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DEPARTAMENTO DE CIÊNCIAS DA VIDA

FACULDADE DE CIÊNCIAS E TECNOLOGIA UNIVERSIDADE DE COIMBRA

Study of a wild rabbit (*Oryctolagus cuniculus*) population Habitat factors related to its spatial distribution

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Dissertação apresentada à Universidade de Coimbra para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Ecologia, ramo de especialização Ecologia Aplicada, realizada sob a orientação científica do Professor Doutor José Paulo Sousa (Universidade de Coimbra) e co-orientação do Professor Alberto Ferreira (IMAR, Universidade de Coimbra).

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RESUMO

O Coelho bravo (Oryctolagus cuniculus) é considerado uma espécie chave na Península Ibérica, embora os seus números tenham caído durante as últimas décadas, o cada vez se torna mais uma preocupação para os ecossistemas mediterrânicos. Alguns esforços têm sido feitos para recuperar as populações de coelho-bravo como o uso de tocas artificiais, gestão de habitat ou acções de repovoamento. O objetivo deste trabalho é avaliar a distribuição das tocas e abundância relativa de coelho-bravo numa reserva de caça como forma a prever a distribuição da espécie nesta zona. Para isso, um modelo GLM foi realizado para explicar quais os parâmetros que são seleccionados pelos coelhos para a construção de tocas. O modelo final foi dado principalmente pela AFS (sistemas agro-florestais), PMC (culturas permanentes), HAA (áreas agrícolas heterogéneas) e negativamente pela ANT (territórios antropogênicos).

Com o método de contagens de latrinas, as abundâncias relativas de coelho bravo foram estimadas e não mostraram uma correlação significativa entre a abundância e a distribuição tocas. Além disso houve uma ligeira diferença (mas não significante) entre a abundância relativa de Coelho bravo estimada em 2010 e 2012.

Este estudo deve ser continuado e melhorado de forma a aumentar o conhecimento da distribuição desta população e num futuro próximo ser possivel melhorar as condições de habitat nesta area com vista a conservação do Coelho bravo.

ABSTRACT

European wild rabbit (*Oryctolagus cuniculus*) is considered a keystone species in the Iberian Peninsula, though its numbers have declined during recent decades, which is a concern for Mediterranean ecosystems. Some efforts are being made to recover populations of wild rabbit, such as the use of artificial warrens, habitat management or restocking actions. The objective of this work is to evaluate warrens distribution and relative abundances of wild rabbit within a hunting reserve in way to predict species distributions. For that, a GLM model was performed to explain which parameters for burrows construction are selected by rabbits. The final model was given mostly by AFS (Agro-forest systems), PMC (Permanent crops), HAA (Heterogeneous agriculture areas) and negatively by ANT (Anthropogenic territories).

With the method of latrine counts, relative abundances of wild rabbit were estimated and there was no significance correlation between relative abundances and distribution of warrens. Also, there was some (but not significant) increase between abundances estimated in 2010 and now in 2012.

This study should be continued and improved to increase knowledge of this population distribution and to in the near future improve habitat conditions in this area.

CHAPTER 1

INTRODUCTION

1. BIOLOGY

1.1. Taxonomy and distribution

The European wild rabbit (*Oryctolagus cuniculus*) is a mammal originated from Iberian Peninsula [1]. It belongs to the order of Lagomorphs, which includes rabbits, pikas and hares and to the Leporidae family, where pikas are not included. Also, two sub-species are recognized: *Oryctolagus cuniculus cuniculus* and *Oryctolagus cuniculus algirus*. Both are brown/grey in colour and about 35 cm long when adult, but they are genetically different and have different weights: *O. c. cuniculus* at 1.50 – 2.00 Kg, whereas *O. c. algirus* weights

Due to the introduction of this mammal in other many parts of the world, there is a distribution of *O. c. cuniculus* over northeast Spain, South France, Occidental Europe and Australia and over southeast Iberian Peninsula, North of Africa, and Portuguese Atlantic islands in the case of *O. c. algirus* [2, 3]⁻ Indeed, in the Iberian Peninsula is formed a contact zone where the two sub-species occurs [3].

1.2. Habitat and Alimentation

For wild rabbit, like all the other species, the best habitat is the one that provides with abundance, all the resources required for survivor and reproduction. Many authors consider rabbit patterns of abundance are likely to be related with habitat variability.

This species live in a wide variety of habitats from agriculture landscapes to woodlands, although they seem to occupy the typical Mediterranean habitat with dry and warm climate and above 1500m [4]. However, they have a wide of requirements such as the presence of open areas with suitable food species, availability of cover protection against predation and soils properties for warrens building [5-7].

According to diverse surveys, they prefer open areas of grassland, farmland and

Mediterranean scrubland. In Fa et al. (1993) rabbit abundances were associated with mixed grassland/matorral and forest/matorral habitats. Furthermore, vertical vegetation structure and composition seems to be favourable for this lagomorph abundance when there are low herbaceous vegetation providing high quality food and a certain amount of tall vegetation offering cover but without hindering movement [6, 8]. Indeed, open areas resulted by prescribed fires, seem to be favourable for rabbits since dense vegetation present before fire, hide its movements [9]. Also, it allows the appearance of a significant area occupied by a high diversity of low herbaceous, mostly gramineous and leguminous, used for alimentation, being related to rabbit abundances [6].

Because its alimentation is composed mainly for vegetation with considerable quantity of cellulose, is difficult to digest and absorb whereby a system of double digestion is required. For this, rabbits make use of coprophagy, a process where rabbit excreted soft feces reached in protein, which are ingested again and will be submitted to a second digestion, giving origin to hard feces [10]. Soft feces are produced in a structure denominated cecum (between small and large intestine) where bacterial flora degrades cellulose and produces these excrements with high concentrations of nutrients and which are ingested again [11]. It allows an efficient nutrient absorption at small intestine level and after that occurs the excretion of hard feces. According to these last authors, this physiological habit of coprophagy benefits rabbits at a nutrition level.

1.3. Reproduction

As we know, rabbits are strongly reproductive species and according to Ward (2005), European wild rabbit is able to reproduce throughout the year, although some conditions as climate and food availability affect reproduction. So, in the Iberian Peninsula the typical breeding season is from mid autumn to the end of spring ^[12].

Each female can reach up to 12 litters per year, although 2-4 is more common.

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The gestation period is in average 31 days and each litter can raise 3-6 young. At birth, the little rabbits are blind and with no fur, but after 1 week the fur grows and with about 13 days old they open their eyes.

The period of maternal dependence rounds 20-30 days ^[4], after that they leave de reproduction warren and start to feed by themselves.

Sexual maturity is reached between four (*O. c. algirus*) and nine (*O. c. cuniculus*) months.

Also, the average life expectancy is up to 10 years ^[1] but in the wild this number is lower, due to predation and other factors.

2. ECOLOGY

2.1 Organization and Social behaviour

Wild rabbit is a species that lives in familiar groups establishing colonies. It has been considered as highly social animals that mix freely within groups but interact less frequently between groups ^[13]. A familiar group consists of up three males and nine females [14], which are joined by juveniles of the year. Sometimes between these familiar groups, exchanges could occur especially with juveniles, forming a set of familiar groups called colony. The colonies form the population of wild rabbit in a certain place, and all this structure is very important to maintain the balance of these species populations. They typically forage in groups to increase the likelihood of detecting and to dilute the impact of predators [15] and their activity is crepuscular, being higher during twilight and night [16].

Different individuals fulfil different roles in a social hierarchy and this situation becomes stronger during mating and breeding season [17, 18]. Within each group there's a dominant male that fertilizes some females, and a female that reproduces in the principal burrow, while the others build smaller burrows around.

The animals from a colony usually use the same alimentation zone around a burrow system.

2.2. Warrens

In order to avoid bad weather conditions and predators, rabbits build warrens, which have an important role as shelter [19] providing a safe breeding place [20]. They are the result of continuous digging by a group of rabbits which can build a structure of high complexity, consisting of a ramified network with several chambers [21], and this could vary between different warrens, i.e. each one could be constituted with one or more burrows (openings) linked through tunnels.

Indeed, warrens are dynamic structures which it construction and occurrence depends on several factors and there are some studies pointing the relation of warrens with other ecological aspects.

Firstly, the presence of these structures is particularly important in open areas with no shrub protection [22, 23] they are frequently congregated along the border of the scrubland with pastures adjacent to the marsh [24]. Also, the colonies are usually where the soils have good drainage capacity and between roots of tall shrubs [25] or along sloping lands with low probability of being affected by heavy rains [26]. Plus, recent studies point the importance of spatial factors such as distance to nearest-neighbour warren (active) and density of warrens within vital area (200m), for those areas where rabbits build warrens [27] and it influence on population dynamics could be different when we're dealing with different and low densities populations. Also, in Barrio and Bueno (2009) is referred that warrens occurrence is more related with high warren neighbouring densities than with the presence of open areas, and that they are more likely to be used if they're close to an active neighbouring warren. So, the use of

spatial variables allows the improvement of predictive models to develop conservation plans.

Concerning soil type, it strongly influences warren structure and spatial distribution and according to some studies, the most favourable for rabbit excavation are sandy soils [28, 29]. Also, these kinds of soil have a low water-holding capacity, which prevents the death of young rabbits by collapsing or flooding of warrens by heavy rains [26, 30]. However, according to other authors, burrows in sandy soils are smaller than in heavy soils [31]. Plus, they are usually build in an elevated position [32] or under supporting elements such as shrubs roots, trees or rocks to stabilize the burrow structure [33].

In recovery programs, artificial warrens are often used as a management measurement to enhance populations [27].

3. IMPORTANCE

3.1. Wild rabbit as a keystone species

Species that are exceptional relative to the rest of the community, in maintaining the organization and diversity of their ecological communities, are considered keystone species [34, 35]. In the particular case of European wild rabbit, it acts as a keystone species in the Iberian ecosystems [36-38] for different reasons.

Firstly, rabbits are critically important to the conservation of the predator community in the Mediterranean [36]. They influence the diet of many predators such as the Red fox (*Vulpes vulpes*), Egyptian Mongoose (*Herpestes ichneumon*), Wild Cat (*Felis sylvestris*), Bonelli's Eagle (*Hieraaetus fasciatus*) and other birds of prey, but more important they have a strong effect on two predator specialists on rabbits in the Mediterranean ecosystem, the Iberian Lynx (*Lynx pardinus*) and the Imperial Eagle

(*Aquila adalberti*) ^[1, 39] which depends on rabbits presence. For instance, it has been calculated that the autumn mean rabbit density required for Iberian lynx residence is 1 rabbit ha⁻¹, while the spring mean rabbit density required for reproduction is 4.6 rabbits ha^{-1 [40].} Therefore, rabbit decline is one of the main causes of the decline and near extinction of these two of the most endangered predators of the world, once it means there's less food for them, reducing their survival and reproductive rates ^[1].

Secondly, this on of the most appealing small game species^[41] for the hunters of the Iberian Peninsula and it's an important rural food source for people who live there. Although due to the decline of rabbits its hunting has been partly substituted in some areas by partridge and large game hunting (e.g. deer), rabbit hunting remains an important cultural and economic activity, with many land-owners and gamekeepers basing their livelihoods upon income from commercial rabbit hunting^[1]. For this reason many hunters and hunting associations have dedicated significant amounts of time and money to rabbit recovery efforts.

Moreover, wild rabbit has an important role in plant communities by preservation of plant species diversity and seed dispersal^[42], contributing to modulate in the Mediterranean landscapes. Finally, it is an interesting native species by it own and just for that, its conservation worth it.

4. DECLINE

Rabbit numbers were once extremely higher in Spain and Portugal. Indeed, this seems to be the explanation for the origin of the name Hispania a latinization of the Phoenician expression i-shepham-im, that could mean "rabbit coast or rabbit island" [43]. However, rabbit numbers suffered a decline over the 20 century, due mainly to human induced mortality, habitat loss and fragmentation and to the appearance of two

rabbit diseases: Myxomatosis and Rabbit Hemorrhagic Disease (RHD) [1, 44].

In the last 30 years rabbit numbers have declined on average by 80% in Spain [45] and about 24% in Portugal between 1995 and 2002 [46]. Moreover and according to Ward (2005), rabbit decline has been uneven, with some areas still containing rabbits at relatively high density but in many areas rabbit populations are extinct or nearing extinction, and many other areas containing rabbit populations at very low density.

4.1. Myxomatosis and Rabbit Haemorrhagic Disease (RHD)

Over the last 50 years the decline of rabbit populations was accentuated with the arrival of these two viral diseases. The additive effect of both diseases has reduced rabbit numbers in most of its historical range, especially in ecologically less- favorable areas [47].

Myxomatosis appeared in Portugal and Spain in the 1950's [1, 48]. First originated in South America where it is endemic in the native Cottontail rabbit (*Syvilagus* sp.), this disease was deliberately introduced in France in 1952 by a farmer keen to eradicate rabbits from his land [1, 49]. It spread rapidly across Europe and was first discovered in the Iberian Peninsula in 1953 where was responsible for killing 90% of the rabbits [45]. This is a viral disease transmitted by direct contact but mainly by fleas and mosquitoes, so it's most prevalent during spring and summer when these insects are more present. It can kill wild rabbits directly or indirectly by increasing susceptibility to predation. Common symptoms are lumps and swellings around the genitals and head possibly progressing to acute conjunctivitis, blindness, loss of appetite and fever, lead animal to death in 13 days. [1]. Following the initial outbreak, mortality rates due to myxomatosis started to decline as the degree of resistance to the disease increased and by the 1980's rabbits showed signs recovery [50]. Still, disease continues to play a major role in the dynamics of rabbit populations [51].

Just when populations in Portugal and Spain were recovering from myxomatosis another devastating rabbit disease arrived – Rabbit Haemorrhagic Disease (RHD)- and had a great impact on it reducing number of wild rabbits again.

RHD was firstly described in Chine in 1984 [52], was detected in Europe in 1987 and spread to Spain and Portugal by 1989 [1], where 55-75% of wild rabbits were killed by this disease [47]. This is also a viral disease but in this case it is mainly spread by direct contact between individuals, not being necessary an insect vector. RHD is most prevalent during winter and spring, and it kills adult rabbits but not young under eight weeks [1]. Although this is poor understood, it is known that rabbits born to immune mothers are temporarily protected by maternal antibodies, and that if infected with RHD at this time young rabbits will gain life-long immunity to the disease [1]. This disease cause haemorrhaging of lungs and lesions in the liver [53] and symptoms include bleeding from the nose and mouth. After infection, RHD has a period of incubation of 24-48 hours and rabbits usually die within 6-24 hours of the onset of fever [1].

Both diseases for being present during almost all year, don't allow a total recovery of wild rabbit populations, once one of these virus can kill an immune individual to the other. However, vaccines against both viral diseases have been developed with higher immunity rates and when used, vaccination allows the control of closed populations or where there's a strong control of populations [1]. Still, these vaccines don't confer complete immunity and both have negative side-effects [54].

4.2. Habitat loss and fragmentation

Habitat loss and fragmentation has been a major cause to the decline of the species numbers, beginning even before the arrival of the two viral diseases. With the economic grow and the rural exodus, changes in the agrarian structure have happened and led to the intensification of the agriculture and livestock farming in certain areas,

and to the underutilization of other vast rural areas where traditional uses ceased to be competitive[55]. Both processes contributed to the substitution of Mediterranean mosaics that characterized the traditional Iberian agricultural landscapes (wild rabbit's most preferred habitat as explained before) by large monospecific homogeneous patches of scrubland and crops [56]. Areas of closed forest provide less food for rabbits than mixed agro-forestry and similarly, large monocultures of crops fail to provide year-round food sources for rabbits and lack vegetation for protection from predators [1]. All this has thus had a negative impact upon the species, contributing to its decline [57] and according to Ward (2005) it has been estimated that 1% of Mediterranean scrub-forest is lost each year to human development.

Farther, much ideal rabbit habitat has been lost in recent years to urbanization and infrastructure development and also to large forest fires in Spain and Portugal.

4.3. Human induced mortality

In Portugal and Spain, a significant number of deaths of this species is strongly correlated with humans by hunting or to protect agriculture [1]. By themselves, these traditional practices were probably sustainable, but when in combination with diseases and habitat loss, they contribute to rabbit decline.

Although some rabbit control is needed, especially in areas where they could cause damage to crops, in some other areas where rabbit declined it is not justified [1].

Moreover, and as we know, wild rabbit is one of the most appreciated species for hunting in Iberian Peninsula, and each year many millions of rabbits have been killed by this activity. Although this practice benefits rabbits when recovery of valuable habitat is a concern to hunters, in other away strongly influences rabbit decline, especially when there's no control.

In Portugal, hunting season is between September and December, in ordered

territories.

4.4. Predation

Predation effect doesn't contribute directly to rabbit decline, once always existed a large number of rabbit's predators even when its populations were in high densities. Also, rabbits evolved in away to counter high predation rates through anti-predator behavior and high reproduction rates [57].

The problem is that the recovery of some rabbit populations that were strongly reduced by the reasons explained before, may be being partly prevented by opportunistic predators [1]. Also, reductions in vegetation by intensive agriculture and forestry may increase the vulnerability of rabbits to these predators by reducing shelter availability.

Moreover, the increase of opportunistic predators has been partly caused by the decrease of top predators due to inappropriate non-selective predator control implemented by hunters and farmers [58].

5. CURRENT STATUS

In spite of *O. cuniculus* be a widespread colonizer and be considered a pest outside its natural range (e.g. Australia) [59], according to IUCN red list of threatened species, this vertebrate fills in the "Near threatened" category [60]. The same classification is in the Portuguese red book of vertebrates by the Portuguese Institute for Nature and Biodiversity Conservation (ICNB) relative to this species in mainland Portugal [61]. This is because, as was been told before, rabbit populations within the natural range have declined an estimated 95% since 1950, and 80% in Spain since 1975 [45, 62] and about 24% in Portugal between 1995 and 2002 [46]. Also, there is no evidence that the decline has escalated in recent years, though threats remain and the

decline is continuing in different regions of the Iberian Peninsula.

6. CONSERVATION – What can be done

As was mentioned before, *O. cuniculus* is referred as a keystone species and also as an important game species, so its conservation should be considered to achieve a sustained rabbit recovery.

Even with a narrow focus on the endangered Iberian Lynx and Iberian Imperial Eagle, widespread rabbit recovery is necessary given the large interconnected areas required to sustain viable meta-populations of these specialist predator species [1].

In order to have populations of rabbits (and rabbit predators) to link up into more continuous distributions in the future, it will be necessary to first stabilize and maintain remnant rabbit populations, and thus to reverse on-going declines and the transient nature of many rabbit populations [1]. For this, is necessary to achieve some specific measurements in a wild rabbit conservation plan for Portuguese populations by 1) planning a management strategy in different geographic levels; 2) monitoring hunting impact; 3) reducing the impacts of, and avoiding rabbit diseases; 4) programing populations reinforcements with local specimens through translocations/reintroductions; 4) protecting and restoring rabbit habitat in current and potential rabbit areas; 5) reducing the short term impact of common rabbit predators, but only where justified and only of non protected species and 6) including hunter associations and other institutions in the management of local populations.

In conservation programmes (in this case *O. cuniculus*) direct and indirect methods are currently used to estimate rabbit abundances and population trends. Direct methods are based on surveys or counts of the animals, while indirect methods are based on the monitoring of animal signs [63](such as latrines, dispersal excrements, scratches and tracks) or even warren censuses.

Dispersal excrements and latrines parameters are usually used to ecology surveys of this specie, once they well reflect the relative abundance of wild rabbit [64] [25] and might be useful to large-scale samplings [25].

Latrines are special communal sites where rabbits deposit excrements for social reasons [65]. They're territorial visual marks and with secretions, used as exchange of information between individuals of one colony and neighbour colonies [65]. Usually they're associated with scratches made mainly by dominant males as a visual exhibition for other males [66].

Moreover, slaughter quotes can be added (when are known) to improve the determination of the abundance index.

7. OBJECTIVES AND WORK HYPOTESIS

Species distribution models are empirical models used to predicted geographic range of a species and relating its occurrence to environmental variables based on statistical or other response surfaces [67]. It has become a useful tool for fundamental ecological and biogeographic research, and for biodiversity management and conservation [68].

For this work it will be performed a species distribution model to evaluate the importance of environmental and spatial parameters in the location of burrows, in way to predict species distribution.

Also, it will be estimated rabbit abundance within the study area and ascertain if it has a relationship with distribution of burrows.

Finally, is intended to manage a rabbit population in a captive breeding area (in way to future restocking actions).

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CHAPTER 2

METHODOLOGY

1. STUDY AREA

This present study was realized between October 2011 and June 2012 in the Associative Hunting Zone (HZ) of "Carvalhal da Azóia" managed by Hunters' Club of Samuel parish as well as in the captive breeding area of the same institution.

This association has about 200 partners and is located in Carvalhal da Azóia, Samuel parish, county of Soure and in the district of Coimbra. In the case of the Hunting Zone (HZ) (study area), it has an area of 3160 hectares and it's divided in two distinct hunting zones, one constituted in 1991 and the second in 2007 (Fig. 1).

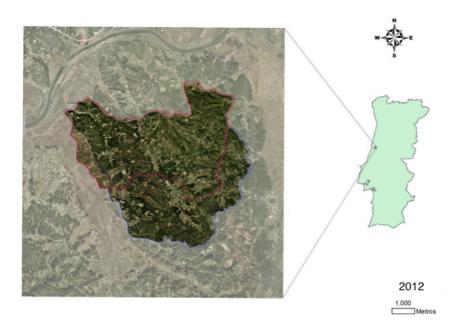


Figure 1 - Study area divided in two zones

Soure county, where the hunting zone is located, is part of the center region, in the lower Mondego sub-region and it goes from sub-wet to wet climate. It belongs to district of Coimbra with an average annual temperature of 15,5°C and with an average annual precipitation of 75,4 mm [69].

The hunting zone consists mainly of forests of dense shrubs, oaks of *Quercus robur*, pines and eucalyptus.

For all the study area were defined 1x1 Km UTM (i.e. Universal Transverse Mercator geographic coordinate system) squares with reference to a military card of the study zone.

The methodology used in the field to collect data in 2010, supported the methodology used in this current research in way to compare some data. In that previous fieldwork, the relative abundance index was determined in all areas within study area and for the warren census was given priority to areas where higher abundances of wild rabbit were indicated.

In this current survey only areas not explored before were submitted to the census of warrens.

2. EVALUATION OF SPATIAL AND ENVIRONMENTAL ELEMENTS RELATED TO WARRENS DISTRIBUTION

As said before, in 2010 warrens census was made only in some areas of the study zone, where relative abundances of wild rabbit were considered more significant. So, in this present work (the second phase of the census) presence of warrens was determined only in remaining areas, namely areas with lower abundances during that previous work .

Between November 2011 and April 2012 was made the warren census by a researcher who looked for it deeply through the field in a total of 21 squares of the hunting area (Fig. 2). In a small part of the research, the mapping was help by a hunter who showed some warrens in the field.

In figure 3 are represented the total number of squares (37, in a total of 45) where warren census was carried out so far (first and second phase of census). Each UTM square was considered totally visited when warrens present there (considering the areas

where access is physically possible) were considered totally found, giving a total of 28 totally visited squares when regarding both phases of research. More 10 UTM squares were visited though weren't totally explored, with the possibility of containing untracked warrens.

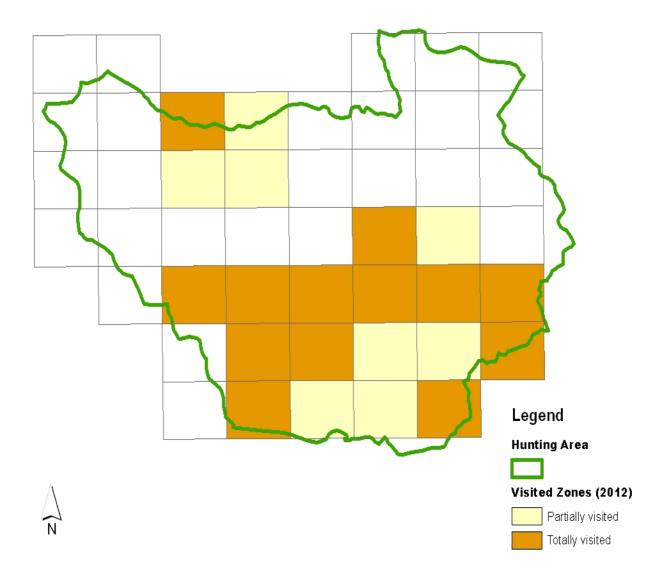


Figure 2 – Study area with 13 totally visited zones and 8 partially visited, both cases visited during practical work for this dissertation.

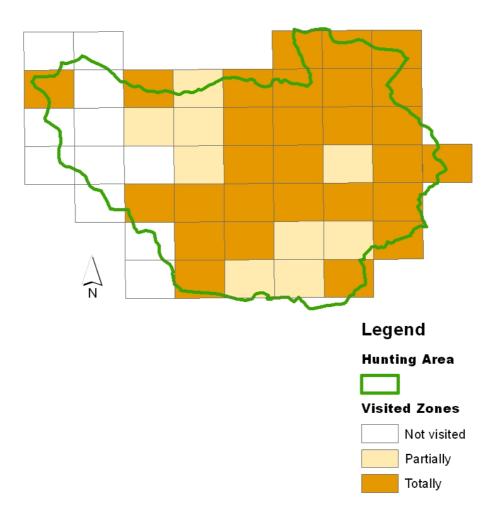


Figure 3 – Study area with the total areas visited so far.

In the field, each burrow (opening) was geo-referenced with a GPS, through the UTM geographic coordinate system which divides the Earth surface in 60 zones and displays coordinates in meters [70]. In all warrens was considered if they're constituted with only one opening or with a group of linked openings (for this was used the criteria of distance between them $\leq 6m$).





Figure 4 – Warren constituted by more than one opening (A). Single opening of a warren (B) Also, it was registered the size (number of openings), the use (if it is active or not), the type of protection associated to burrow (slope, rocks, roots or shrubs), its diameter (the biggest and the smallest) and the soil type (compacted or sandy) associated. Each burrow was considered used (active) when some evidence of wild rabbit activity was present (excrements, scratches, clean paths) and unused when it had webs or vegetation in the entrance and absence of wild rabbit evidence activity [26]. Plus, it was registered in the area the percentage of herbaceous cover and shrub cover with classes from 0 to 4 (Table I), mean height of shrub cover and for percentage of tree cover it was directly measured using a densitometer.

 Table I – Respective percentage of vegetation cover to each class.

Classes				
0	[0]			
1	[1-25]			
2	[26-50]			
3	[51-75]			
4	[76-100]			

2.1. Model Construction

Other environmental and spatial parameters were considerer to evaluate the responsible factors in the spatial distribution of warrens. These parameters were collected having in count the scales homerange and landscape (Table II). Some of these variables needed to be calculated in GIS through the software ArcGIS 10.0 ®.

Hydrographic and road network were created in base of a digital *layer* of military card in GIS, where lines were traced in water systems and roads (primary and secondary). Elevation was created through tracing lines of altimetry and points elevation, through a military card too. Distance to near hydrographic and road feature was obtained through centre points of 200 x 200 squares (i. e. distance between centre point and near feature) and elevation values for each square were taken from these

points also.

Different uses of soil and vegetation structure were taken from GIS also. The map of the study zone was primary classified having in count the Corine Land Cover 2006 (CLC2006), creating a layer of polygons with different patches for different soils, concerning 3rd level of classification of CLC2006 and by photointerpretation of an aerial photo.

After this last classification, the 200 x 200 m squares were also used to calculate percentage of type of soil occupation for each of them. These squares have these dimensions once wild rabbit distance dispersion are within that extension [71].

For reasons of model simplification, CLC2006 nomenclature was reclassified for the 1st level of classification (Table III).

CODE	INDEPENDENT VARIABLES	SCALE
ELEV HN RN	<i>Environmental variables</i> Elevation Distance to near hydrographic feature Distance to near road feature	Landscape
CURS TURBD RNTR CONSAR DTMC ITMC VIN ORCH OLIVE PRMPAST TMCAPPC CLSY	Use of Soil Continuous urban system Discontinuous urban system Road and train network and associated areas Construction areas Dried temporary crops Irrigated temporary crops Vineyards Orchards Olives Permanent pastures Temporary crops and/or associated pastures and permanent crops Cultural systems	Homerange
ANASNA AGRFOR HRDFOR	Agriculture with natural and semi-natural areas Agro-forests systems Hardwood forests	
CNFOR MXFOR	Coniferous forests Mix Forests	

Table II – Independent variables taken from GIS	Table II –	Independent	variables	taken	from	GIS
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BUSH	Bushes	
SCLVEG	Sclerophyllous vegetation	
OPNFNP	Open forests and new plantations	
SPVEG	Sparse vegetation	
MARSH	Marshes	

Table III – Reclassification of CLC2006 nomenclature to the 1st level of classification for de use of soil, with respective variable used in the model.

Nomenclature CLC (2006)	Reclassification	Variable	
Continuous urban system Discontinuous urban system Road and train network and associated areas Construction areas	Anthropogenic Territories	ANT	
Dried temporary crops Irrigated temporary crops	Temporary Crops	ТМС	
Vineyards Orchards Olives	Permanent Crops	РМС	
Permanent pastures	Permanent Pastures	РМР	
Temporary crops and/or associated pastures and permanent crops Crop systems Agriculture with natural and semi-natural areas	Heterogeneous agriculture areas	HAA	
Agro-forests systems	Agro-forests systems	AFS	
Hardwood forests Coniferous forests Mix Forests	Forests	FOR	
Natural herbaceous vegetation Bushes Sclerophyllous vegetation	Herbaceous and shrub vegetation	HSV	
Open forests and new plantations	Open forests and new plantations	OPF	
Sparse vegetation	Sparse vegetation	VEG	

For the model of burrows distribution, a GLM was performed having as response

variable number of burrows by 200m square, only for the ones that overlay totally prospected zones (Fig.3). So, the independent variables were taken only for these same areas too.

GLM was performed in *Brodgar 2.6.6* and all the other statistics tests in Statsoft Statistica 7.

3. SURVEY OF RELATIVE ABUNDANCE OF WILD RABBIT POPULATION

Within study area, a census of the relative abundance of wild rabbit colonies was surveyed during June of 2012, i.e. after breeding season, and to minimize the occurrence of excrements washed by rain. For this, in each 1x1 Km UTM squares mentioned before, was defined a point to draw 500m linear transects (Fig. 4). These points were defined in 2010, taking in consideration the road network (primary roads and if possible, secondary roads for having less human interference) in way to in the future, turn easier to do and compare the same study.

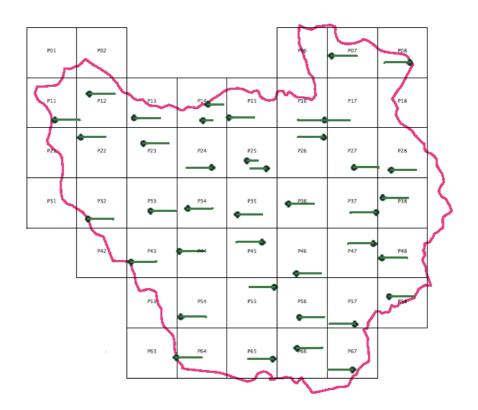


Figure 5 - Location of 500 m linear transects for each 1x1 Km UTM square within study area.

Within hunting zone (study area) were defined 44 UTM squares but only 38 transects were defined because in some squares, the area occupied by hunting zone was minimal (less than 25%) (Fig 3). Since in two points (P14 and P25) the area didn't allow passage where the transect was drawn before, was defined other point within same UTM square, to continue the transect.

All transects were slowly walked on foot with 2m vision in both sides in way to be possible to identify and count all the evidences of wild rabbit presence (latrines, scratches, pellets, warrens, and direct observation of individuals) (Fig. 5). Also, to improve data registration, each transect was divided in 5 parts with 100m each one, measured by steps. For this, the observer calibrated and recorded the number of steps to walk 100m in plane soil, and later in the field was gave a border of 25 and 50 steps to offset the irregularity of the area.





Figure 6 - Evidences of wild rabbit presence. Photo of a scratche in the field (A) and a latrine example (B).

Disperse excrements were organized in 3 categorical groups regarding their number (Table III).

Ex
(1-5)
(6-10)
(11-15)

Table IV – Number of excrements divided in three categorical groups: Ex (1.5), Ex (6-10), Ex (11 -15).

For all the segments was registered also by observation of zone the use of soil as an identifying parameter, and it was divided in some categories exposed in Table IV. After, this was used to check for differences in relative abundances of wild rabbit between distinct uses of soil.

Data were collected by direct observation of the area where the observer walked along transect while filled a field form presented in Annex I. Later, data were processed to draw a map in GIS with the distribution of relative abundances of wild rabbit through the hunting zone, having as criteria the number of latrines by transect (500m). Only number of latrines evidence was used to draw this abundance map, once it was previously used as an index to estimate rabbit abundance [72] and appear to be a useful indirect estimator of rabbit abundance in large-scale studies, despite the potential problems with decay rates or age of rabbit pellets [25]. Thus, it will better reflect the relative abundance of wild rabbit.

Code	Use of soil
UNC-FALL	Uncultivated/Fallow
CRO-IRR	Crop Irrigation
CRO-DRY	Dried Crop
BUSH	Bush
EUC-WSF	Eucalypt with "sub-forest"
EUC-NSF	Eucalypt without "sub-forest"
PIN-WSF	Pinewood with "sub-forest"
PIN-NSF	Pinewood without "sub-forest"
MF	Mixed Forest

Table V – Use of soil

In way to standardize data, numbers of latrines were classified in 4 categories (A-Rare, B-Low, C-Medium and D-High). For this classification was used the criteria of dividing 38 (number of transects) by 4 (number of desired classes) giving 9,5, and data were classified in ascending with 9 or 10 cases for each class (depending if there was equal numbers to get in the same class) (Table V).

Class	Reclassification
А	Rare
В	Low
С	Medium
D	High

 Table VI - Reclassification of standardized classes of latrines.

4. MONITORING A CAPTIVE BREEDING AREA

The monitoring actions took place in the captive breeding area located in Carvalhal da Azóia, which belongs to the Hunters' Association of Samuel parish.

This captive area is organized in 6 subdivisions and in 3 of them there are 3 feeding areas (Fig. 6).

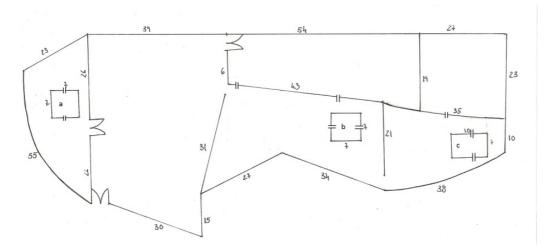


Figure 7 – Captive breeding area scheme with 6 subdivisions and with 3 feeding areas (a,b,c).

This practical work took place between October 2011 and July 2012. For reasons of incompatibility in availability of different interveners and for occurrence of diseases, there was no visit to the captive area in February, March and May 2012.

So, between each capture there was a period of about 30 days in way to study population dynamics and possible disturbances that can influence it.

The capture of animals to collect data occurred in the feeding areas, where one night before the capture, the doors of these areas were closed with a mechanism in way to only open in one direction, i.e. the individuals can only enter to feeding but they can't get out (Fig. 7).



Figure 8 – Feeding zone within captive breeding area (A) with the gateway detail (B).

After capture, all sampling data were recorded and for that some procedures were followed:

- Identification of the captured animal by putting an earring with a number or by recording its number if it's already marked.
- Observation of the animal sex and after that, analyse of characters of it reproductive state: in case of male its external testicles and in case of female if its mammary glandules are visible, if there's presence of embryos trough touching and if there's lack of fur in the abdomen.
- Measurement of the animal head, body (from the tip of snout to the end of tail) and right hind paw with a plastic tape-measure, and animal weighing with an electronic balance by suspending a dark bag.
- Examination of animal physical conditions: look for wounds and evidences of diseases (like myxomatosis and RHD). In case of wounds, it must be treated and in case of diseases indications, the animal should be slaughtered to avoid contamination.
- Vaccination for myxomatosis and RHD in case of no vaccinated animals.

For this dissertation there was no enough data to take conclusions about population dynamics during these months of data record, for reasons of animal diseases, captures with few animals and because of no visits in some months. **CHAPTER 3**

RESULTS

1. EVALUATION OF SPATIAL AND ENVIRONMENTAL ELEMENTS RELATED TO WARRENS DISTRIBUTION

During the census (2012) 73 warrens (Table V) with a total of 130 burrows (openings) were found, which are represented in figure 8, giving a total of 274 burrows when it includes the ones of 2010.

during fieldwork for this dissertation (2012)			
	Total	Last	
Total		projection	

181

274

73

130

Warrens

Burrows

Table VII – Total number of found warrens and burrows(openings) with reference to the ones projectedduring fieldwork for this dissertation (2012)

The minimum number was 1 opening per warren (122 occurrences) and the maximum was 7 (2 occurrences) (Table VI). Plus, the average number was 1,8 openings per warren.

Openings	1	2	3	4	5	6	7
Warrens	37	22	8	3	0	1	1

 Table VIII – Total number of warrens for each number of openings (from 1 to 7 openings)

Also, from the 130 burrows projected, 75 (57,7%) were in use and 55 (42,3%) weren't; In relation to protection, 122 (93,8%) were associated to elements of protection (like slope to protect warrens by rain or like shrubs against predation) and 8 (6,2) were considered unprotected. Regarding soil type, in most cases (66,9%) were considered sandy and in 43 (33,1%) were considered compact soils (Table VII).

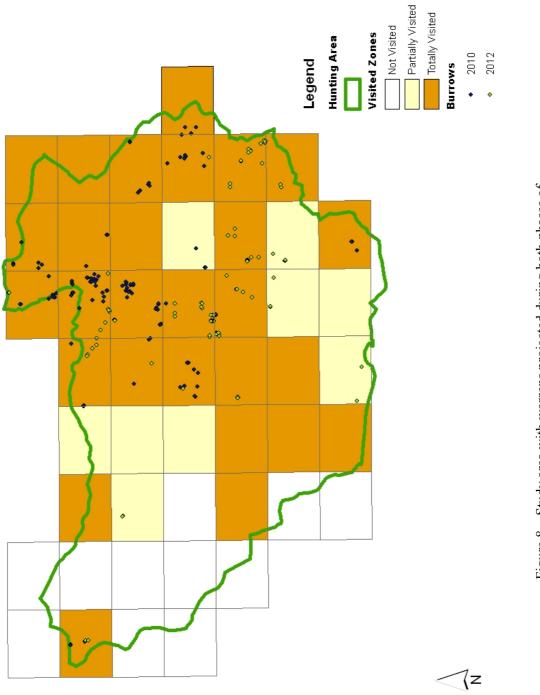


Figure 8 – Study area with warrens projected during both phases of warrens census (2010 and 2012)

	Used		Protected		Soil type	
	Yes	No	Yes	No	Sandy	Compact
Ν	75	55	122	8	87	43
%	57,7	42,3	93,8	6,2	66,9	33,1

Table IX – Burrows that in a total of 276 were considered in use and protected, and also the ones for each soil type.

Regarding the 130 burrows, in table VIII are presented the mean percentage of tree cover, and the average number for larger and smaller diameter.

Table X – Average percentage of tree cover and average size of larger (D) and smaller (d) diameter of burrows (N=130)

Mean Cover	Mean D	Mean d	
(%)	(m)		
53,03	21,4	16,1	

1.1. Model construction to evaluate burrows distribution

To analyse warrens distribution, was performed a GLM with a Poisson distribution once dependent variable isn't normal distributed (Fig. 9).

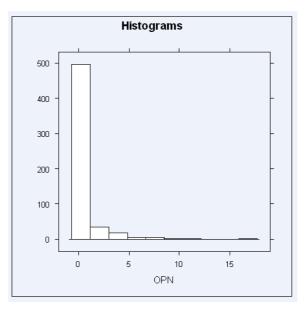


Figure 9 – Histogram for response variable OPN (number of openings)

Before the analysis, some variables were transformed to get better distributions, such as square root transformation for HN, RN, ANT, AFS, OPF and VEG, and cubic root transformation for PMC.

Also, to check collinearity between variables, variance inflation factor (VIF) analysis was used. A high VIF value is a serious indication of collinearity because it means that the variation in the response variable is explained well by the other variables. So, VIF eliminates environmental variables that are collinear and leaves only the variables that contain unique information [73]. For this model, variable FOR was eliminated with the higher VIF value (18.99) and after that, VIF values of the other variables decreased (Table X).

	With FOR	Without FOR
OPN	1.03	1.03
HN	1.09	1.09
RN	1.12	1.11
ELEV	1.16	1.15
ANT	2.14	1.18
ТМС	8.00	1.18
РМС	1.48	1.07
HAA	6.12	1.17
AFS	2.77	1.03
FOR	18.99	-
HSV	7.54	1.07
OPF	4.54	1.13
VEG	1.40	1.06

Table XI – VIF values with variable FOR and after its removal

The Model was given by the equation:

 $Y1 \sim 1 + HN + RN + ELEV + ANT + TMC + PMC + HAA + AFS + HSV + OPF + VEG$

Model was selected with the AIC (Akaike Information Criteria) (lowest AIC is

selected) with a forward selection method.

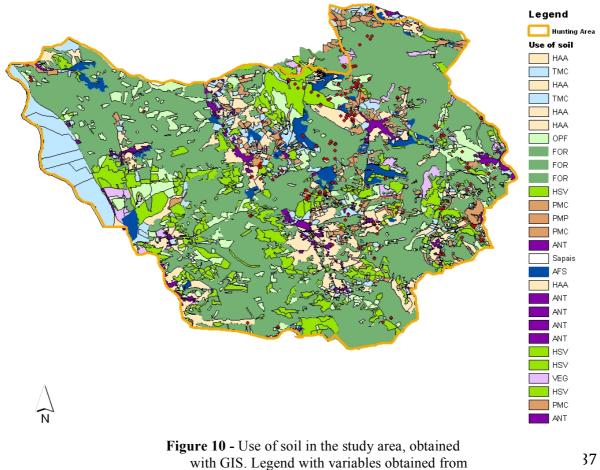
The final model was given by:

$$Y1 \sim ELEV + PMC + AFS + ANT + HN + HAA + RN$$

and selected variables are presented in Table XI. The variable which better explains the dependent one (number of burrows by 200m square) is AFS (agro-forest systems) followed by PMC (permanent crops). ANT (anthropogenic territories) and RN (road network) are inversly related with dependent variable.

Table XII - Selected variables for the model with respective significance values.

Coefficients							
Intersept	ELEV	PMC	AFS	ANT	HN	HAA	RN
-1.73889	0.01026	0.88983	0.90925	-1.82750	0.03445	0.79775	-0.02962



CLC2006 reclassification.

2. SURVEY OF RELATIVE ABUNDANCE OF WILD RABBIT POPULATION

In table IX are presented the descriptive analysis related to the evidences of wild rabbit presence collected along transects in the 38 total areas for the survey of relative abundance of wild rabbit. The evidence with higher number of observations (193) per transect (500m), was the category |1-5| of disperse excrements with an average of 7,3 observations/transect. This category is followed by number of latrines, to which belongs a maximum number of observations/transect of 73 and with an average of 3,6 counts/transect.

	Ν	Mean	Min	Max	Stand. Dev.
Ex (1-5)	38	7,30	0	193	10,8
Ex (6-10)	38	3,48	0	103	6,2
Ex (11-15)	38	2,28	0	63	4,3
LAT	38	3,59	1	73	5,3
SCRAT	38	2,63	0	54	4,2
DO	38	0,04	0	2	0,2

 Table XIII – Descriptive analysis for each evidence category of wild rabbit

For estimation of relative abundance of wild rabbit only was used the latrine evidence for reasons of better reflect relative density (as explained before), for being the more representative after Ex |1-5| and in order to compare results with relative abundance estimated in 2010 (where latrine evidence was used). Although Ex |1-5| represents a higher number of observations/transect than LAT, it also has a higher standard deviation. On the other hand, standard deviation of LAT category is lower and so data are more consistent.

2.1. Maps of relative abundance of wild rabbit

In all transects there was presence of latrines, as figure 11 shows, and higher abundances are mostly distributed across the north area of hunting zone.

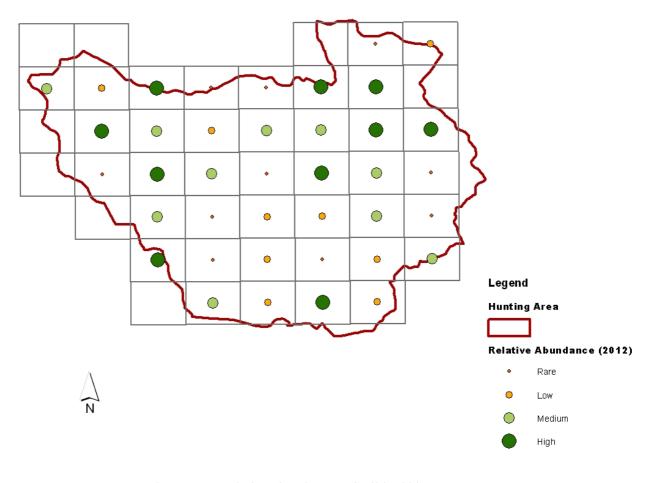


Figure 11 – Relative abundances of wild rabbit presented in 4 classes of abundance distributed within study area, in 38 UTM squares where transects were performed.

To analyse differences between relative abundances of wild rabbit estimated in the study area in 2010 and in this last case (2012), a paired-sampled t-test was performed. It revealed there were no significant differences between both cases (t= 1,75; p=0,08). Indeed, in figure 12 we can see no big differences between both; only in 5 UTM squares of hunting area abundances increased and there were no cases of

decrease.

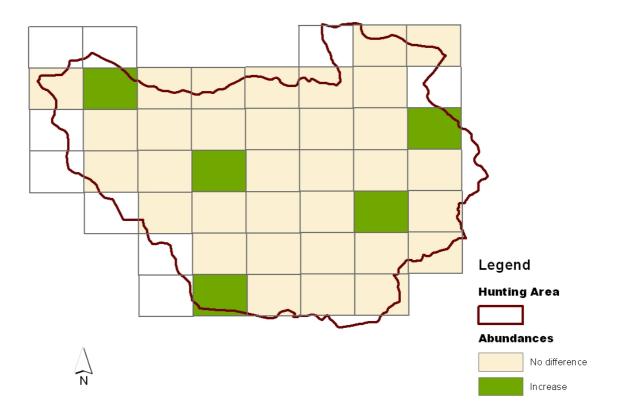


Figure 12 – Differences in estimated relative abundance of wild rabbit between 2010 and 2012.

2.2. Influence of "Use of soil" in disperse latrines

Use of soil registered in each segment of the 500m transects, was used to analyse if it influences distribution of latrines. Although assumption for normal distribution is violated (*Shapiro-Wilk test:* W= 0,68; p=0) an one-way ANOVA was used. There was a possibility of go on through a non-parametic test (*Kruskal-Wallis*) but according to Zar (2010) it needs similar distributions and variances and it doesn't occur (*Levene's test:* p= 0,000001). However, analysis of variance is a robust test, meaning that error probabilities are not always seriously altered by violation of the test's assumptions [74]. So, one-way ANOVA was implemented with the results of significant differences between means in number of latrines in different uses of soil (F=4,45; p=0,00006). After that was performed a post-hoc *Tukey test* to check the significant differences between means of different types of use of soil. "Bush" was the only variable which presented significant differences with Eucalypt without "sub-forest", uncultivated lands and with mixed forests (Table XII). In table XIII are presented the average number of latrines for these variables with results of significant differences.

Table XIV – Probabilities of *Tukey test* for variables that presented significant differences with variable BUSH

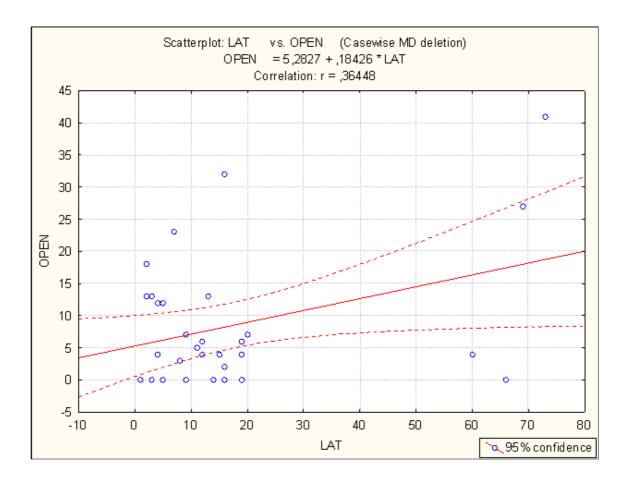
	EUC-WSF	UNC-FALL	MF
BUSH	0,00016	0,001366	0,036886

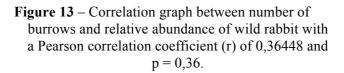
Table XV – Mean of latrines found for bushes, eucalypt without "sub-forest", uncultivated lands and mixed forests.

	BUSH	EUC-WSF	UNC-FALL	MF
Mean	6,36	1	0,71	1,73

3. ANALYSIS OF RELATION BETWEEN NUMBER OF BURROWS AND RELATIVE ABUNDANCE OF WILD RABBIT

To analyse at whether number of opening warrens and relative abundance of wild rabbit are in a certain way associated, was performed a correlation between these to variables from data in each totally prospected UTM square. The analysis shows that they're not significant correlated at p = 0,36 and in figure 8 we can see that they're just slightly positively correlated (r = 0,36448; r varies from -1 to 1), with some tendency of less number of burrows when abundances are lower.





CHAPTER 4

DISCUSSION

4.1. BURROWS DISTRIBUTION MODEL

Warrens are a key factor in population dynamics of European rabbit and can influence stability and persistence in long term [27]. So it becomes a useful and also necessary "tool" to include in population distributions studies of wild rabbit. Plus, according to Palomares (2001), the higher number of burrows, the more valid is the approach to rabbit numbers.

During this study, diverse parameters associated with burrows were registered *in situ* and one of the most evident was the protection associated to burrows - 93,8% of cases. This means that warrens are in most cases associated with shrubs, roots, rocks or with some degree of slope, which is an important issue to take in count when using artificial warrens as a management tool.

To perform a predictive model of wild rabbit distribution along the study area, number of burrows was used but only for the totally prospected areas. All variables were taken from 200 x 200 squares (that overlay with totally prospected zones) because represents vital area for wild rabbit [27].

Final model showed that Agro-forests systems (AFS) are the variable that better explains burrows distribution in the hunting area, followed by permanent crops (PMC) and heterogeneous agriculture areas (HAA). These kind of results agrees with previous studies where open areas for feeding in transition with shelter areas are the "selected" zones for rabbits [5], and this happens in Agro-forest systems where forest provide shelter and areas linked to agricultures gives the opportunity for feeding. This could be supported for collected data *in situ*, for instance the mean percentage of tree cover associated with burrows (53%), in the way that for shelter they use areas with intermediate cover (in this case trees). Namely, for warrens construction they don't use not only open areas but also areas with too dense vegetation, because with this value of

44

cover tree, it doesn't mean that there isn't lower vegetation but also means that there's no too dense lower vegetation, once cover trees doesn't allow sunlight to enter totally in the forest. Indeed, Rolan and Real (2011) showed that dense vegetation appeared negatively correlated with abundance.

In case of permanent crops, perhaps wild rabbit uses these areas because of food supply since this kind of crops are usually associated with herbaceous vegetation (specially olives) but also because of less perturbation, once it no suffer constant changes and consequently we could look at this like less fragmentation in that area, and so wild rabbit are more likely to occur [75]. In addition and besides of providing food, HAA for being heterogeneous could provide different kind of areas and resources, for instance different kind of soils that are an important parameter to build warrens as explained before or maybe for the possibility of find transitions zones (like in the case of AFS). Indeed, regarding figure 10 (burrows and use of soil) it's possible to see that prospected burrows are more distributed across northeast and centre of the study area, where we see a "blur" of different patches of use of soil (heterogeneity) with agriculture and herbaceous systems for feeding, and shrub and forests for shelter. Plus, according to Barrio and Bueno (2009) warrens occurrence is also related with high warren neighbouring densities, which could explain these higher densities in some areas.

The fact of ANT (anthropogenic territories) and RN (Road network) negatively explain distribution of burrows, is an expected result once it increase fragmentation and perturbation.

With these results, we can realize that location and characterization of natural warrens could be an important step to optimizing the conservation programs (for instance in use of artificial warrens) since they're a key factor in population dynamics of European rabbit and can influence stability and persistence in the long term [27].

4.2. RELATIVE ABUNDANCE OF WILD RABBIT POPULATION

Analysing relative abundances of wild rabbit and regarding figure 11, there is a small pattern in north part of hunting area, where seems to have higher abundances in relation to the south. This could be related to many factors such as use of soil, to where usually hunters release animals from the captive breeding area or to warrens distribution (which was thinking as an hypothesis).

In that way, a test to check for correlations was performed but as shown in the results, they're not significantly correlated, besides of a slightly relation occur. However, the explanation for this could be the fact that wild rabbit build its warrens in some area but it mainly drops its excrements in some remote area, perhaps in the feeding zones or close to this. Actually, according to Palomares (2001) rabbits defecate more on feeding grounds than in resting places.

Regarding results between average numbers of latrines by transect and according different kinds of "habitat", bushes are the case with more number of latrines per transect. This can be explained with the fact of it provides shelter [6] and the lower mean for uncultivated lands is perchance explained by low aliment supply and by the fact that open areas turn rabbits vulnerable to predation.

The fact of eucalypt "without sub forests" has a lower number of latrines could be an issue to go deeply, since it's an actual problem regarding increase of this plantations sometimes with damage to other types of vegetation.

3. IMPORTANCE TO RECOVERY PROGRAMS

This study brings new data and new important information to take managements decisions in the future for the hunting zone. Instead of going right to restocking actions, is beneficial go through habitat management first, since it can enhance rabbit populations itself [76, 77].

Diverse hunter associations and other institutions have adopted some conservation measures where the decline of the species is verified. For instance the implementation of hunting monitoring programmes in hunting zones such as the evaluation of the importance of environmental [75] and spatial parameters [27] and also its importance to the location and use of burrows; the estimation of rabbit abundance [46] and the management of rabbit populations in captive breeding areas [78] followed by restocking actions. All these procedures aim to have in the end, an evaluation of the populations' states and with that, make an implementation of essentials measures to the habitat management in way to achieve the best recovery and stabilization of rabbit populations.

In this case of wild rabbit, it is important that two sides, science and hunting games, walk in the same direction, because one needs the other and with cooperation they can easily continue the way by their interests and the final goals are the same, indeed.

However, this is only part of the research beginnings and besides of the remaining UTM squares that need to be explored to complete this part of collection data, more different data is essential to increase knowledge of this area and of this rabbit population, for instance, which kind of predators occupy this same zone

CHAPTER 5

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CHAPTER 6

ANNEXES

ANEXO I

Coelho bravo (Oryctolagus cuniculus) Abundância

Observador:_____ Data:_____ Quadrícula:____

Segmento	Uso do Solo	Ex (1-5)	Ex (6-10)	Ex (11-16)	LAT	PEG	OD

Code	Use of soil
UNC-FALL	Uncultivated/Fallow
CRO-IRR	Crop Irrigation
CRO-DRY	Dried Crop
BUSH	Bush
EUC-WSF	Eucalypt with "sub-forest"
EUC-NSF	Eucalypt without "sub-forest"
PIN-WSF	Pinewood with "sub-forest"
PIN-NSF	Pinewood without "sub-forest"
MF	Mixed Forest

ANEXO II

Elevation for the hunting area with centre points of 200x200 squares to exctrar elevation values for the model

