as a preferred nesting tree, because they can support the weight of nests and are easier to land on and take off from (Donázar *et al.* 2002, Moreno-Opo *et al.* 2012).

Since this study was carried out inside the limits of a current and historical breeding area for the Cinereous Vulture, the effects of anthropogenic disturbance were likely to be uniformly weak (Table 1) in contrast to other areas where they are an important predictor (Morán-López *et al.* 2006a), thus the results should be interpreted in this context. Therefore, although distance from tracks and roads to the nest was not a very important negative predictor, road access is restricted in many areas of the study area, and the roads which cross the study area are secondary roads with little car transit. In the same way, since the study area has low human population density, the distance from villages between occupied and unoccupied areas is also less marked (Table 1).

With regard to habitat nest-selection for colonial or semi-colonial raptor species, conspecific attraction may play an important role in breeding spatial distribution (Sergio & Penteriani 2005, Limiñana *et al.* 2011). Indeed, the first model confirmed that the proximity to the nearest nest was an important predictor in determining nest-location, and therefore, most of the predicted suitable areas were located surrounding pre-existing vulture nests (Fig. 2a). Moreover, this model had a higher AUC value, lower AICc value and no spatial autocorrelation among residuals, thus suggesting that the aggregated spatial pattern of the nests should be considered in any predictive model for Cinereous Vulture. Model 2 can also be considered for predicting the spread of the colony in the long term, because this model allows the identification of places far away from the current existing nests (Fig. 2b). However, in the light of the aforementioned reasons, we believe that the model that included the distance to the nearest nest is more appropriate, particularly in order to predict the immediate colonization of new territories. Therefore, for this semi-colonial species, increasing the availability of preferred habitats in the periphery of the existing nests might be more efficient in ensuring the occupancy of given areas when designing areas for conservation priority.

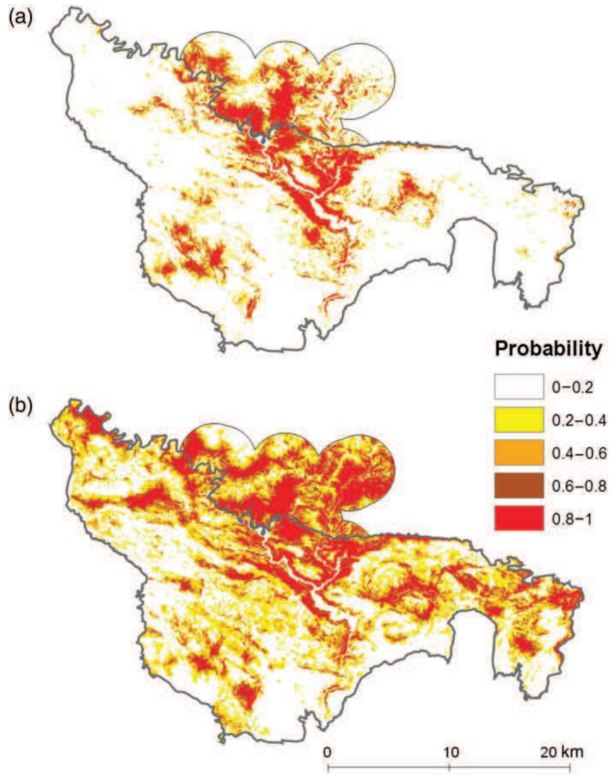

Figure 2. Probability of presence of Cinereous Vultures derived from logistic regression at a 50 × 50 m resolution taken into account the distance from the nearest nest (a) or without this variable (b).

Predictive species distribution models have been used in a wide range of applications including regional biodiversity assessments, conservation biology, wildlife management and conservation planning (Rodríguez *et al.* 2007). However, the lack of fine-grain predictive maps may result in their low effectiveness for designing further protected areas (López-López *et al.* 2007, Fernández & Gurrutxaga 2010). In the light of these considerations, our predictive maps may be valuable for the local management of the Cinereous Vulture. For instance, here we have shown that many favourable areas are situated outside the protected limits of the Natural Park, but are inside the Guadiato-Bembézar 'Site of Community Importance' for the Natura 2000 network (Fig. 2). In these conservation priority areas for the Cinereous Vulture, the implementation of spatial and temporal buffer zones around potentially sensitive areas (breeding sites) where disturbances are limited or prohibited (Margalida *et al.* 2011), and the preservation of large vegetation patches with extensive coverage of Cork Oak should favour the establishment of new breeding pairs. Lastly, the probability maps may also be very helpful in identifying sites for the construction of artificial nests, although microhabitat variables such as tree species and tree height would be necessary to select the exact sites for these.

The Cinereous Vulture can be considered as an habitat indicator species owing to its nest-site selection in areas with high conservation status that are important to many other species (Moreno-Opo *et al.* 2012). The predictive map that included the distance to the nearest neighbour also revealed that less than 8% of the study area had a higher probability value of occupancy than 0.8 (Fig. 2a), which can be considered a reasonable threshold value, because the odds are higher than 4:1 (Real *et al.* 2006). These low values of suitable habitat inside the breeding range imply that the conservation of these best suitable areas is a major concern in order to minimize the negative impact of habitat loss. The scale used in our experiment (50-m resolution) permits the delimitation of protection areas in which the occurrence of target species is more probable, thus allowing the application of fine-scale management decisions.

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