Inês Sofia Mendes Carneiro

Territorial and Social Behaviour of the Pyrenean desman (*Galemys pyrenaicus*) assessed from Scat Deposition

Dissertação de mestrado em Ecologia,
apresentada ao Departamento de Ciências da Vida da Faculdade de Ciências e Tecnologia da Universidade de Coimbra.

Julho 2016

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Universidade de Coimbra

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Dissertação apresentada à Universidade de Coimbra para cumprimento dos requisitos necessários à obtenção do grau de mestre em Ecologia, realizada sob a orientação científica do Professor Doutor Paulo Gama Mota (Departamento de Ciências da Vida, Faculdade de Ciências e Tecnologia, Universidade de Coimbra) e do Doutor Lorenzo Quaglietta (Centro de Investigação em Biodiversidade e Recursos Genéticos)

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Cover image:

Galemys pyrenaicus illustration.

Author: Claude Guerineau

Source: Dessins Desman Des Pyrénées Marie- Claude MC Guerineau, 2016, at: http://abela11.fr/

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Resumo

Os ecossistemas aquáticos são conhecidos pela sua notável biodiversidade, contendo cerca de um terço de espécies restritas a este habitat (Charbonnel et al., 2015). No entanto, encontram-se entre os habitats mais ameaçados do mundo, devido essencialmente a actividades antropogénicas que afectam gravemente a biodiversidade aquática (Biffi et al., 2016; Charbonnel et al., 2015). Nestes casos, espécies raras de carácter endémico e de reduzido tamanho populacional são particularmente importantes para a biologia de conservação, dada a sua vulnerabilidade (Charbonnel et al., 2015; Melero, Aymerich, Luque-Larena, & Gosàlbez, 2012).

Dentro das espécies aquáticas raras, *Galemys pyrenaicus* é um dos mamíferos Europeus menos conhecido do público em geral e dentro da comunidade científica (Charbonnel et al., 2015; Melero et al., 2012). O seu estatuto vulnerável, associado à falta de conhecimentos sobre a ecologia e comportamento da espécie, tem-se revelado um dos maiores desafios contemporâneos à conservação e gestão da mesma para muitos cientistas (Melero et al., 2012). Estudos anteriores, focados na selecção de habitat da toupeira-de-água a uma escala fina, apresentam alguns problemas como a definição de escalas grosseiras para identificação de associações ambientais finas e a falta de inferência estatística (Biffi et al., 2016; Charbonnel et al., 2015).

Considerando os problemas descritos, o nosso projecto tenta complementar a informação existente sobre a selecção de habitat da toupeira-de-água usando descritores a duas diferentes escalas espaciais (micro-habitat – 0,5 m^2 – e transecto - ~200-600m). Os objectivos principais deste estudo são 1) estudar os padrões que determinam quais as variáveis ambientais que mais influenciam o comportamento de deposição de excrementos por parte da toupeira-de-água, a duas escalas diferentes; e 2) perceber a importância ecológica das variáveis de habitat seleccionadas para a deposição de dejectos como “recursos-chave” para determinar a presença de toupeira-de-água. Para isso, testámos a influência das variáveis: presença de amieiro, localização nas margens ou leito, exposição do substrato, velocidade da água e a presença de musgo (variáveis ambientais e biológicas de escala fina). Também testámos a influência das variáveis: percentagem de cobertura, “spraintability”, largura do leito, velocidade da água, percentagem de “pool” e percentagem de “riffle” (variáveis ambientais e biológicas de larga escala) na abundância de dejectos de toupeira-de-água encontrados por km de transecto.

Verificámos que a uma escala mais fina a toupeira- de-água depositou os seus excrementos principalmente em locais não expostos, localizados nas margens do rio, perto de locais de grande velocidade da água. Ao contrário do que era esperado, a presença de amieiro não resultou ser determinante na selecção feita pela espécie. A presença de musgo demonstrou um efeito inconsistente da variável. À escala do transecto, o uso do habitat local pela toupeira baseado na distribuição dos seus dejectos parece influenciado pela heterogeneidade de substrato. Estes resultados serão importantes para perceber quais as características de habitat mais importantes para a toupeira-de-água, o que poderá permitir inferências sobre a comunicação e organização social da espécie.

Palavras-chave: ecossistemas aquáticos, espécies em perigo, espécies endémicas, *Galemys pyrenaicus*, selecção de habitat, comportamento animal, comunicação, organização social.

Abstract

Freshwater environments are known for its notable biodiversity, holding about one third of vertebrate species restricted to this ecosystem (Charbonnel et al., 2015). However, they are amongst the most threatened habitats in the world due to human activities that cause alterations of the natural river conditions and strongly affect aquatic biodiversity (Biffi et al., 2016; Charbonnel et al., 2015). In these environments, rare species with small population sizes and especially endemic species are of particular interest for conservation biology due to their vulnerability to extinction (Charbonnel et al., 2015; Melero et al., 2012).

Among rare freshwater species the Pyrenean desman (*Galemys pyrenaicus*) is one of the less known European mammals to the general public (Charbonnel et al., 2015; Melero et al., 2012) and within the scientific community. Its vulnerable status together with an almost complete lack of knowledge regarding their ecology and behaviour has made their conservation and management a contemporary challenge for many scientists (Melero et al., 2012). Previous studies have investigated the habitat preferences of Pyrenean desman at small- site scale but they present some problems like the definition of scales too coarse to identify finer habitat associations and the lack of statistical inference (Biffi et al., 2016; Charbonnel et al., 2015).

Taking into account the described problems, our project tries to complement the information existent on Pyrenean desman habitat preferences using descriptors at two different scales (small-site scale – 0,5m^2 – and a larger scale - ~200-600m). The main objectives of this study were 1) to study the patterns that determine which environmental factors mostly influence the scat deposition behaviour of the Pyrenean desman at two different scales 2) to understand the ecological importance of the habitat variables selected for scat deposition as key resources for determining the Pyrenean desmans' presence. This was achieved by testing the influence of the variables presence of alder, bank or bed localization, substrate exposure, water speed and presence of musk (small-scale environmental and biological variables). We also tested the influence of the variables: percentage of coverage, spraintability, riverbed width, water speed, percentage of pool and percentage of riffle (large scale environmental and biological variables).

We verified that at a small-site scale, Pyrenean desman preferentially selected as habitat requirements non-exposed sites, preferably at riverbanks near locations of high river flow. Contrary to what was expected, alder presence was not determinative for Pyrenean desman selection. Musk revealed inconsistent variable effect, with its significance varying a lot. At a larger scale, the use of local habitat by the Pyrenean desman appears to be driven by higher spraintability with transects with abundant emergent items and greater percentage of substrate heterogeneity preferably selected. These results will be important also to help understanding which habitat characteristics are important to the Pyrenean desman, which may draw clues on communication and social organization of the species.

Keyword: aquatic ecosystems, endangered species, endemic species, *Galemys pyrenaicus*, habitat selection, animal behaviour, communication, social organization.

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1 Introduction

Freshwater environments are known for its notable biodiversity, holding about one third of vertebrate species restricted to this ecosystem (Charbonnel et al., 2015). However, they are amongst the most threatened habitats in the world due to human activities that cause alterations of the natural river conditions and strongly affect aquatic biodiversity (Biffi et al., 2016; Charbonnel et al., 2015). In these environments, rare species with small population sizes and especially endemic species are of particular interest for conservation biology due to their vulnerability to extinction (Charbonnel et al., 2015; Melero et al., 2012). Extinction rates of freshwater fauna are extremely high with around 15 000 species worldwide already extinct (Charbonnel et al., 2015).

Among rare freshwater species the Pyrenean desman (*Galemys pyrenaicus*) is one of the less known European mammals to the general public (Charbonnel et al., 2015; Melero, Aymerich, Santulli, & Gosàlbez, 2014) and within the scientific community. This is mainly because of difficulties in its studies due to lack of capture licenses' approval and easily scat misidentification when surveys are based in recording indirect signs without non-genetic confirmation leading to false presences or absences (Charbonnel et al., 2015; Melero et al., 2014). Its vulnerable status together with an almost complete lack of knowledge regarding their ecology and behaviour has made their conservation and management a contemporary challenge for many scientists (Melero et al., 2012).

Several aspects of the biology and conservation of the species have been addressed in recent decades including studies on its distribution, e.g.: Bertrand 1993a, Queiroz et al. 1998, Aymerich et al. 2000, Palomo and Gisbert 2002; morphology, e.g.: Richard 1986, Richard and Michaud 1975 ; diet, e.g.: Bertrand 1993b, Castién & Gonsálbez 1995; general biology, e.g.: Richard 1986;

reproduction, e.g.: Castién 1994; and captive behaviour, e.g.: Richard 1986, Queiroz and Almada 1993 (Melero et al., 2012). Yet, basic knowledge such as distribution range and habitat preferences are still incomplete for this species (Charbonnel et al., 2015).

1.1 Study species characterization

1.1.1 Taxonomy and Evolution

The Pyrenean desman (*Galemys pyrenaicus)* also known as Iberian desman is classified within the Talpidae family, subfamily Desmaniae and it was first described by Etienne Geoffroy Saint-Hilaire in 1811 (Marcos, 2004) (Table 1).

Table 1 - Taxonomic position of the study species: Galemys pyrenaicus.

Classification	
Kingdom	Animalia
Phylum	Chordata
Subphylum	Vertebrata
Class	Mammalia
Order	Soricomorpha
Family	Talpidae
Genus	*Galemys*
Species	*Galemys pyrenaicus*
Subspecies	*Galemys pyrenaicus rufulus*

In the past, Desmaniae was represented by a higher number of species with a large geographic distribution but currently besides Pyrenean desman (*Galemys pyrenaicus*) the only representative of this sub-family is the Russian desman (*Desmana moschata*) (Silva, 2001).

The phylogenetic relationship of this species (Figure 1) with the family Talpidae can be traced to the Eocene however it has always been questioned due to the highly distinct morphology of desmans from other members of the Talpidae (Cabria, Rubines, Gómez-Moliner, & Zardoya, 2006). Recent studies based on the desman's mitochondrial genome confirmed its position and also showed the close phylogenetic relationship between *Desmana* and *Galemys*, admitting the morphological evidences that grouped both genera within Desmaninae subfamily (Cabria et al., 2006).

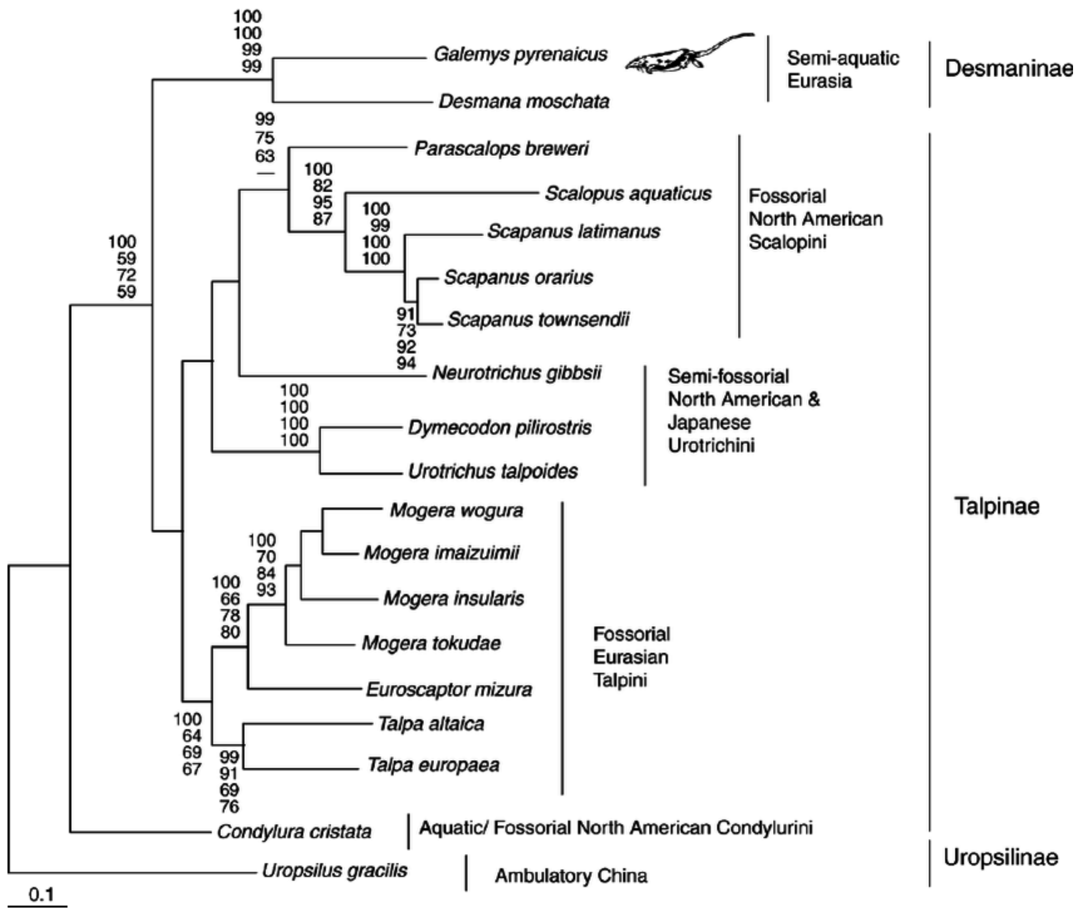


Figure 1- Phylogenetic relationships of Talpidae based on mitochondrial cytochrome b gene sequence data (from: Cabria et al. 2006)

1.1.2 Species Morphology

Galemys pyrenaicus lives associated with aquatic habitats and exhibits a highly specialized morphology (Cabria et al., 2006). The hydrodynamic shape of their body seems appropriate to decrease water resistance and progression effort while moving (Queiroz, 1996).

It is a small mammal, with a body length between 15-25cm and about 70g of weight (Marcos, 2004; Queiroz, 1996).

Desman is covered with a dense and glossy dark-brown fur which is silvery-grey in the abdomen (Marcos, 2004; Queiroz, 1996). This fur is responsible for retaining air which provides an excellent protection against water and cold (thermal isolation) and also provides buoyancy (Marcos, 2004; Richard, 1985). The feet, tail and snout are almost devoid of hairs (Marcos, 2004).

The hind legs of Pyrenean desman are large, wide, and with webbed feet responsible for water propulsion (Queiroz, A. Bertrand, & Khakhin., 1996; Richard, 1985). Forelegs are short and narrow with sharp and long claws possibly used to ward off rocks (Richard, 1985). The tail is long and flattened at the tip and it has an important role in the equilibrium and water propulsion (Queiroz, 1996). At the bare of the tail desmans presents musk glands (Marcos, 2004).

Pyrenean desman does not have an acute sense of vision since its eyes are very small. Instead it presents a long, mobile and very developed snout with highly complex vibrissae and Eimer's organs. These structures have an important role for the perception of objects and preys and rely on tactile and olfactory senses which apparently are used by desmans to explore its habitat (Marcos, 2004; Queiroz, 1996).

It is not easy to distinguish males from females at naked eye due to the lack of sexual dimorphism (body size or colouration) (González-Esteban, Villate, & Castién, 2003; Vidal, Perez-Serra, & Pla, 2010). However, studies from González-Esteban et al. 2003 revealed the possibility to distinguish them through examination and palpation of the urinary papilla. Regardless the age or reproductive cycle males show a hard pelvic arch not present in females.

Age is also a difficult criterion to access based on external biometric parameters (body mass and length) as desmans' population show a high degree of uniformity in body parameters(González-Esteban, Villate, Castién, Rey, & Gosálbez, 2002). The most recent criterion developed was also proposed by González-Esteban et al. 2002 and it estimates age based on dental wear by examining the growth rings on dental sections and the wear of the upper canine tooth.

1.1.3 Geographic distribution

At present, *Galemys pyrenaicus* has a restricted geographic distribution limited to the Pyrenees (Andorra, France and Spain) and to high altitude areas of the North Iberian Peninsula, more precisely at northern and central Spain and northern Portugal (ICN, 2005; Marcos, 2004; Queiroz et al., 1996).

Due to its habitat requirements, desman's distribution is patchy with some populations being currently isolated (Nores et al., 1998). It is consider that there is no connection between the Pyrenean and the North Iberian populations and that the populations from Cordilheira Central in Spain are also very isolated (ICN, 2005). One of the greatest threats for the sustainability of animal populations is the fragmentation of habitats and the reduction of effective population sizes. Isolation can also favour the process of morphological differentiation within the species' distribution area and because of this, there are some authors proposing the existence of two distinct subspecies of *Galemys pyrenaicus*: *Galemys pyrenaicus rufulus* (the variety form from Iberian Peninsula) and *Galemys pyrenaicus pyrenaicus* (the typical form from the Pyrenees) (González-Esteban, Castién, & Gosálbez, 1999). However, there is no clear differentiation reported between the two possible subspecies (González-Esteban et al., 1999).

In Portugal, desman occurs in the northern and central mountain ranges with its southern distribution coinciding with Serra da Estrela and the most suitable areas for its presence being Bragança, Vila Real, Braga and Viana do Castelo districts (Queiroz et al. 1998; Queiroz et al. 1996).

In terms of river basins, the species seems to occupy all the main watersheds at North of Douro river (Minho, Âncora, Lima, Neiva, Cávado, Ave and Leça river basins) and the main sub-basins of Douro river (Sabor and Tua basins) (Queiroz et al. 1998). However the species seems rare in the innermost watersheds (Teja's stream, Côa River, Mós' stream, Aguiar's stream and Águeda River) and in the medium and superior sections of Vouga and Mondego river basins' and at the upper sections of the Zêzere River (Tejo basin) (ICN, 2005; Queiroz et al., 1996).

Galemys pyrenaicus in Portugal (Figure 2) occurs in 4 protected areas of the north and centre of the country: Peneda-Gerês National Park, Alvão Natural Park, Montesinho Natural Park and Serra da Estrela Natural Park (Queiroz et al. 1996; Queiroz et al. 1998).

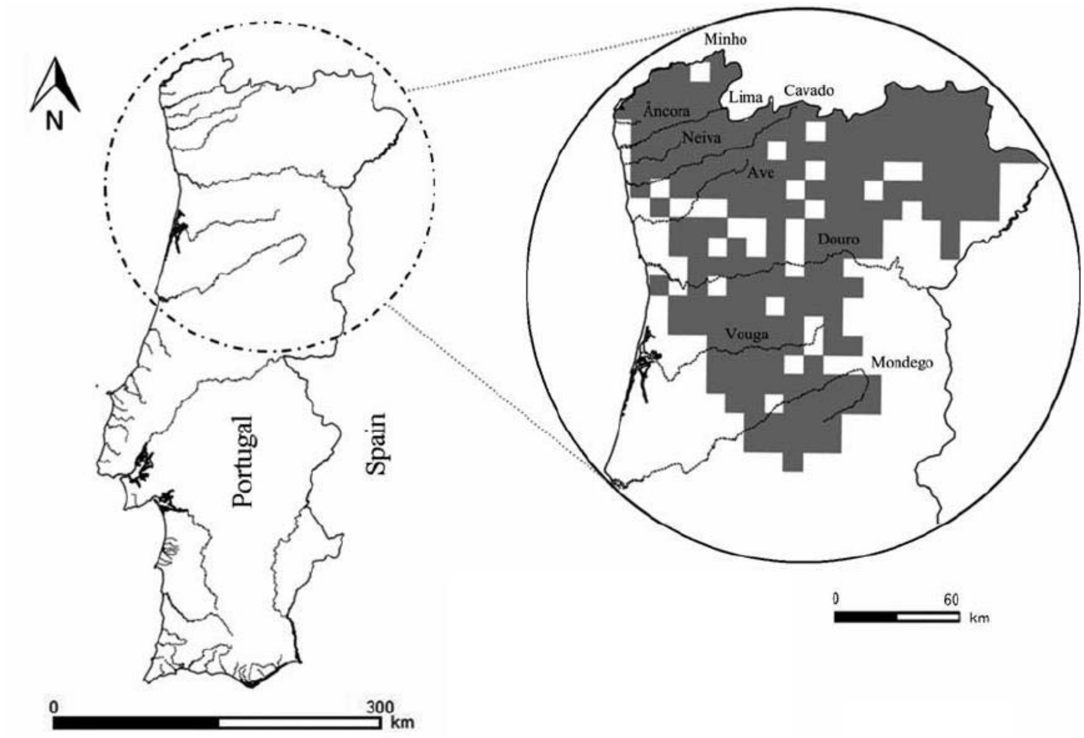


Figure 2 - Map representing Galemys pyrenaicus distribution in Portugal based on studies from 1990 to 1996 (10x10 km UTM) (Pedroso & Chora, 2014).

1.1.4 Ecology

1.1.4.1 Habitat

Pyrenean desman is strictly associated and dependent of aquatic habitats (aquatic and riparian corridor) (ICN, 2005; Marcos, 2004). According to the scientific literature desmans supposedly occupies habitats where there is cold, permanent flowing and highly oxygenated and turbulent water (typical characteristics from trout zones) (Esteban & Iglesias, 2012; Marcos, 2004; Queiroz et al., 1996). Normally, these places are located between 10 and 1300 m of altitude and they usually present regular flow (with drought flow higher than 100 l/s), water velocity higher than $0.2ms^{-1}$ (Nores et al., 1998), good alternation between hydro morphological microhabitats (riffle, run and pool zones) and a riverbed substrate mainly composed by material of high granulometry such as: cobbles and boulders (Esteban & Iglesias, 2012; Queiroz et al., 1996). Within these conditions, Pyrenean desman can inhabit stretches ranging from small mountain rivers, especially the upper sections, to mid reaches, and even canals of water mills (Marcos, 2004).

These are the main requirements to identify the distribution of the potential area for the species. At more detailed scale, the minimum requirements for desman's presence seem to be essentially: water quality (which determines food availability) and the high preservation of banks which is important to shelter maintenance (Esteban & Iglesias, 2012; Ramalhinho & Tavares, 1989) that is why *Galemys pyrenaicus* is frequently referred as a bio-indicator species (Queiroz et al., 1996).

The species appears to prefer unpolluted streams however there are records of its presence in moderately polluted sites suggesting that desman has a certain tolerance to pollution (Marcos, 2004).

Bank preservation is of extreme importance due to the existence of stonewalls and riparian vegetation like ash (*Fraxinus excelsior*) and alder (*Alnus glutinosa*). Their exposed roots together with the available rocks create good shelters and allow access to crevices located under the banks, which desman uses as nests (Marcos, 2004). Pyrenean desman unlike other species of the family Talpidae does not dig tunnels with numerous galleries, it digs very simple tunnels or just facilitates access without the need to move much soil (Esteban & Iglesias, 2012).

The available scientific data does not indicate the presence of the species in rivers or streams of excessive depth, high sedimentation and/or lack of river bank shelters along considerable extensions. Other unsuitable habitats include watercourses of intermittent nature that are physically or ecologically isolated; small coastal streams flowing directly into the sea; sections of rivers that show a high degree of pollution (organic or chemical); or lentic habitats, such as dams and natural ponds at high altitude (Queiroz et al., 1998).

1.1.4.2 Feeding activity

Desman feeds predominantly on aquatic benthonic macroinvertebrates' species ecologically sensible to contamination. This explains its preference for unpolluted, fast-flowing streams as they usually present high prey abundance and richness (Esteban & Iglesias, 2012).

Studies on desman's diet show a high specialization of it for some groups of Trichoptera, Plecoptera, Ephemeroptera and Diptera but generally Trichoptera and Ephemeroptera are found in higher quantities (Esteban & Iglesias, 2012; Marcos, 2004). This is because® Trichoptera larvae are large and immobile prey and Ephemeroptera larvae are very abundant, despite its small size (Castién & Gonsálbez, 1995; Esteban &

Iglesias, 2012). The prey selection is based on the need to obtain large quantity of biomass in proportion to the time spent searching for food because desman is a small species with high energetic needs to obtain homeotermia. In this case, Trichoptera is the group that contributes the most for Pyrenean desman biomass (Castién & Gonsálbez, 1995; Esteban & Iglesias, 2012).

1.1.4.3 Reproduction

Pyrenean desman's reproductive behaviour is largely unknown but it is thought that the reproductive period occurs between January and July (Marcos, 2004). Male spermatogenesis probably starts in November and from January to May it is possible to find sexually active males. Oestrus in females begins in January and its reproductive period lasts from February to May with first pregnant females appearing in February and the last in June (Marcos, 2004). The gestation period lasts for about 30 days with the birth of the young occurring from March to July (ICN, 2005). Usually, the average litter size is around 3 or 4 (ICN, 2005). Sexual maturity is reached one year after the birth (ICN, 2005). Pyrenean desman's reproductive life lasts just 1 to 2 years, with total female sterilization being frequent after one or two reproductions, with few individuals outlasting the 3 years of life (Nores et al., 2002). This also constitutes a limitation factor for the reproductive ability of the species (Marcos, 2004).

1.1.5 Behaviour

One of the most unknown aspects of the species biology is its behavioural ecology particularly how individuals use and interact in space and time (Melero et al., 2012). The social organization and activity patterns of *Galemys pyrenaicus* has only been investigated in a few studies conducted by David Stone two decades ago: Stone & Gorman, 1985; Stone, 1985, 1987a, 1987b and recently by Melero et al., 2012, 2014.

However, there is an evident lack of knowledge in what concerns to desman social and spatiotemporal behaviour which compromises management and conservation plans for its population.

1.1.5.1 Social organization and Home range occupancy

First studies concerning social behaviour and home range occupancy has shown that Pyrenean desman confines itself to relatively constant home ranges to which it shows a strong fidelity. Individuals were first thought to occupy ranges of 200m for males and 100m for females (Richard & Viallard, 1969) however, some work developed later by David Stone (Stone & Gorman, 1985; Stone, 1987a, 1987b) revealed greater ranges for all individuals with males occupying a medium range of 429m and females a medium range of 301m. The most common pattern of spatial organization observed was the sedentary lifestyle constituted by pairs of resident adult males and females living in the same section of the stream but utilizing separated nest sites. In these cases, female's home range was always enclosed within the male's range (Stone & Gorman, 1985; Stone, 1985, 1987a, 1987b) .

In contrast to these, transient desmans were juveniles or solitary adult individuals which did not always exhibit site fidelity and were regularly seen to change their ranges. The average home ranges for juveniles were 250m and for adults 572m (Stone, 1985, 1987b).

The behaviour of males and females at the border areas of their respective ranges was also noticeably different with males spending most of their time swimming across the stream, with little associated diving and feeding behaviour while females were frequently observed feeding. Juveniles displayed a similar pattern to that of the resident adult females (Stone, 1985, 1987b).

According to all of these observations Stone, 1985, 1987b stated that there are several factors from the behaviour of individual desmans which suggest that their spatial organization is a form of territoriality proposing that the repetitive patrolling behaviour of males at border areas provide evidences of territorial demarcation and defence. However, recent studies also related to social and space-use behaviour contradict the idea of the species being territorial and avoiding conspecifics, defending that Pyrenean desman socio-spatial organization is community-based, with non-exclusive or permanent territories and home range shared between individuals (Aymerich, Fernández, & Gonsálbez, 2013; Melero et al., 2012).

Resting sites may play an important role in the social organization of the species playing a role in individual protection and resting behaviour but also in communication between the species (Melero et al., 2012). Stone, 1985 and Melero et al., 2012 refer their importance but they also have different ideas on how individuals occupy their shelters.

Stone, 1985 defends that sedentary and transient individuals always use separated rest sites and even within the pairs of sedentary individuals it was never observed their sharing. This emphasizes the theory defended by Stone that Pyrenean desman is a territorial species which avoids mutual aggressive encounters.

On the other hand, Melero et al., 2012 observed that resting sites are commonly used by pairs of individuals regardless of their age or sex and that they are shared simultaneously by conspecific adults of the same or opposite sex. This agrees with the idea that desman is not a solitary and aggressive species. Melero et al., 2012 also adds that the continuous use of resting sites by subsequent desmans suggest that these may constitute a key resource for the species.

1.1.5.2 Patterns of activity

Concerning activity patterns, Pyrenean desman is believed to present a biphasic pattern of activity primarily nocturnal, with individuals being active just after the 22:00h for about 7 hours. A secondary brief period is also evident mostly during the afternoon lasting between 2 to 4 hours (at least during summer months) (Stone & Gorman, 1985; Stone, 1985).

Earlier studies on captive desmans performed by Richard 1985b also verified the biphasic period of activity of the species for most of the year (April to December). However, during the remaining months he observed that the usual pattern was altered and desman's activity became mostly diurnal. The activity of both sexes decreased during the months of September, October and November (probably due to the poor weather as Pyrenean desman is affected by rainfall and temperatures) (Stone, 1987a).

According to Stone, 1987a, paired resident adults were characterized by the consistent biphasic pattern, as well as the juveniles, exploring their entire range in a 24-hour period. Solitary desman instead exploit its range in a 48-hour period in which one-half of their range is visited during an initial 24-hour period.

In terms of daily activity, Melero et al., 2014 conclusions are more or less consistent with those of Stone, 1987a, 1987b, referring that individuals presented a bimodal activity pattern in spring with one primary nocturnal activity bout and a short one during the afternoon. However this pattern changed during autumn to a trimodal rhythm with individuals including one or two nocturnal resting bouts and reducing their diurnal activity to a single, shorter bout. This shift in rhythm is supposed to be related with an individual's ability to adapt their behaviour to the duration of the night in different seasons.

The primary nocturnal behaviour referred both by David Stone and Yolanda Melero may be related to the prey availability, since most invertebrate drift occurs during the night (Marcos, 2004).

1.1.5.3 Scat deposition and Scent Marking

Pyrenean desmans' detection based on indirect traces like scats' deposition has been largely used in studies of the species' distribution (Queiroz, 1996). These studies refer that the majority of scats is deposited on rocks or vegetation (essentially roots) emergent from the riverbanks (Biffi et al., 2016; Pedroso & Chora, 2014) or riverbed (Queiroz, 1996; Queiroz et al., 1998). They are also found close to the water level (usually between 10 and 30 cm of height and distance from water) and the majority of them are possibly located in sheltered places near indentations and holes' entrances, but sometimes they are also detected in exposed places (Queiroz, 1996; Queiroz et al., 1998). *Galemys pyrenaicus'* scats can be isolated or in latrines (Queiroz, 1996; Queiroz et al., 1998). In general, latrines are places used to deposit scent-marks, which consists of faeces, urine and/or secretions of scent glands (Almeida, Barrientos, Merino-Aguirre, & Angeler, 2012). Border latrines usually have a function in territory maintenance and acts as information sites for the other members of a population mostly about the use of resources which is also a reflection of the habitat quality and suitability (Almeida et al., 2012; Sillero-Zubiri & Macdonald, 1998).

Although it is believed that Pyrenean desmans leave their scats both for excretion and communication, formal assessments of this topic are missing. The only studies that refer to scent-marking in *Galemys pyrenaicus* are the Stone's studies on social organization behaviour of the species: Stone & Gorman, 1985; Stone, 1985, 1987a, 1987b. In his studies he refers that desmans show high familiarity with the boundaries of their range by daily following a routine pattern of movements which served for the continual renewal of faecal and sub-caudal scent marks at strategic positions. More recently, Melero et al., 2014 also states evidences of indirect communication between individuals by means of scent-marks deposition.

1.1.6 Accompany Fauna and Predators

There are some aquatic and semi-aquatic vertebrates that share habitat with Pyrenean desman. The best known are: brown trout *Salmo trutta*, viperine snake *Natrix Maura*, the white-throated dipper *Cinclus cinclus*, the Eurasian water shrew *Neomys fodiens*, the water vole *Arvicola sapidus* and the Eurasian otter *Lutra lutra* (Melero et al. 2014). Most of the species described co-habit friendly with Pyrenean desman but others are occasional predators of *Galemys pyrenaicus* (Melero et al., 2014).

Only in the last two decades has it been shown that Pyrenean desman is prey to several species of fish, birds and other mammals. Some examples include: the pike *Esox lucius*, the grey heron *Ardea cinera*, the little egret *Egretta garzetta*, the white stork *Ciconia ciconia*, the barn owl *Tyto alba*, the buzzard B*uteo buteo*, the stoat *Mustela erminia*, the weasel *Mustela nivalis*, the beech marten *Martes foina* and also the American mink *Mustela vison* (Marcos, 2004). Despite all these generalist predators, the otter *Lutra lutra* is considered one of the most frequent and major predators (Fernández-López, Fernández-González, & Fernández-Menéndez, 2014). However there are no conclusive evidences to date (Queiroz et al., 1996).

1.1.7 Status and Threats

It is hard to obtain precise estimates on Pyrenean desman’s population size (Fernandes, Herrero, Aulagnier, & Amori, 2008). However, some studies had been conducted in France, Spain and Portugal using radio-tracking following successful captures of the individuals in water courses with favourable habitat conditions. The results show that Pyrenean desman's densities are naturally low (around 5 to 10 individuals per kilometre) with estimated lower densities in less favourable habitats (Fernandes et al., 2008; ICN, 2005). In Portugal, studies developed in Sabor's and Paiva's rivers estimated that are less than 10 000 mature individuals divided into small isolated subpopulations with around 6 resident individuals per kilometre (Chora & Quaresma, 2001; ICN, 2005; Pedroso & Chora, 2014).

In general, Pyrenean desman's populations are considered in regression either in the context of population dimensions or in what concerns to global and national distribution area being pointed situations of high population's fragmentation and serious population's decline as evidence of the high risk of the species' extinction (ICN, 2005). Besides Quaglietta & Beja, unpublished data, few surveys have been conducted in Portugal since 90's everything points to a progressive regression of the species along the East (inland), West and South (coastal) boundaries of the species distribution area (ICN, 2005; Pedroso & Chora, 2014).

Due to the high decreasing population levels and the increasing threats to the species, in Portugal Pyrenean desman is protected under the law: DL nº 140/99 and DL nº 49/05 of the Habitats Directive 92/43/CEE, and DL nº 316/89 of the Bern Convention) and is classified as Vulnerable (VU) by the Portuguese Red Data Book (Fernandes et al., 2008; Pedroso & Chora, 2014). The fact that Pyrenean desman confines itself to a specific habitat within a restricted area makes it more vulnerable to every action and/or activity that causes changes in the aquatic systems and its denaturalization and consequently in food availability (Marcos, 2004; Pedroso & Chora, 2014). The major threats to the species are essentially: dam's construction (which is considered the most significant threat), water organic and chemical pollution, riverbanks' and natural riverine vegetation's destruction, restriction of water flow and gravel/sand extractions (ICN, 2005; Pedroso & Chora, 2014; Queiroz et al., 2005). In addition to these, there are factors that affect directly the species or populations causing mortality like: the use of nests, poisons and explosives as fishing methods or the direct persecution from fishermen (ICN, 2005; Pedroso & Chora, 2014; Queiroz et al., 2005). Pyrenean desman's conservation has been a much discussed topic because of the urgent need to take actions to counteract the species decrease. The actions proposed include: appropriate management of water courses, habitat restoration, improvement of knowledge about the species ecology and behaviour and the use of desman as a flagship species to promote river conservation amongst the public (Fernandes et al., 2008).

1.2 Study framework/importance

Previous studies have investigated the habitat preferences of Pyrenean desman at small spatial scale in France, Spain and Portugal. From these studies, some river characteristics have been reported as preferred by the species, however, these studies are rather old or consist of "grey literature" (Biffi et al., 2016; Melero et al., 2014). These preliminary data helped in planning new studies that are arising as the interest in these species' conservation increases but there are still a lack of information on desmans' distribution, general biology and ecology with very incomplete knowledge on basic subjects like species' distribution range and habitat preferences (Charbonnel et al., 2015; Melero et al., 2012, 2014). Other problem within the studies of the species' distribution range and habitat preferences is the lack of certainty on the quality of the presence-absence data based on indirect signs, since DNA analysis was only applied very recently to faeces confirmation (Charbonnel et al., 2015). Also, the large scales used in most of the studies seem too coarse to identify finer habitat associations because they did not take into account the particular features of the freshwater environments. Lack of statistical inference is also noticeable with most of the studies being based on descriptive observations (Biffi et al., 2016; Charbonnel et al., 2015).

Taking into account the described problems, my thesis project tries to complement the information existent on Pyrenean desman's habitat variables preferably selected for scat deposition by using two different scales. I believe that this is crucial to clarify the species ecology behaviour and to improve the design of on-going future research, management and conservation actions.

1.2.1 Objectives

The main objectives of this study were 1) to determine the ecological variables that may be related to scat deposition in Pyrenean desman 2) to make a quantitative assessment of their relative importance, in order to produce predictive models of these species ecological preferences and space use. This was achieved by testing the influence of factors such as the presence of alder, bank or bed localization, substrate exposure, water speed and presence of musk (small-scale environmental and biological variables) on Pyrenean desman scats' presence and on its abundance.

Based on the limited, available literature (Ramalhinho & Tavares 1989; Queiroz et al. 1998; Melero et al. 2012; Charbonnel et al. 2015; Biffi et al. 2016), we expected desmans to deposit their scats mainly in non-exposed sites with presence of alder, near high river flow and probably, with no presence of musk coverage in the substrate. Concerning the preference for riverbanks or riverbed we could expect both as Queiroz et al., 1998 results indicate more scat deposition in the riverbed while Biffi et al., 2016; ICN, 2014a; Pedroso & Chora, 2014 referred the opposite.

We also tested the influence of the variables: percentage of coverage, spraintability (which corresponds to the percentage of substrate available for scat deposition), riverbed width, water speed, percentage of pool and percentage of riffle (large scale environmental and biological variables) on the abundance of Pyrenean desman scats' found per km of transect. Based on the available literature (Ramalhinho & Tavares 1989; Queiroz et al. 1998; Melero et al. 2012; Charbonnel et al. 2015; Biffi et al. 2016) we expected a high abundance index of Pyrenean desman scats for an intermediate percentage of coverage, high values of spraintability, narrower riverbed, high water flow, low percentage of pool and finally a high percentage of riffles.

These results will be important to our understanding of the habitat characteristics that are important to the Pyrenean desman. This will allow us to formulate and test hypothesis on communication and social organization of the species.

2 Methodology

2.1 Study area

This study was performed in the Sabor's and Tua's basins, which are considered the main tributaries of the right bank of Douro's river, and secondarily in some rivers and streams from Paiva's basin. Sabor's watershed is considered the biggest Douro's sub-basin in national territory and it covers: Bragança, Macedo de Cavaleiros, Vimioso, Miranda do Douro, Mogadouro, Alfândega da Fé, Carrazeda de Ansiães, Vila Flôr and Torre de Moncorvo (Queiroz et al., 1998). Tua's watershed is the second biggest Douro's sub-basin and it includes the municipalities: Vinhais, Bragança, Macedo de Cavaleiros, Mirandela, Chaves, Valpaços, Vila Flôr, Carrazeda de Ansiães, Vila Pouca de Aguiar, Murça and Alijó (Queiroz et al., 1998). As Tua's watershed, Paiva's watershed is also classified as the second biggest Douro's sub-basin but from the left bank of the river. It covers: Castelo de Paiva, Cinfães, Arouca, S. Pedro do Sul, Castro de Aire, Vila Nova de Paiva, Viseu, Moimenta da Beira, Satão and Sernancelhe (Queiroz et al., 1998). These three areas were all considered as places of Pyrenean desman's presence confirmed during the distribution studies established by Queiroz et al., 1998

Each of the study areas are characterized below (Figure 3):

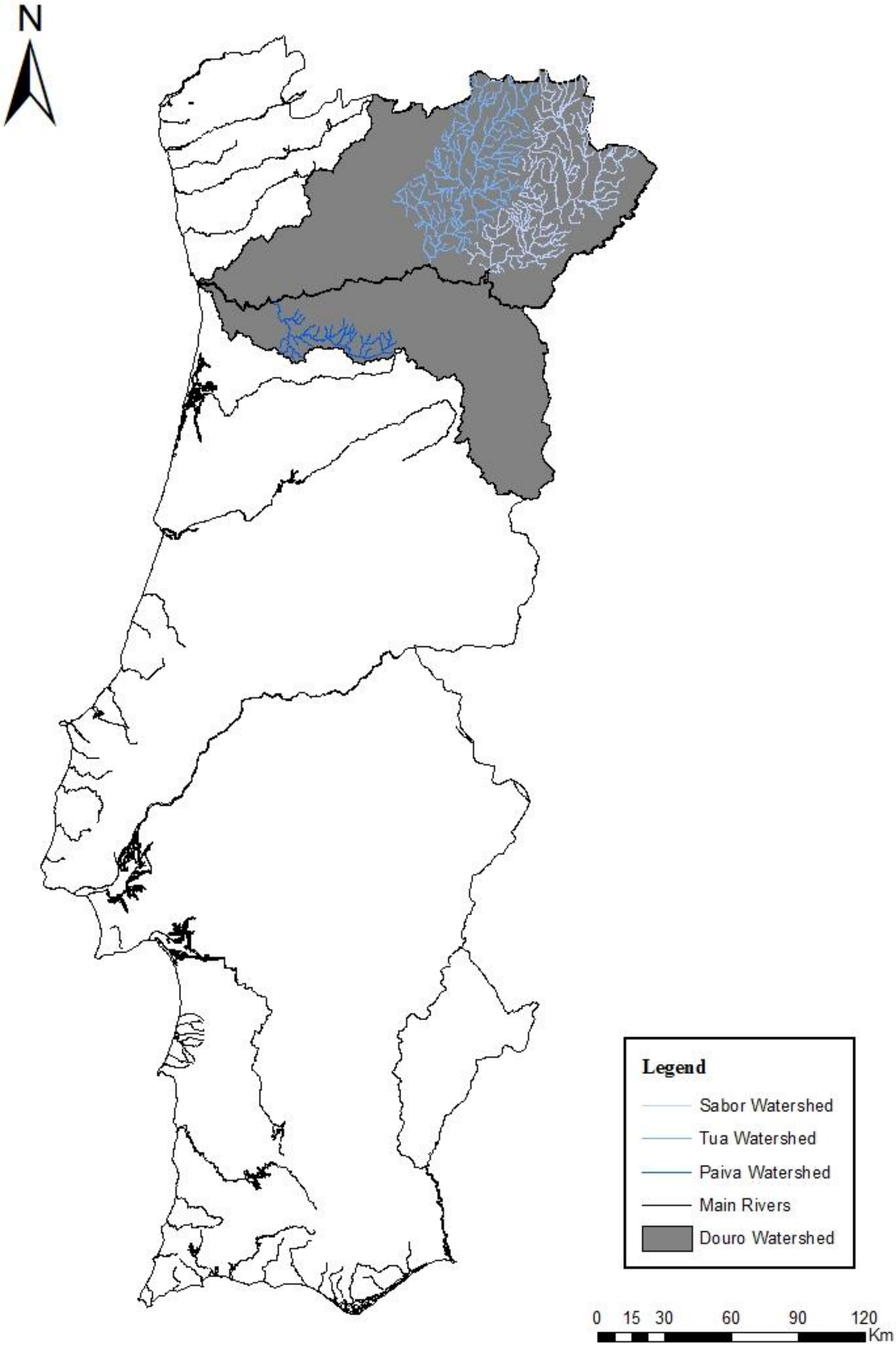


Figure 3 - Overview of the study area. The three different sub-basins sampled: Sabor, Tua and Paiva's are part of the Douro watershed and are represented in different colours.

2.1.1 Sabor's Watersheed

Sabor River flows from Spain, 2km away from Portuguese border (Serra de Montesinho) and drains an area of approximately 3868 km^2, being that 3453 km^2 (87% of the total area) are located in Portuguese territory. Its main tributary is Maçãs River but there are others equally important: Vilariça's stream, Azibo River, Fervença River, Angueira River, Onor's river, Vale de Moinhos' stream and also S. Pedro's stream (Queiroz et al., 1998). Sabor's basin is part of one of the biggest geomorphological units from the Iberian Peninsula – Hesperian Massif – and it is characterized by the presence of granite, schist, quartzite and metamorphic rocks (Nunes, 2015) being schist the dominant. Its altitude gradient ranges between 100m (mouth of the Sabor River) and 1100m (Hills of Bornes and Nogueira) and the annual rainfall gradient ranges from the 500mm to 1000mm. In general, the total annual precipitation increases in direct association with the altitude and due to these characteristics climate is predominantly Mediterranean with Continental influence (Nunes, 2015).Mean annual temperature ranges between 10ºC and 16ºC (Parque Natural de Montesinho, 2016) and considering the thermicity index, the site has two distinct bioclimatic belts: Meso-mediterranean and Supra-mediterranean zones (Sabor: Trás-os-Montes, 2012).

Sabor's basin reveals an irregular character, concentrating the highest flows between December and March, due to the values of the precipitation. From July to September the average values of the flow are quite low and sometimes even null during the years of marked drought (A. Nunes, 2015).

Land cover is dominated (>80%) by Mediterranean oak forests, mainly cork oaks (*Quercus suber*), juniper (*Juniperus oxycedrus* var. *lagunae*) and holm (*Quercus rotundifolia*) which are the endemic formations of main interests. But the most important vegetation of the Sabor's Basin is the riparian flora represented by the endemic *Antirrhinum lopesianum* existent in the rocky scarps and by the *Petrorrhagia saxifraga, Festuca duriotagana*, and thickets of boxwood *Buxus sempervirens* (ICN, 2014b). It is also visible the presence of olive groves and other permanent crops, and arable cropland and pastures (Sabor: Trás-os-Montes, 2012).

Most of the Baixo Sabor is included in the Rede Natura 2000 within the Special Protection Area (SPA) of the rivers Sabor and Maçãs, classified under the European Directive 79/409/EEC, and the Sites of Community Importance (SCI) of the rivers Sabor and Maçãs and of Morais, classified under the and 92/43/EEC (Sabor: Trás-os-Montes, 2012). The classification as SPA was mostly because of the populations of birds existent in the area, like: golden eagle (*Aquila chrysaetos*), Bonelli's eagle (*Hieraaetus fasciatus*), and Egyptian vulture (*Neophron percnopterus*). Classification as SCI was due to the presence of a large number of habitats and species of conservation concern as the wolf (*Canis lupus*) (Sabor: Trás-os-Montes, 2012).

In general, the good quality of water, the good conservation status of the riverbanks and the existence of a preserved ecologic *continuum* makes this a very important place to every fauna associated with the aquatic environment, especially to our study species *Galemys pyrenaicus*. However, Sabor's watershed is characterized by the presence of Baixo Sabor's dam which is considered one of the main threats to the habitats and aquatic populations of the area because it caused the submersion of an important stretch of the river and besides this, many are the hydraulic enterprises in their tributaries.

2.1.2 Tua's Watersheed

Tua River results from the conjoining between Tuela and Rabaçal rivers. These last two rivers have their source in Spain, with Tuela river flowing from Zamora and covering all Bragança's county and Rabaçal river, flowing from Galiza and entering in Portugal near Vinhais countil (Beira, 2014; Ferreiro, 2007). The conjoining occurs 4km North of Mirandela (Beira, 2014; Ferreiro, 2007). Tua's watershed has a total dimension of 3093km^2 with Tua River occupying an extension of 56.5km (Queiroz et al., 1998). It drains in average 12 counties being the biggest in terms of occupied area: Vinhais (23%), Mirandela (21%) and Valpaços (17%) (Moreira, 2013). Its main tributaries are Rabaçal, Tuela and Tinhela rivers (Moreira, 2013). The area covered by Tua's watershed have an average height of 509m (Moreira, 2013). The landscape is diverse and characterized by a variety of lithological and geological structures that are the basis of the reliefs' diversity. The basin is mainly marked by mountain areas but also by plateaus, especially in Tua's base area, and embedded valleys where it is remarkable the presence of quartzite outcrops (Parque Natural Regional do Vale do Tua, 2013).

The mean annual rainfall ranges from 700mm to 1000mm, irregularly distributed along the year while mean annual temperature varies between 7ºC and 16ºC (Mendes, 2005). Thermal and rainfall annual range together with the North-South orientation of the valley (which confers greater exposure to insolation) determines the existence of microclimates with typical Sub-Mediterranean vegetation (Caracterização Física | Rota da Terra Fria, 2016) where domains species like: holm (*Quercus rotundifolia),* juniper (*Juniperus oxycedrus* var. *lagunae*), and the Portuguese oak (*Quercus faginea*) as well as cork oaks (*Quercus suber*) (S. Nunes, 2003). In the brushwood it's visible mainly: rockrose (*Cistus ladanifer*), *Cistus psilosepalus, Cistus crispus* and rosemary *(Rosmarinus officinalis*) (S. Nunes, 2003). It is also an area used for agriculture and grazing and in lower areas stands out the irrigated agriculture, olive groves, almond groves and vineyards (S. Nunes, 2003).

The Natural Regional Park of Tua's Valley is designated as protected area under the law decree nº 142/2008 from July 24th (Parque Natural Regional do Vale do Tua, 2013) and presents a numerous and diverse fauna. Due to its rare and endangered character the following species are considered as noteworthy: *Lampetra planerii, Cobitis calderoni, Oenanthe leucura, Aquila fasciata* and *Rhinolophus euryale;* but the most emblematic are: *Bufo bufo, Lutra lutra, Microtus cabrera* and our study species *Galemys pyrenaicus.*

In general, the rivers included in Tua's watershed are considered of good quality, however there are records of some punctual pollution mainly of industrial source and also from pig farms and due to the lack of Industrial Water Treatment water quality is getting compromised. Another major threat to the water quality and obviously to the aquatic fauna is the construction of hydraulic infrastructures like the Foz Tua's dam.

2.1.3 Paiva's Watersheed

Paiva's river flows from Nave's plateau, in Serra de Leomil, Moimenta da Beira county (Riopaiva, o mais belo rio de Portugal, 2010). It has an extension of 110km and drains an area of approximately 795,185km^2 covering partially the counties: Arouca, Castelo de Paiva, Castro Daire, Cinfães, Moimenta da Beira, São Pedro do Sul, Sátão, Sernacelhe, Vila Nova de Paiva and Viseu (Riopaiva, o mais belo rio de Portugal, 2010). Its main tributaries are Covo, Paivô and Ardena rivers but there are others more secondary but also important, like: Vidoeira, Paivó and Mau rivers and also Tenente stream (Queiroz et al., 1998). Paiva's river basin is characterised by a Temperate

Mediterranean climate with an average annual temperature of 13ºC and an average annual precipitation higher than 1000 mm (Pinto, 2013). The river and its tributaries make their route mainly on the Schist - Greywacke complex, being schist and granitic formations the predominant in the area (Pinto, 2013). The altitude gradient of Paiva's basin ranges between 100 and 800m and it is conditioned by the surrounding relief forms (Pinto, 2013). In the initial section, the watercourse runs through a plateau where vegetation of Continental character is predominant (ICN, 2014a). In the medium section, due to the river orientation, the high slope of the sheds, and the domain of schist substrate, vegetation presents a Thermo-Mediterranean character with slopes covered by pine and eucalyptus plantations, scrublands, oaks and cork oaks (ICN, 2014a). At the end section, sheds have high coverage and good vegetation density, revealing an Atlantic character (ICN, 2014a).

In general, it presents well preserved riparian vegetation with alders (*Alnus glutinosa*) forming gallery and bordered by fragmentary oaks (*Quercus robur*). It also should be noted the presence of the endemic species *Anarrhinum longipedicellatum* (ICN, 2014a). Paiva's River is classified as Site of Community Importance (SIC) included in Rede Natura 2000 territory and it was considered one of the best rivers in Europe in terms of water quality, assuming big importance to the conservation of riparian and aquatic fauna like: otter (*Lutra lutra*), Schreiber's green lizard (*Lacerta schreiberi*) and also to our study species Pyrenean desman (*Galemys pyrenaicus*) (ICN, 2014a). However, threats to the water quality in the area are increasing due to the implementation of hydraulic enterprises, dams' construction and other factors related with the development of industrial and touristic activities (ICN, 2014a).

2.2 Sampling

In 2014, 129 transects were visited, 19 from Paiva's basin, 82 from Sabor's basin and 28 from the Tua's basin while in 2015: 169 were visited being 71 belonging to the Sabor's basin and 98 from the Tua's basin (Table 2; Figure 4)

Table 2 - Number of transects visited per river basin and by year

	Year		
River Basin	**2014**	**2015**	**Transects per Basin**
Paiva	19		19
Sabor	82	71	153
Tua	28	98	126
Transects per Year	**129**	**169**	**298**

From the 129 sites visited in 2014, 112 were sampled and 17 were not because they were dry while in 2015 only 95 of the 169 sites visited were sampled and 74 were dry (Table 3; Figure 5).

Table 3 – Number of transects visited that were sampled and the number of transects dry per year

Site Code Count	Year		
	2014	**2015**	**Total Sites Sampled/Dry**
Sampled	112	95	207
Dry	17	74	91
Total Sites Visited	**129**	**169**	**298**

From the 112 sites sampled in 2014: 6 belonged to Paiva's watershed, 79 to Sabor's watershed and 27 to Tua's watershed. In 2015, from the total of 95 sites sampled, 57 were from the Sabor's watershed and 38 from Tua's watershed (Table 4).

Table 4 - Number of Sites sampled by watershed per year.

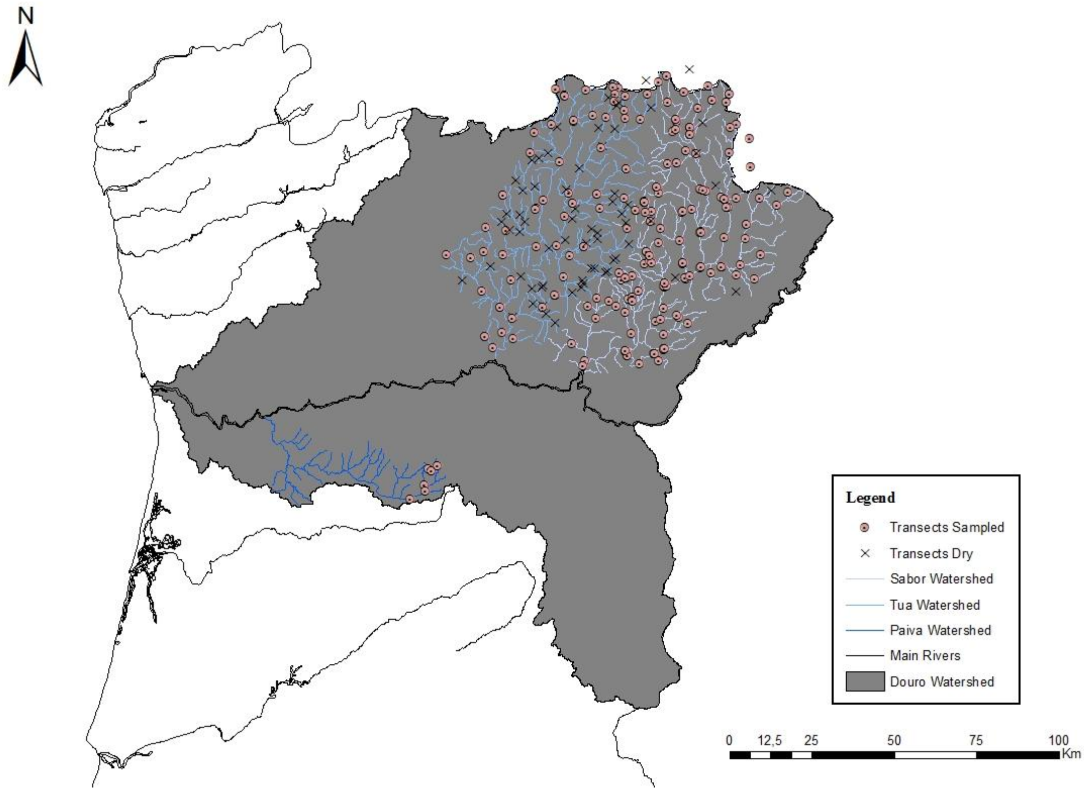
River Watershed	Year		
	2014	**2015**	**Sites Sampled per Watershed**
Paiva	6	-	6
Sabor	79	57	136
Tua	27	38	65
Sites Sampled per Year	112	95	207

Transects were selected based on the specie's distribution studies developed twenty years ago by Queiroz et al. 1998 which record a Pyrenean desman's presence confirmed of 82% in Tua's basin, 74% in Sabor's basin and 89% in Paiva's basin.

Namely, having access to the data of Queiroz et al. 1998, we re-visited all the sites sampled twenty years ago by Queiroz and collaborators. To these, we added new sites, to increase the final sample size and have a more homogeneous and detailed sampling, particularly of Sabor and Tua watersheds. Site selection criteria were similar to those used by Queiroz et al., 1998, namely suitable habitat conditions and ease of access. Additional sites, sampled for other aquatic vertebrate species within the framework of companion studies (Ferreira, Filipe, Bardos, Magalhães, & Beja, 2016) and presenting suitable habitats for the Pyrenean desman, were also added.

To ensure independence among sampling and to avoid spatial autocorrelation transects selected were separated between them by a minimum distance of 500m, especially if they located in the same river.

Figure 4 - Overview of the study area with representation of transects sampled (signalled with a circle) and transects found dry (signalled using a cross).



2.3 Scat Survey

Scat survey was carried out between May and September of 2014 and May and October of 2015. This period is characterized by a typical dry season with little fluctuating water levels and low rainfall. This is important to minimize variations in the sign detection probabilities due to removal of faeces by rising water levels or washing by rain (in other words, to limit false absences), and to facilitate exploration because the rivers are easier to prospect (Ajo & Cosío, 2009; Charbonnel et al., 2015).

Searches for Pyrenean desman faeces were conducted along river transects of approximately 600m which approximately matches the mean home range of the species (Charbonnel et al., 2015). Nores et al., 2012 refer that this is the minimum distance necessary to guarantee a 95% probability of finding desman evidence. However, some other studies defend that a distance of 200-250m is sufficient to detect desman's evidence if a previous selection of the favourable habitat is done (Queiroz et al., 1998).

We tried to accomplished the 600 m because we wanted to cover the maximum range possible used by Pyrenean desman in order to describe the preferable microhabitat characteristics of the sites where they leave their scats but sometimes, depending on the transect characteristics it was impossible to fulfil this distance.

In general, each transect was visited once during each year, but there were 29 sites repeated from 2014 to 2015 and even during the same year (see Appendix 1, Table 34). The main reason for the repeated visits was: to re-sample sites were the species was known to occur twenty years ago but genetic analyses of the current dataset did not provide substantial results (i.e., there were some failures in the DNA amplification). River transects were waded by pairs of skilled observers. There were also some data collected by observers from UTAD (University of Trás-os-Montes e Alto Douro) which had a previous formation in desman scats' identification, were also considered. We tried to limit the number of observers as much as possible during each visit and at least the most experienced observer was present during all the field survey periods to minimize the observer bias and to ensure a correct and faster identification of the desman scats. Each observer was responsible for inspecting a specific riverbank side and also the substrate along the streambed. They used a flashlight to meticulously examine every emergent stone, root or trunk of the riverbed and also cavities or potential shelter places in the banks. These are the favourable places for prospection because they are referred as the typical places where Pyrenean desman leaves its scats (Silva, 2001). When rivers were too deep to search by walk, the observers used a float to examine the riverbanks in order to get information about scats left and/or characterize the type of habitat. Otherwise this would not be considered because of difficulties in progression. Usually the river transects were waded upstream to prevent washing the scats.

Pyrenean desman scat's identification was based mainly in texture, colour, smell and size. Usually they present an irregular cylindrical shape and a grainy texture, due to the remains of chitin from macroinvertebrates exoskeleton (Queiroz et al., 1998). These traces of chitin are visible at light that is also other reason why it is important to use a flashlight during the field survey. When scats are fresh they are black with a dry green or brownish tone and they normally look oily (Queiroz et al., 1998). They also present a very typical musky smell that almost entirely disappears as the scat gets older and dry (Queiroz et al., 1998). The size of the scats ranges the 15 to 25mm of length and 3 to 5 mm of width (Queiroz et al., 1998; Silva, 2001). They could appear isolated (Figure 6) or in groups of two or more scats (Figure 7). Usually, groups with more than three scats together are called latrines (Queiroz et al., 1998; Silva, 2001), and they are described as places regularly used by species for marking behavior.

laboratory confirmation.

Figure 5 - Pyrenean desman isolated scat.

Figure 6 - Pyrenean desman latrine.

All feaces detected and suspected of being left by Pyrenean desman were collected and stored in plastic tubes with 96% alcool, labelled, and frozen for posterior laboratory confirmation.

2.4 Measurements: Marking Site and Habitat characterization

2.4.1 Marking Site Characterization

The marking site of the “True Sites”: which are the sites with signals of the Pyrenean desman’s presence and also “Discrete” and “Random Sites” which are sites randomly selected that describe places of Pyrenean desman’s absence, was characterized by noting in a paper sheet a series of environmental and biological variables measured at micro-habitat scale (~0,5m^2). All the sites characterized along each transect were geo-referenced.

2.4.1.1 Scat Characterization:

Number of scats observed was determined by counting the number of scats at the site of detection. When at the same site more than one scat was found, it was only consider as part of the same count the scats with similar characteristics based on the opinion of the most experienced observer. We also assigned a probability of belonging to Pyrenean desman to each collected scat. This percentage was defined by the most experienced observer based on his previous knowlegde when compared to the observations of the morphology, length and smell of the scat in the field.

2.4.1.2 Description of the Substrate and Hydrological conditions:

A set of variables was selected according to previous studies on the Pyrenean desman’s habitat preferences (Ajo & Cosío, 2009; Charbonnel et al., 2015; Marcos, 2004; Queiroz et al., 1998) that linked species presence to river characteristics (both water and banks) at a small scale. Physical parameters like substrate and hydrological conditions were measured and described in ~0,5 m^2 area where we found desman signs for the “True Sites”, and where points were selected as “Random”/ “Discrete Sites”

(sites of scat abscence). The variables recorded varied between 2014 to 2015. In 2014 twelve variables were noted: marking site, musk, exposed, otter, cm distance to H_2O, cm height to H_2O, cm distance to bank, habitat (riffle, run, pool), speed, cm depth, m width and bank or bed. In addition to these variables, in 2015 nine other variables were (making a total of 20 variables): wall, slope of the marking site, scat position, shading, coverage, alder, cm bank's height, bank's slope and also spraintability. All the variables recorded for the description of the site are described below:

- ***Marking Site***

Describes the type of substrate found at the sampling site (~0,5m^2) using the categories: pebble (64-128mm), cobble (128-256mm), boulder (256-512mm), rock (>512mm), outcrop, ground, roots, branch and trunk.

- ***Musk***

Binary variable indicating presence or absense of musk covering the substrate at the sampling site.

- ***Wall***

Binary variable indicating if the marked substrate was part of a bank wall or not.

- ***Slope***

This variable was measured using an Android application "Angle Meter" which calculate the approximate angle or slope oh the substrate's surface sampled. It was defined by the following categories:

1- Between 0º and 20º;
2- Between 20º and 40º;

3- Between 40º and 70º;

4- Between 70º and 90º;

5- More than 90º;

- ***Otter***

Indicates the presence (1) or absence (0) of otter scats within the area defined as sampled site ($0.5m^2$).

- ***Scat's position***

This variable defines the position of the scat (for "True Sites"), or of the "Random"/ "Discrete Site" point, in relation to the current. The position is defined in favour of the current and it is described using the categories: up, middle or down the current (Figure 8).

Figure 7 - Scheme representative of the Scat Position evaluation in relation to the river current. (1) corresponds to the up position; (2) marks the middle position and (3) down position

- ***Height to H_2O(cm)***

This variable is numeric and refers to the height measured from the scat (in case of the “True Sites”) or from the “Random”/ “Discrete” point to the water surface. This was measured using a ruler and reported in cm.

- ***Distance to H_2O(cm)***

This variable is numeric and refers to the distance (horizontal) measured from the scat (in case of the “True Sites”) or from the point described as “Random”/ “Discrete” to the water surface. The variable was measured using a ruler and it was described in cm.

- ***Distance to Bank(cm)***

Refers to the distance measured from the scat (in case of the “True Sites”) or from the point described as “Random”/”Discrete” to the closest bankside. It was defined in cm and measured with the help of a ruler of one meter.

- ***Shading/ Coverage***

These variables were estimated individually as a percentage of the area of the marking site within one meter radius shaded/covered by the riverbank vegetation. They were classified using the estimated percentages: 0%; 25%; 50%; 75% and >. 0% was used to describe places with no shade or uncovered while > was used to characterize sites almost completely shaded or covered by vegetation.

- ***Exposed***

Refers to the scat (in case of the “True Sites”) or to the point described as “Random”/”Discrete” and it was classified using 0 when they were hidden or non exposed; 0.5 when they were partially exposed and 1 when they were totally exposed.

- ***Habitat***

Habitat was described considering the riverbed characteristics near the sampled site. It was defined using one of the three following categories:

Riffle – shallow section with fast flowing current;

Run – area with fast flow, that runs smoother than riffles and is also deper;

Pool – area with greater depths and slower speed;

- ***Depth(cm)***

Water depth was estimated near the sampling site using a ruler of one meter. A category was then attributed following the described criteria:

Low – if the water depth was between 0 and 50cm;

Medium – if water depth was between 50cm and 1m;

High – if the water depth was higher than 1m;

- ***Speed***

This varible describes the water velocity near the sampling site and it was estimated using the categories:

1 – Null/almost null: when there was no perceptible water movement;

2 – Weak: records of low speed flow;

3 – Medium/strong;

- ***Alder Presence or Absence***

This variable signals the presence (1) or absence(0) of alder (*Alnus glutinosa*) in the $0.5m^2$ area around the scat.

- ***Width(m)***

Width was estimated measuring the horizontal distance of the riverbed from the marking site to the nearest riverbanks using a ruler of one meter. A category was then attributed following the described criteria:

Narrow – if the riverbed width was less than 2 m;

Medium – if the riverbed width was between 2 and 8 m;

Large - if the riverbed was larger than 8 meters;

- ***Bank or Bed***

Indicates the place where the sampled site was located. We used the categories 1 – when the marking site belonged to the bank and 2 – when the marking site was at the riverbed.

- ***Bank's Height***

This variable indicates an estimated measure of the bank's height near the sampled site in cm.

- ***Bank's Slope***

Bank's Slope was estimated based on the following categories:

1- 0-20: almost no slope existent (the bank seems part of the riverbed);
2- 20-40: slightly inclined;
3- 40-70: rather inclined;
4- 70-90: vertical;
5- More than 90: excavated;

- ***Spraintability***

Refer to the percentage of substrate available for desman's scat deposition (i.e. presence of emergent items and cavities, diversity of substrate types) near the sampled site. Lower percentages mean that the substrate is very homogeny and so there's few substrate available for scat deposition, while high percentages mean high heterogeneity. The categories attributed are described below:

1- Less than 5%;
2- 5-19%;
3- 20-39%;
4- 40-69%;
5- 70-100%;

2.4.1.3 “Discrete” and “Random Sites” Selection

“Discrete” and “Random Sites” were selected according to the following methods:

“Discrete Sites” were two points randomly selected within a 10 meters’ radius from the site where Pyrenean desman scats were found. We used an Android application “Random Number” to help to define the distance and the direction (up/down) at which each “Discrete Site” was from the True marking site. The point randomly selected was only accepted if no Pyrenean desman presence was detected nearby (i.e., in a $\leq$ 2m radius).

“Random Sites” were selected using an Android application “Random reminder” which randomly produced an alarm during the time we were wading along the transect. The alarm was adjusted to ring with a frequency that allowed to obtain at least ~10 random points per transect. These points were also accepted only if no Pyrenean desman’s scat presence was detected nearby.

2.4.2 General Habitat Characterization

At the end of the sampling the general habitat characteristics of the transect were noted. Some variables collected were similar to the ones used for description of the substrate and hydrological conditions at small scale (area where the scat was found) but now applied to a larger scale (sampling transect). Variables collected were the same for both years and they were mainly riverbank and riverbed descriptive variables (Table 5).

Table 5 – General habitat variables used to describe the riverbank and riverbed of the transects sampled.

Category	Code	Description
Riverbanks	**%Rocksbank**	Estimated % of rocks found in the riverbanks. It includes all the substrate with >512mm of surface's length. The % was defined by categories: 0, 25, 50, 75, >.
	%Stonesbank	Estimated % of stones found in the riverbanks. It includes all the substrate <512 mm of surface's length (pebbles, cobbles and boulders). The % was defined by categories: 0, 25, 50, 75, >.
	%Groundbank	Estimated % of the riverbanks covered by gravel sediment. The % was defined by categories: 0, 25, 50, 75, >.
	%Sandbank	Estimated % of the riverbanks covered by fine sediment. The % was defined by categories: 0, 25, 50, 75, >.
	%Wall	Estimated % of wall found in the riverbanks. The % was defined by categories: 0, 25, 50, 75, >.
	Spraintability	Estimated % of heterogeneity of substrate available for scat deposition (i.e. emerging items and cavities and diversity of substrate). **1:** <5%; **2:** 5-19%; **3:** 20-39%; **4:** 40-69%; **5:** 70-100%.
	%Shading	Estimated % of the river shaded by the riverbank vegetation. The % was defined by categories: 0, 25, 50, 75, >.
	%Coverage	Estimated % of the river covered by the riverbank vegetation. The % was defined by categories: 0, 25, 50, 75, >.
Riverbed	**mWidth**	Estimated average of the riverbed's width in meters.
	cmDepth	Estimated average of the riverbed's depth in meters.
	Speed	Estimated average of the river's water speed. It was defined by: 1 – null/almost null; 2- weak; 3- medium/strong;
	%Riffle	Estimated average % of the riverbed with turbulent fast water units with rapid and shallow flow.
	%Run	Estimated average % of the riverbed with non-turbulent fast water units of shallow gradient that flows uniformly.
	%Pool	Estimated % of the riverbed with slow water units of deep flow.
	%Mud	Estimated % of the riverbed covered with mud. The % was defined by categories: 0, 25, 50, 75, >.
	%Sand/Gravel	Estimated % of the riverbed covered with fine sediment (sand) or gravel. The % was defined by categories: 0, 25, 50, 75, >.
	%Pebble	Estimated % of the riverbed covered with pebble (64-128mm). The % was defined by categories: 0, 25, 50, 75, >.
	%Cobble	Estimated % of the riverbed covered with cobble (128-256 mm). The % was defined by categories: 0, 25, 50, 75, >.
	%Boulder	Estimated % of the riverbed covered with boulder (256-512 mm). The % was defined by categories: 0, 25, 50, 75, >.
	%Outcrop	Estimated % of the riverbed covered with substrate with >512mm of surface's length. The % was defined by categories: 0, 25, 50, 75, >.
Other	**Otter**	Indicates the presence (1) or absence (0) of otter traces in the riverbank or riverbed.

Another variable annotated was the number of plausible Pyrenean desman's scats found along the transect. This number was confirmed after by genetic analyses. We calculated the kilometric abundance index (KAI) dividing the number of Pyrenean desman's scats found by the distance in kilometres covered in each transects.

2.5 Scat confirmation

Desman excrements are not easy to differentiate. Some are smaller than usual and may be confounded with those of shrews, namely: *Neomys sp.*, or *Crocidura russula*, which also occur at the same type of habitat (Marcos, 2004). In addition, scats of other semiaquatic insectivorous vertebrates, including birds, like the white-throat dipper (*Cinclus cinclus*), can also be easily confounded with those of the Pyrenean desman. Thus, a laboratory confirmation is of great importance. Genetic analysis provides a reliable and non-invasive method to easily distinguish the faeces of the Pyrenean desman from those of other ecologically related species (Gillet et al., 2014).

In laboratory, genomic DNA from faecal samples was extracted using the Stool Mini Kit (Quiagen Inc., Hilden, Germany), following the manufacturer's instructions (Charbonnel et al., 2015; Gillet et al., 2014). DNA extractions was conducted in a separate room with a UV-sterilised platform where no Pyrenean desman tissue samples were previously treated (Charbonnel et al., 2015; Gillet et al., 2014), to avoid contaminations. The species identification process was developed by CIBIO (Research Center in Biodiversity and Genetic Resources) members of the group ConGen at CIBIO facilities. First, a small cytochrome b fragment of approximately 400bp was amplified by nested PCR, using specific primers (GPYRF1: 5'- TTGTAGAATGGAKCTGAGG-3', GPYRF2: 5'-TTCCTTCACGAAACAGGATC-3' and GPYRR1: 5'-GTCGGCTGCTAAAAGTCAGAATA-3') (Charbonnel et al., 2015). This is suitable for the amplification of DNA extracted from faeces because this DNA is often degraded and has low quality (Gillet et al., 2014). Before the nested PCR, single PCRs were carried out using 0.1 µM of each primer (forward primer GPYRF1 and reverse primer GPYRR1), 0.34µl of dNTPs, 2.5mM of $MgCl_2$, 1X GoTaq® buffer reaction (Promega

Inc., Madison, USA), 1U® GoTaq DNA polymerase (Promega Inc., Madison, USA) and approximately 20-30ng of DNA in a final volume of 17 µl (Gillet et al., 2014). Amplifications were performed in a thermal cycler VWR Unocycler using one activation step at 94 º C for 5 min followed by 40 cycles (denaturation at 94º C for 50 s, annealing at 52º C for 45 s, extension at 72º C for 45 s) and final extension step at 72º C for 10 min (Gillet et al., 2014).

For the nested PCR, 0.3 µl of the previous PCR products was used as DNA template, with addiction of the GPYRF2 as forward primer instead of the GPYRF1 (Gillet et al., 2014). PCR products were then sequenced on an Applied Biosystems® 3730 DNA analyser and verified using CHROMASPRO v 1.5 (Charbonnel et al., 2015; Gillet et al., 2014). After that, sequences were submitted to the BLAST® functionality which is available on the NCBI website: http://blast.ncbi.nlm.nih.gov (Charbonnel et al., 2015; Gillet et al., 2014).

2.6 Statistical analysis

All scats confirmed by the genetic validation were considered for analysis. However, the process of confirmation is slow and expensive. Thus, in order to have a more representative sample (N) we also included in the analysis the scats with >70% of certainty of being *Galemys*, attributed in field by the most experienced observer in Pyrenean desman scats' identification. These scats were included after checking the photos taken in field and usually the majority of them were located in sites with confirmed scats or belong to the same latrine as other confirmed scats.

2.6.1 Marking Site Characterization

To understand which microhabitat variables related to substrate and hydrological conditions of the river were preferentially selected by Pyrenean desman, two types of models were performed based on presence/absence and the number of spraints respectively, using as response variables: (1) scats' presence (named as "Chosen") and (2) abundance (called "Nspraints"). Each model was tested separately for the two types of absence data: (1) the "Discrete Sites" and (2) the "Random Sites", because they presented different spatial relation with the "True Sites". The two types of analysis were separately tested for 2015 and for both years together: 2014 + 2015, due to the reduced number of variables recorded in 2014.

Some of the variables were not considered for all the analyses. Variables like: otter, cm height to H_2O, cm distance to H_2O, cm distance to bank, collected in both years, were not included because they presented a high number of missing values in comparison to the other variables of interest. Habitat was also excluded because it showed a strong correlation (see Appendix 1, Table 35) with the explanatory variable speed and the last was preferably selected because it was more representative and caused the model to be more efficient (lower AIC). The variables: cm bank's height and bank's slope, only collected in 2015, were not considered because when we tested all the variables applying a Pearson's correlation test they presented strong correlation values see (see Appendix 1, Table 35) between them and both with the variable bank or bed. Bank or bed was preferably selected because in addition to the correlation problems cm bank's height and bank's slope variables also presented high number of missing values. Shading and coverage, collected in 2015, were also highly correlated (see Appendix 1, Table 35), as indicated by the Pearson's correlation test because of its closer ecological relationship so, they were tested individually. However, none of the models converged when either shading or coverage were included, so they also were not considered for the models. Similarly to shading and coverage, none of the models converged when we added independently the following variables: wall, slope, scat's position, spraintability (all collected only in 2015), depth (cm), width (m) or marking site (collected during both years), therefore they were not included as part of any model.

2.6.1.1 Presence of scats

Analyses of the 2015data

For the analysis data using "Discrete Sites" as the absence points we accessed the effect of the explanatory variables: exposed, speed and alder in the dependent variable: presence of scat deposition, represented by the name "Chosen", with a Generalized Linear Mixed Model (GLMM) with binomial distribution (presence (1)/absence (0)). This type of family is consistent with the data's distribution. We used "Site.Code" as random effect to test the effect of the explanatory variables independently of the transect to which they belonged and also "Gen.Code" to account for possible individual variation among scat samples. When using "Random Sites" as the absence points we accessed the effect of the variables: exposed, speed, alder, musk, and bank or bed also in the dependent variable: presence of scat deposition ("Chosen") by applying a Generalized Linear Mixed Model (GLMM) with binomial distribution (presence (1)/absence (0)) and only with "Site.Code" as random effect. Although the explanatory variables musk and bank or bed were not excluded at the beginning of the analysis, they were not considered in the model using "Discrete Sites" because when included the model showed lack of convergence. However, when they were added to the analysis with "Random Sites" the model ran perfectly.

Analyses of the 2014+2015 data

For the 2014+2015 data analysis the procedures were similar to the described for the 2015. When using the "Discrete Sites" as absence points we accessed the effect of the explanatory variables: musk, exposed and speed in the dependent variable: presence of scat deposition ("Chosen") also with a Generalized Linear Mixed Model (GLMM) with binomial distribution (presence (1)/absence (0)). The random factors considered were: "Site.Code", "Gen.Code" and also "Year" in order to exclude the effect of the variability that could exist between the two years. For the analysis using "Random Sites" the model tested was equal to the described above, with the exception of "Gen.Code" as random factor. The explanatory variable bank or bed caused the lack of convergence when added to the model so it was excluded from the analysis for both models tested using the 2014+2015 data.

We performed model selection based on the measure of goodness of fit: Akaike's information criterion (AIC) for all the analysis performed. We used a set of models considering different combination of predictors and we measure the ΔAIC and the wAIC to identify the single or several "best models" in explaining the variance of the response variable: presence or absence of scat deposition. The "best models" were all those with ΔAIC values ≤ 2or with sum of cumulative weights (wAIC) ≥ 95%. The null model (model with no predictors) was never among the "best models" so all the predictors selected by the model had an explanatory power.

Finally, all the "best models" were model averaged to quantify the effect sizes of the predictors based on the parameter estimates and to translate the results into a more conventional statistical approach. We also computed the relative importance (RI) of the predictors to see their contribution to the "best models".

2.6.1.2 Scats' abundance

Analyses of the 2015 data

The models performed were similar to the described above for scats' presence analysis; the main differences between them are the response variable used and also the distribution family applied. For the analysis of the 2015 data using "Discrete Sites" as the absence points we accessed the effect of the explanatory variables: exposed, speed and musk in the dependent variable: abundance of scats, represented by the name "Nspraints", with a Generalized Linear Mixed Model (GLMM) using a Poisson distribution. This type of family is consistent with the data's distribution. We used "Site.Code" and "Gen.Code" as random effects. For "Random Sites" data we accessed the effect of the variables: exposed, speed, alder, musk and bank or bed also in the dependent variable: abundance of scats ("Nspraints") by applying a Generalized Linear Mixed Model (GLMM) with Poisson distribution. Only "Site.Code" was used as random factor. The explanatory variables bank or bed and alder were not included in the model using "Discrete Sites" because the model did not converge but when using "Random Sites" the convergence problem was not found.

Analyses of the 2014+2015 data

For the 2014+2015 data analysis, when using the "Discrete Sites" as absence points we accessed the effect of the explanatory variables: exposed, speed, bank or bed and musk in the dependent variable: abundance of scats ("Nspraints") also using Generalized Linear Mixed Model (GLMM) with Poisson distribution. The random factors considered were: "Site.Code", "Gen.Code" and also "Year". For the analysis using "Random Sites" data, we tested the same explanatory variables referred above and it was also applied a Generalized Linear Mixed Model (GLMM) with Poisson distribution using abundance of scats ("Nspraints") as response variable. The random factors used were: "Site.Code" and "Year".

Both analyses applied for different years were subjected to model selection based on the Akaike's information criterion (AIC) to identify the single or several "best models" in explaining the scats' abundance. The null model was never among the "best models". In the end, all the "best models" were model averaged to quantify the effect sizes and we also computed the relative importance (RI) of the predictors.

2.6.2 General Habitat Characterization

In the analysis of the effect of general habitat variables in the kilometric abundance index (KAI) the response variable presented high variance and in order to avoid it we log transformed it. We then applied a LM (Linear Model) with Gaussian distribution to test the effect of the explanatory variables: %coverage, speed, %spraintability, mwidth, %pool and %riffle in the response variable KAI, named as "indexkm" (log transformed). The variables: %rockbank, %stonebank, %groundbank, %sandbank, %wall and otter were tested but they were not considered in the final model because they showed p-values near 1 which indicate that they are perfectly non-significant. Furthermore, when compared the AIC of the model including this variables and the one without them, the last showed more efficiency (less AIC). The other variables: %mud, %sand/gravel, %pebble, %cobble, %boulder and %outcrop showed some high correlation values (see Appendix 1, Table 36) when Pearson's correlation test was applied. However, when including these variables independently (without correlated variables) they also showed p-values near 1 and increased the AIC of the model, so they were not included. According to the Pearson's correlation test, shading and coverage were also highly correlated (see Appendix 1, Table 36), as well as mwidth – cmdepth and %riffle – %run (see Appendix 1, Table 36). So, they were tested independently and the variables considered best for the model were: coverage, mwidth and %riffle. The model was then subjected to model selection based on the Akaike's information criterion (AIC). The "best models" were also subjected to model averaging and we also computed the relative importance (RI) of the predictors.

All statistical analyses were performed in R software (R Development Core Team 2015) using: "lme4" (Bates et al., 2014) and "MuMin" (Barton, 2009) packages, except for general habitat characterization where: "car" (Fox et al., 2011) and "MuMin" (Barton, 2009) packages were used.

3 Results

3.1 Survey results

From all the sites sampled, we verified *Galemys*' presence in a total of 48 sites, 23 in 2014 and 30 during 2015 (Figure 9), which indicates that the species' presence was confirmed in 5 sites for both years (see Appendix 1, Table 37).

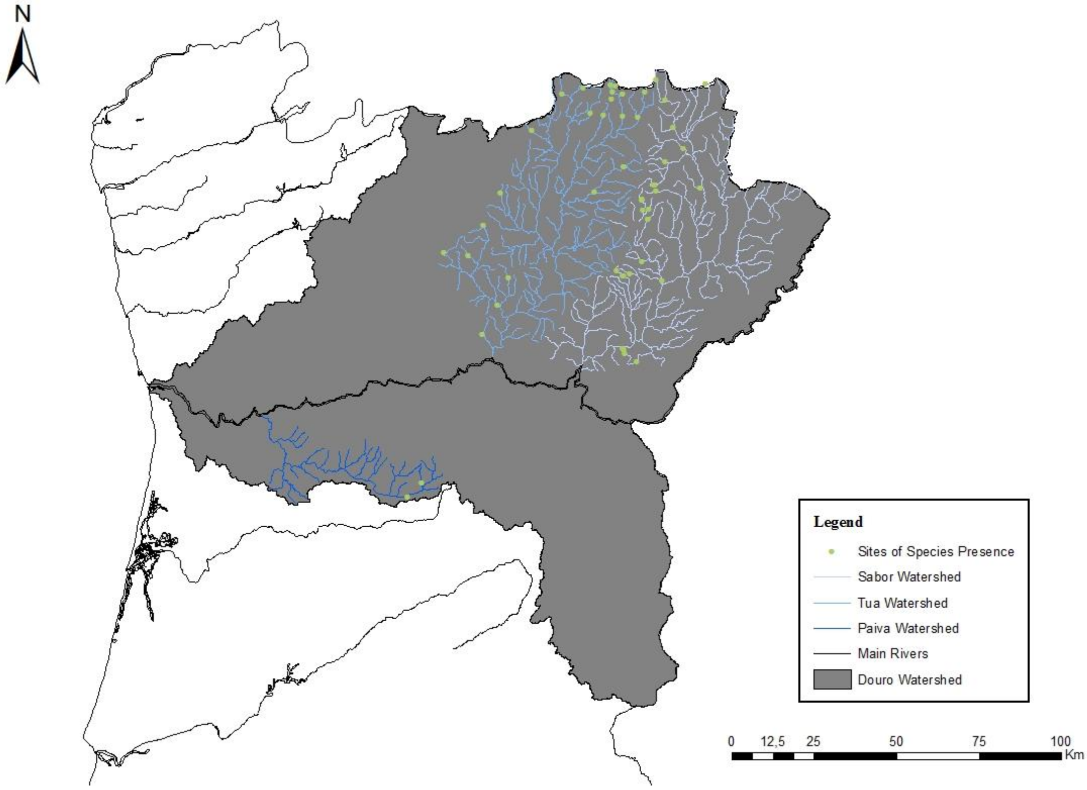


Figure 8 - Overview of the study area with green points representing the sites of confirmed Pyrenean desman's presence.

A total of 351 *Galemys pyrenaicus*' scats were considered in the study, with 111 of them (52 from 2014 and 59 from 2015) confirmed by the genetic analysis and about 240 (44 from 2014 and 196 from 2015) with percentage higher than 70% (Table 6).

Table 6 - Number of scats considered in the study (confirmed and %higher than 70) divided per year.

N scats Considered			
Year	**% higher than 70**	**Confirmed**	**Total per Year**
2014	44	52	96
2015	196	59	255
Total N	240	111	351

3.2 Marking Site Characterization

3.2.1 Presence of scats

Analyses of the 2015data

For data using "Discrete Sites" as absence points, AIC-based model selection found two statistically best models (Table 7) for predicting the Pyrenean desman scats' presence ("Chosen"), with the null model not being among the best models (ΔAIC=304.14). From the variables considered in the best models only alder (Figure 10, c)) and exposed (Figure 10, b)) showed high importance (RI for predictors=1) in explaining the presence/absence of scat deposition, whereas the variable speed (Figure 10, a)) had a lower effect (RI=0.65). Effect sizes of the model averaged predictors (Table 8) indicate that exposed negatively affected (p=0.019) the scats' presence and the latter is associated with the presence of alder (p=0.129) and water speed (p=0.339).

For the data using "Random Sites" as absence points, AIC-based model selection also found one statistically best model (Table 10). The null model was not part of the best models (ΔAIC=595.52). Best model showed high importance of the variables exposed (Figure 11, b)), alder (Figure 11, e)), speed (Figure 11, c)) and bank or bed (Figure 11, a)) (RI of the first three predictors=1; RI bank or bed=0.99) and lower importance of the variable musk (Figure 11, d)) (RI=0.85). Exposed and the category riverbed (bank or bed 2) showed negative effect (p=<0.001; p=0.004, respectively) on scats' presence while speed (p=<0.001), musk (p=0.113) and presence of alder (p=0.354) were positively associated with the scats' presence (Table 11).

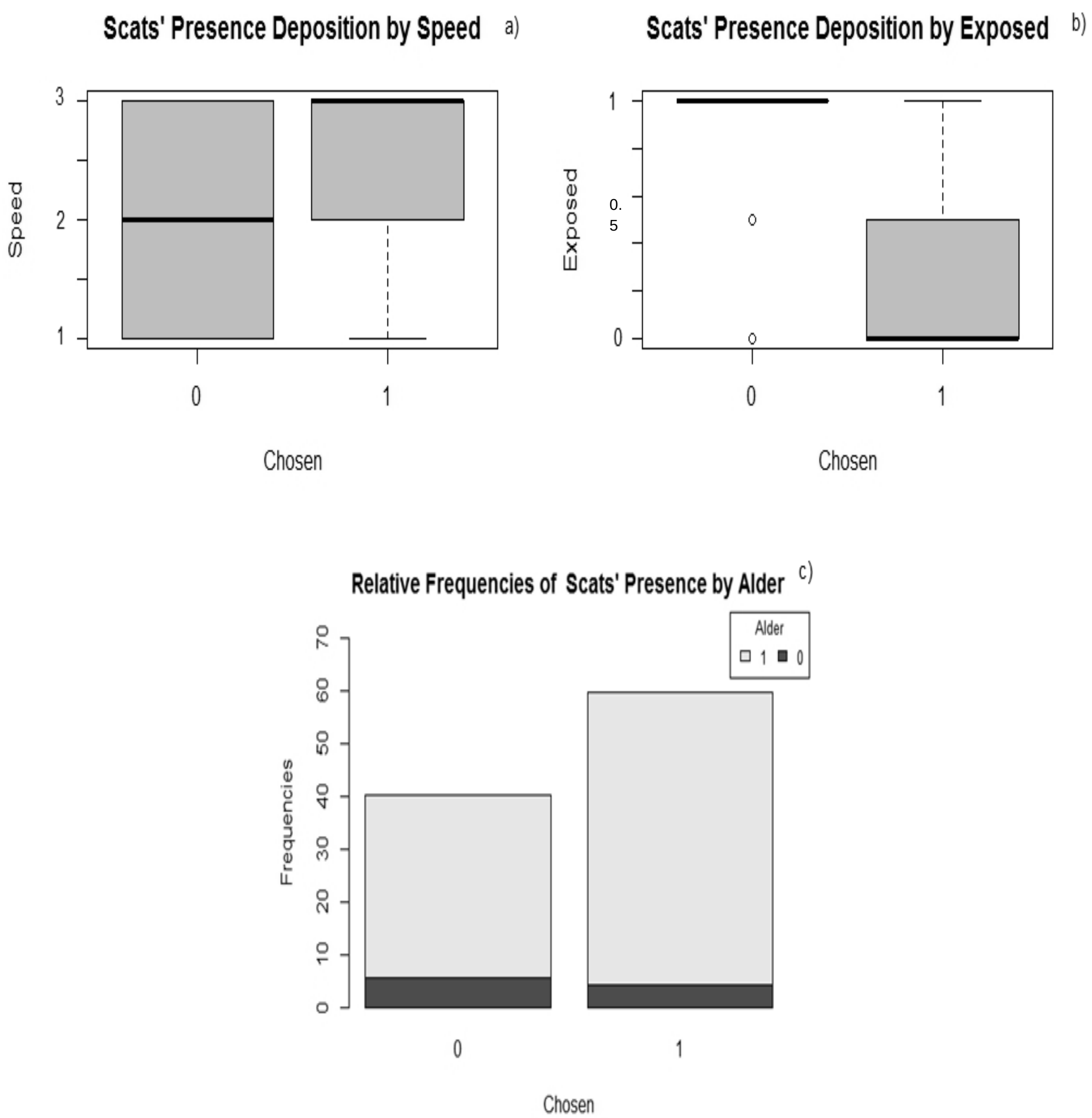


Figure 9 – Data exploration of the response variable: scats' presence (named as "Chosen") in relation to the variables: Speed, Alder and Exposed, integrated in the model using 2015 data with "Discrete Sites" as absence points. a) Variation for the variable speed according to scats' presence (1) or absence (0); b) Variation for the variable exposed in relation to scats' presence (1) or absence (0); c) Relative frequency of the presence (1) or absence (0) of Alder for places of scats' presence (1) or absence (0).

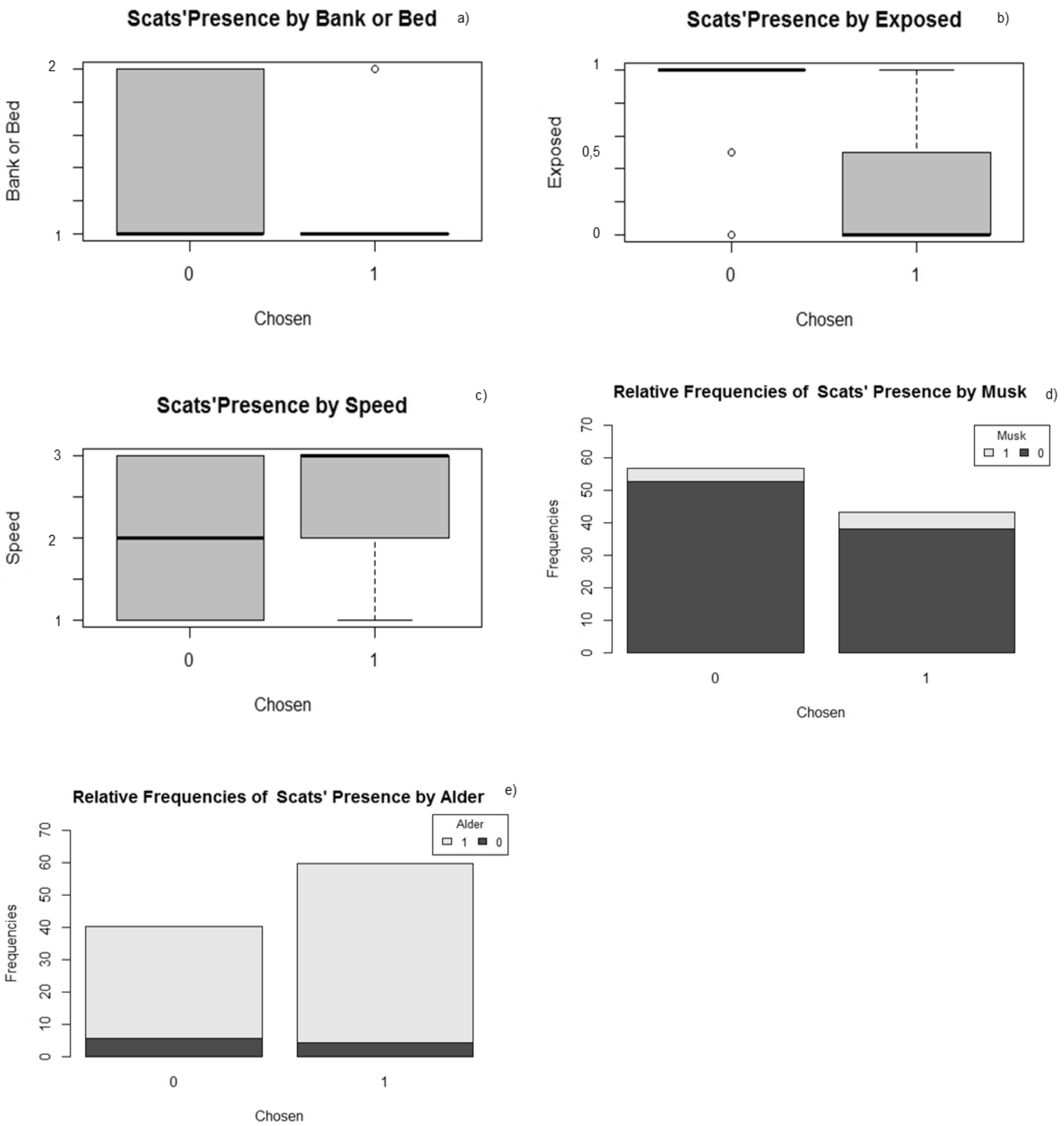


Figure 10 – Data exploration of the response variable: scats' presence (named as "Chosen") in relation to the variables: Bank or Bed, Exposed, Speed, Alder and Musk, integrated in the model using 2015 data with "Random Sites" as absence points. a) frequency of the variable bank or bed in relation to scats' presence (1) or absence (0); b) variance for the variable exposed in relation to scats' presence (1) or absence (0); c) variance of the variable speed according to scats' presence (1) or absence (0); d) and e) relative frequencies of the presence (1) or absence (0) of Alder and Musk, respectively, for places of scats' presence (1) or absence (0).

Table 7 - Best models (ΔAIC < 2) for prediction of the Pyrenean desman scats' presence using 2015 data with “Discrete Sites” as absence points obtained after the AIC-based model selection. Best models are in bold and underlined.

Component models					
Variables	**Df**	**logLik**	**AIC**	**ΔAIC**	**Weight**
<u>Alder + Exposed + Speed</u>	**<u>6</u>**	**<u>-71.89</u>**	**<u>155.79</u>**	**<u>0.00</u>**	**<u>0.65</u>**
<u>Alder + Exposed</u>	**<u>5</u>**	**<u>-73.53</u>**	**<u>157.07</u>**	**<u>1.28</u>**	**<u>0.35</u>**
Exposed + Speed	5	-113.82	237.64	81.86	0.00
Exposed	4	-138.80	285.60	129.82	0.00
Alder + Speed	5	-160.24	330.48	174.69	0.00
Alder	4	-164.29	336.57	180.79	0.00
Speed	4	-198.18	404.36	248.58	0.00
(Null)	3	-226.96	459.93	304.14	0.00

Table 8 - Output for the average model of the best models resultant of the predictions for scats' presence using 2015 data with “Discrete Sites” as absence points. Significant results in bold.

Model-averaged coefficients					
	Estimate	**Std. Error**	**Adjusted SE**	**z value**	**Pr (> |z|)**
(Intercept)	10.3273	6.4812	6.5117	1.586	0.113
Exposed	-20.6357	8.7570	8.8001	2.345	**0.019**
Alder 1	3.2255	2.1146	2.1250	0.956	0.129
Speed	0.6397	0.6672	0.6689	1.518	0.339

Table 9 - Relative importance (RI) of the predictors resultant from model-averaging of the GLMM for scats' presence using 2015 data with "Discrete Sites" as absence points.

Relative variable importance			
	Alder	**Exposed**	**Speed**
Importance	1	1	0.65

Table 10 - Best models (ΔAIC < 2) for prediction of the Pyrenean desman scats' presence using 2015 data with "Random Sites" as absence points obtained after the AIC-based model selection. Best models are in bold and underlined.

Component models:					
Variables	**Df**	**logLik**	**AIC**	**ΔAIC**	**Weight**
<u>Alder + Bank or Bed + Musk +Exposed + Speed</u>	**<u>7</u>**	**<u>-94.03</u>**	**<u>202.06</u>**	**<u>0.00</u>**	**<u>0.84</u>**
Alder + Bank or Bed +Exposed + Speed	6	-96.77	205.53	3.47	0.15
Alder + Musk +Exposed + Speed	6	-99.84	211.68	9.62	0.01
Alder +Exposed + Speed	5	-102.79	215.57	13.51	0.00
Alder + Bank or Bed + Musk +Exposed	6	-102.40	216.80	14.74	0.00
Bank or Bed + Musk +Exposed + Speed	6	-104.82	221.64	19.58	0.00
Alder + Musk +Exposed	5	-106.93	223.86	21.80	0.00
Bank or Bed +Exposed + Speed	5	-107.46	224.93	22.87	0.00
Alder + Bank or Bed +Exposed	5	-108.37	226.74	24.68	0.00
Musk +Exposed + Speed	5	-111.67	233.34	31.28	0.00
Alder +Exposed	4	-112.97	233.93	31.88	0.00
Exposed + Speed	4	-114.39	236.77	34.72	0.00
Bank or Bed + Musk +Exposed	5	-151.01	312.02	109.96	0.00
Musk +Exposed	4	-156.54	321.08	119.03	0.00
Bank or Bed +Exposed	4	-157.05	322.09	120.04	0.00
Exposed	3	-162.81	331.61	129.56	0.00
Alder + Bank or Bed + Speed	5	-210.05	430.09	228.04	0.00
Alder + Bank or Bed + Musk + Speed	6	-209.88	431.75	229.69	0.00
Alder + Speed	4	-218.58	445.15	243.09	0.00
Alder + Musk + Speed	5	-218.30	446.59	244.53	0.00
Alder + Bank or Bed + Musk	5	-226.31	462.62	260.57	0.00
Alder + Bank or Bed	4	-228.77	465.54	263.48	0.00
Alder + Musk	4	-233.63	475.25	273.19	0.00
Alder	3	-236.18	478.37	276.31	0.00
Bank or Bed + Speed	4	-243.02	494.04	291.99	0.00
Bank or Bed + Musk + Speed	5	-243.02	496.04	293.98	0.00
Speed	3	-259.46	524.93	322.87	0.00
Musk + Speed	4	-259.44	526.88	324.83	0.00
Bank or Bed + Musk	4	-279.90	567.81	365.75	0.00
Bank or Bed	3	-281.73	569.46	367.40	0.00
Musk	3	-239.94	593.88	391.82	0.00
(Null)	2	-295.76	595.52	393.46	0.00

Table 11 - Output for the average model of the best models resultant of the GLMM for scats' presence using 2015 data with "Random Sites" as absence points. Significant results in bold.

Model-averaged coefficients					
	Estimate	**Std. Error**	**Adjusted SE**	**z value**	**Pr (> |z|)**
(Intercept)	2.3421	1.4178	1.4222	1.647	**0.099**
Exposed	-10.0780	1.8219	1.8276	5.514	**< 0.001**
Alder 1	1.0290	1.1070	1.1105	0.927	0.354
Speed	1.5475	0.4411	0.4425	3.497	**< 0.001**
Musk 1	1.3875	0.8735	0.8750	1.586	0.113
Bank or Bed 2	-1.8824	0.6471	0.6490	2.901	**0.004**

Table 12 - Relative importance (RI) from model-averaging of the GLMM for scats' presence using 2015 data with "Random Sites" as absence points.

Relative variable importance					
	Exposed	**Alder**	**Speed**	**Bank or Bed**	**Musk**
Importance	1	1	1	0.99	0.85

Analyses of the 2014+2015 data

Using "Discrete Sites" as absence points, AIC-based model selection found two statistically best models (Table 13) and the null model was not among the best models (ΔAIC=333.95). The variables exposed (Figure 12, a)) and speed (Figure 12, b); RI for both predictors=1) showed high importance in explaining the presence/absence of scat deposition, whereas the variable musk (Figure 12, c); RI=0.27) showed the least importance. Effect sizes of the model averaged predictors indicated that variables exposed (p=<0.001) and musk (p=0.910) negatively affected the scats' presence and the latter is associated with faster water speed (p=<0.001) (Table 14).

For the data using "Random Sites" as absence points, AIC-based model selection found only one statistically best-model (Table 16). The null model was not part of the best models (ΔAIC=522.74). The variables included in the best model all showed high importance in explaining the scats' presence/absence: exposed (Figure 13, a); RI=1), speed (Figure 13, b); RI=1) and musk (Figure 13, c); RI=0.97). Scats' presence was negatively associate with the variable exposed (p=<0.001) whereas speed (p=<0.001) and musk presented a positive effect (p=0.047) (Table 17).

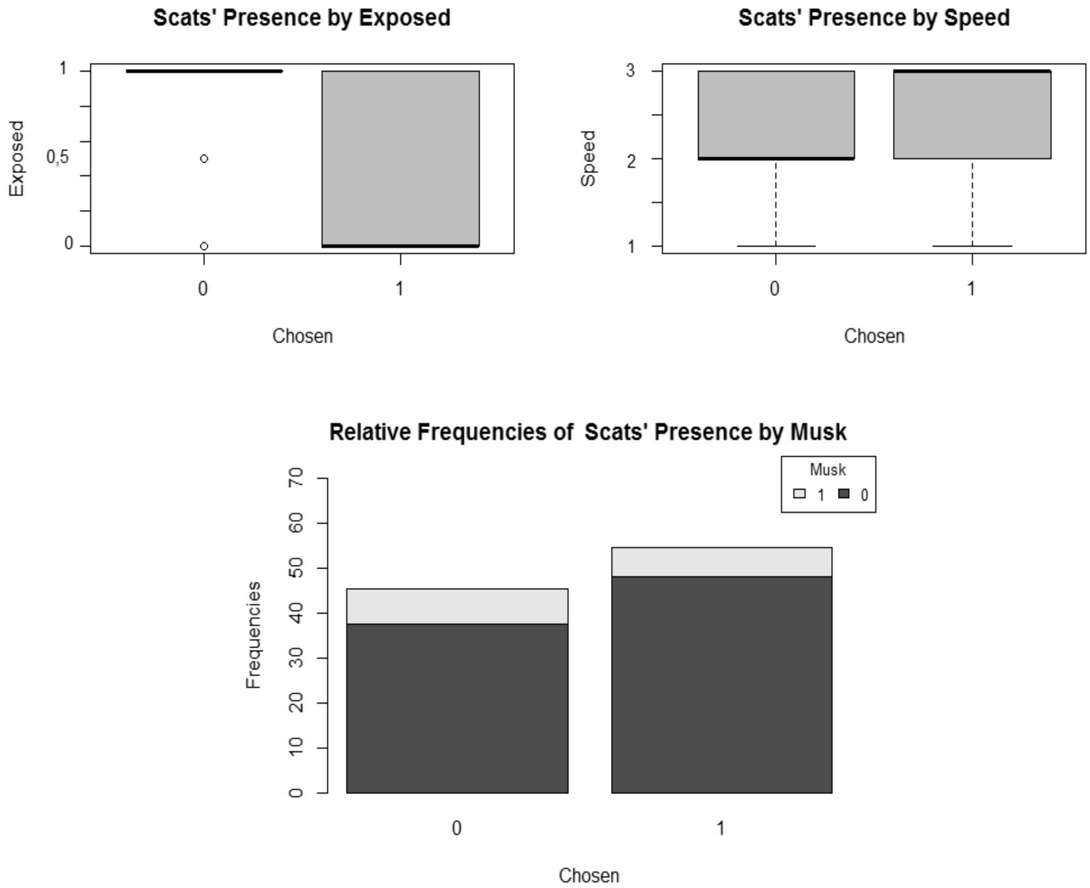


Figure 11- Data exploration of the response variable: scats' presence ("Chosen") in relation to the variables: Exposed, Speed and Musk, integrated in the model using 2014+2015 data with "Discrete Sites" as absence points. a) Variation for the variable exposed in relation to scats' presence (1) or absence (0); b) Variation for the variable speed according to scats' presence (1) or absence (0); c) Relative frequency of the presence (1) or absence (0) of Alder for places of scats' presence (1) or absence (0).

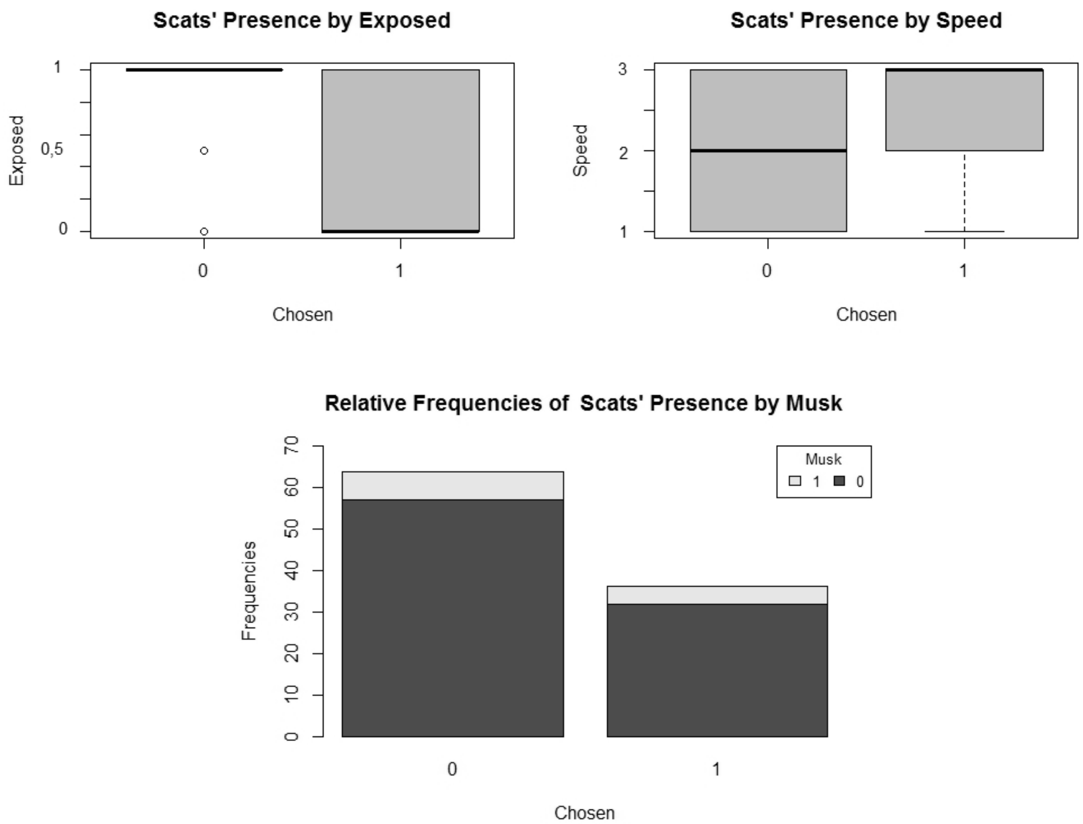


Figure 12 - Data exploration of the response variable: scats' presence ("Chosen") in relation to the variables: Exposed, Speed and Musk, integrated in the model using 2014+2015 data with "Random Sites" as absence points. a) Variation for the variable exposed in relation to scats' presence (1) or absence (0); b) Variation for the variable speed according to scats' presence (1) or absence (0); c) Relative frequency of the presence (1) or absence (0) of Alder for places of scats' presence (1) or absence (0).

Table 13 - Best models (ΔAIC < 2) for prediction of the Pyrenean desman scats' presence using 2014+2015 data with “Discrete Sites” as absence points obtained after the AIC-based model selection. Best models are in bold and underlined.

Component models:					
	Df	**logLik**	**AIC**	**ΔAIC**	**Weight**
<u>Exposed + Speed</u>	**<u>6</u>**	**<u>-173.09</u>**	**<u>358.18</u>**	**<u>0.00</u>**	**<u>0.73</u>**
<u>Musk + Exposed + Speed</u>	**<u>7</u>**	**<u>-173.07</u>**	**<u>360.14</u>**	**<u>1.95</u>**	**<u>0.27</u>**
Exposed	5	-202.62	415.25	57.06	0.00
Musk + Exposed	6	-202.42	416.83	58.65	0.00
Musk + Speed	6	-303.28	618.56	260.38	0.00
Speed	5	-307.26	624.53	266.34	0.00
Musk	5	-340.16	690.31	332.13	0.00
(Null)	4	-342.07	692.13	333.95	0.00

Table 14 - Output for the average model of the best models resultant of the GLMM for scats' presence using 2014+2015 data with “Discrete Sites” as absence points. Significant results in bold.

Model-averaged coefficients					
	Estimate	**Std. Error**	**Adjusted SE**	**z value**	**Pr (> |z|)**
(Intercept)	2.28278	0.69647	0.69832	3.269	**0.001**
Exposed	-6.22258	0.73464	0.73659	8.448	**<0.001**
Speed	0.95578	0.24180	0.24244	3.942	**<0.001**
Musk 1	-0.02349	0.20773	0.20826	0.113	0.910

Table 15 - Relative importance (RI) from model-averaging of the GLMM for scats' presence using 2014+2015 data with "Discrete Sites" as absence points.

Relative variable importance			
	Exposed	**Speed**	**Musk**
Importance	1	1	0.27

Table 16 - Best models (ΔAIC < 2) for prediction of the Pyrenean desman scats' presence using 2014+2015 data with “Random Sites” as absence points obtained after the AIC-based model selection. Best models are in bold and underlined.

Component models:					
	Df	**logLik**	**AIC**	**ΔAIC**	**Weight**
<u>Musk + Exposed + Speed</u>	**<u>6</u>**	**<u>-203.82</u>**	**<u>419.64</u>**	**<u>0.00</u>**	**<u>0.97</u>**
Exposed + Speed	5	-208.42	426.83	7.19	0.03
Musk + Exposed	5	-258.06	526.12	106.48	0.00
Exposed	4	-268.02	544.05	124.41	0.00
Speed	4	-405.28	818.55	398.91	0.00
Musk + Speed	5	-405.15	820.30	400.66	0.00
Musk	4	-466.27	940.54	520.90	0.00
(Null)	3	-468.19	942.37	522.74	0.00

Table 17 - Output for the average model of the best models resultant of the GLMM for scats' presence using 2014+2015 data with “Random Sites” as absence points. Significant results in bold.

Model-averaged coefficients					
	Estimate	**Std. Error**	**Adjusted SE**	**z value**	**Pr (> |z|)**
(Intercept)	1.2802	0.5936	0.5946	2.741	0.055
Exposed	-7.1302	0.6555	0.6566	10.859	**<0.001**
Speed	1.3229	0.2344	0.2348	5.634	**<0.001**
Musk 1	0.8347	0.4201	0.4207	1.984	**0.047**

Table 18 - Relative importance (RI) from model-averaging of the GLMM for scats' presence using 2014+2015 data with "Random Sites" as absence points.

Relative variable importance			
	Exposed	**Speed**	**Musk**
Importance	1	1	0.97

3.2.2 Scats' abundance

Analyses of the 2015data

For data using "Discrete Sites" as absence points, AIC-based model selection found two best models (Table 19) for predicting the Pyrenean desman scats' abundance ("Nspraints"), with the null model not being among the best models ($\Delta AIC=665.18$). The variables exposed (Figure 14, a)) and speed (Figure 14, c)) were present in both models so they had the highest importance (RI for predictors=1) while the variable musk (only present in one of the models) (Figure 14, b)) revealed lower importance (RI=0.39). Effect sizes of the model averaged predictors (Table 20) indicated a negative effect of the variable exposed in the scats' abundance ($p=<0.001$) while variables speed ($p=<0.001$) and the presence of musk ($p=0.615$) showed a positive association.

For the data using "Random Sites" as absence points, AIC-based model selection also found only one best model (Table 22) and the null model was not part of the best models ($\Delta AIC=1636.61$). All the variables included in the model showed high importance: alder (Figure 15, a); RI=1), exposed (Figure 15, c); RI=1), musk (Figure 15, d); RI=1), speed (Figure 15, e); RI=1) and bank or bed (Figure 15, b); RI=0.96). Exposed and the category riverbed (bank or bed 2) showed negative effect ($p=<0.001$; $p=0.019$, respectively) on scats' abundance while water speed ($p=<0.001$), presence of musk ($p=<0.001$), and presence of alder nearby ($p=0.178$) presented positive association with the response variable in test (Table 23).

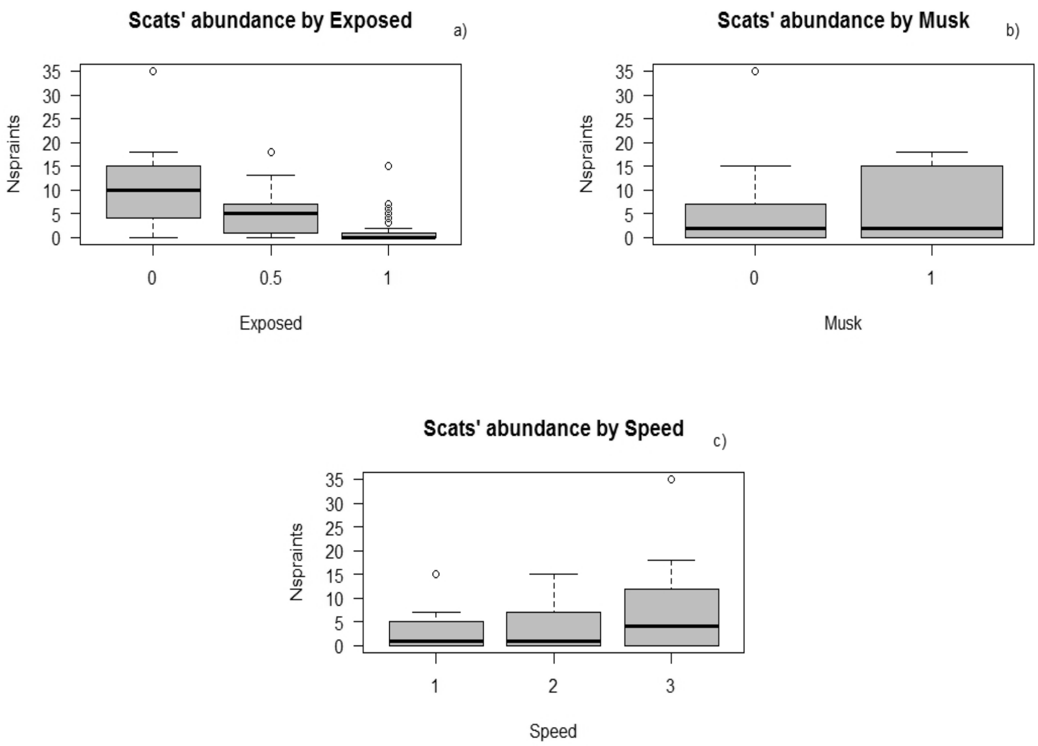


Figure 13 - Data exploration of the response variable: scats' abundance ("Naspraints") in relation to the variables: Exposed, Musk and Speed, integrated in the model using 2015 data with "Discrete Sites" as absence points. a) variation of the abundance of scats in relation to the exposed categories (0- non-exposed; 0.5- partially exposed; 1 – exposed; b) variation of the abundance of scats in relation to the presence (1) or absence (0) of musk; c) variation of the abundance of scats in relation to the different categories of speed (1- null/almost null; 2- weak; 3- medium/strong).

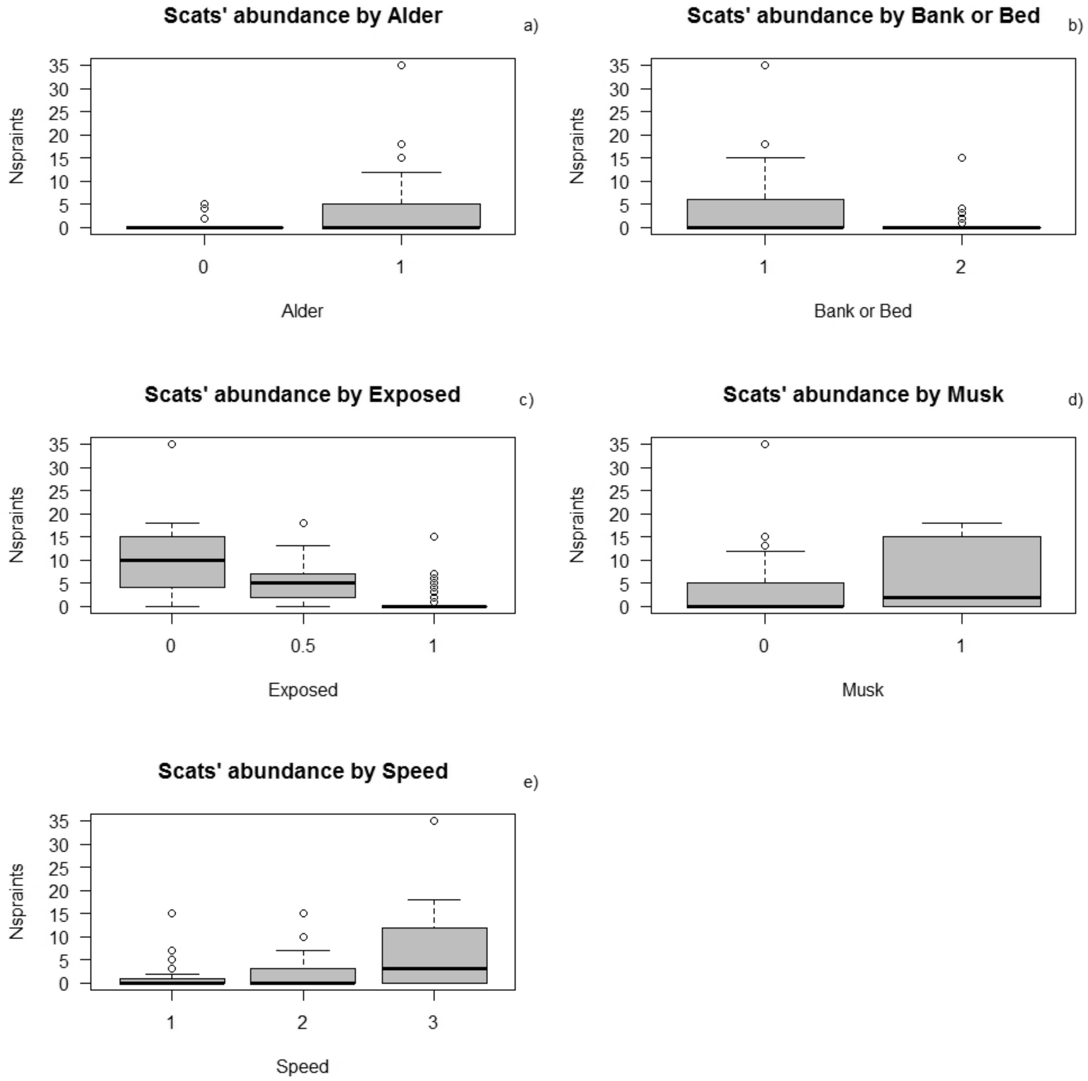


Figure 14 - Data exploration of the response variable: scats' abundance ("Naspraints") in relation to the variables: Alder, Bank or Bed, Exposed, Musk and Speed, integrated in the model using 2015 data with "Random Sites" as absence points. a) variation of the abundance of scats in relation to the alder presence (1) or absence (0); b) variation of the abundance of scats in relation to the place where it is located (1- bank; 2- riverbed); c) variation of the abundance of scats in relation to the exposed categories (0- non-exposed; 0.5- partially exposed; 1 – exposed; d) variation of the abundance of scats in relation to the presence (1) or absence (0) of musk; e) variation of the abundance of scats in relation to the different categories of speed (1- null/almost null; 2- weak; 3- medium/strong).

Table 19 - Best models (ΔAIC < 2) for prediction of the Pyrenean desman scats' abundance using 2015 data with "Discrete Sites" as absence points obtained after the AIC-based model selection. Best models are in bold and underlined.

Component models:					
	Df	**logLik**	**AIC**	**ΔAIC**	**Weight**
Exposed + Speed	5	-637.21	1284.41	0.00	0.61
Musk + Exposed + Speed	6	-636.67	1285.33	0.92	0.39
Musk + Exposed	5	-708.74	1427.47	143.06	0.00
Exposed	4	-711.12	1430.23	145.82	0.00
Musk + Speed	5	-879.96	1769.92	485.51	0.00
Speed	4	-885.88	1779.76	495.35	0.00
Musk	4	-969.30	1946.61	662.20	0.00
(Null)	3	-971.79	1949.59	665.18	0.00

Table 20 - Output for the average model of the best models resultant of the GLMM for scats' abundance using 2015 data with "Discrete Sites" as absence points. Significant results in bold.

Model-averaged coefficients					
	Estimate	**Std. Error**	**Adjusted SE**	**z value**	**Pr (> |z|)**
(Intercept)	-0.3180	0.3386	0.3400	0.935	0.350
Exposed	-2.5125	0.1480	0.1486	16.910	**<0.001**
Speed	0.8569	0.1162	0.1167	7.346	**<0.001**
Musk 1	0.0606	0.1202	0.1205	0.503	0.615

Table 21 - Relative importance (RI) from model-averaging of the GLMM for scats' abundance using 2015 data with "Discrete Sites" as absence points.

Relative variable importance			
	Exposed	**Speed**	**Musk**
Importance	1	1	0.39

Table 22 - Best models (ΔAIC < 2) for prediction of the Pyrenean desman scats' abundance using 2015 data with "Random Sites" as absence points obtained after the AIC-based model selection. Best models are in bold and underlined.

Component models:					
	Df	**logLik**	**AIC**	**ΔAIC**	**Weight**
Alder + Bank or Bed + Musk + Exposed + Speed	**7**	**-647.23**	**1308.46**	**0.00**	**0.96**
Alder + Musk + Exposed + Speed	6	-651.35	1314.70	6.24	0.96
Alder + Bank or Bed + Musk + Exposed	6	-671.53	1355.07	46.61	0.00
Alder + Musk + Exposed	5	-676.57	1363.14	54.68	0.00
Alder + Bank or Bed + Exposed + Speed	6	-675.72	1363.44	54.98	0.00
Alder + Exposed + Speed	5	-685.86	1381.71	73.26	0.00
Alder + Bank or Bed + Exposed	5	-721.77	1453.55	145.09	0.00
Alder + Exposed	4	-731.28	1470.56	162.10	0.00
Bank or Bed + Musk + Exposed + Speed	6	-782.46	1576.93	268.47	0.00
Musk + Exposed + Speed	5	-790.15	1590.29	281.83	0.00
Bank or Bed + Exposed + Speed	5	-810.36	1630.71	322.25	0.00
Exposed + Speed	4	-826.88	1661.76	353.31	0.00
Bank or Bed + Musk + Exposed	5	-857.95	1725.91	417.45	0.00
Musk + Exposed	4	-867.32	1742.65	434.19	0.00
Bank or Bed + Exposed	4	-904.34	1816.68	508.22	0.00
Exposed	3	-920.14	1846.28	537.82	0.00
Alder + Bank or Bed + Musk + Speed	6	-984.14	1980.28	671.82	0.00
Alder + Musk + Speed	5	-1001.97	2013.94	705.48	0.00
Alder + Bank or Bed + Speed	5	-1009.67	2029.35	720.89	0.00
Alder + Speed	4	-1018.63	2045.27	736.81	0.00
Alder + Bank or Bed + Musk	5	-1107.29	2224.57	916.11	0.00
Alder + Bank or Bed	4	-1110.28	2228.56	920.11	0.00
Alder + Musk	4	-1125.47	2258.93	950.47	0.00
Alder	3	-1126.58	2259.17	950.71	0.00
Bank or Bed + Musk + Speed	5	-1208.83	2427.67	1119.21	0.00
Bank or Bed + Speed	4	-1240.30	2488.60	1180.14	0.00
Musk + Speed	4	-1255.06	2518.12	1209.66	0.00
Speed	3	-1271.45	2548.89	1240.44	0.00
Bank or Bed + Musk	4	-1422.60	2853.19	1544.74	0.00
Bank or Bed	3	-1427.38	2860.76	1552.30	0.00
Musk	3	-1468.82	2943.64	1635.18	0.00
(Null)	2	-1470.53	2945.07	1636.61	0.00

Table 23 - Output for the average model of the best models resultant of the GLMM for scats' abundance using 2015 data with "Random Sites" as absence points. Significant results in bold.

Model-averaged coefficients					
	Estimate	**Std. Error**	**Adjusted SE**	**z value**	**Pr (> |z|)**
(Intercept)	-0.30779	0.39553	0.39676	0.776	0.438
Exposed	-2.41119	0.11288	0.11323	21.294	**<0.001**
Speed	0.58646	0.08604	0.08631	6.795	**<0.001**
Alder 1	0.27620	0.20428	0.20492	1.348	0.178
Musk 1	1.02273	0.13320	0.13361	7.654	**<0.001**
Bank or Bed 2	-0.35208	0.14909	0.14944	2.356	**0.019**

Table 24 - Relative importance (RI) from model-averaging of the GLMM for scats' abundance using 2015 data with "Random Sites" as absence points.

Relative variable importance					
	Alder	**Exposed**	**Musk**	**Speed**	**Bank or Bed**
Importance	1	1	1	1	0.96

Analyses of the 2014+2015 data

Using "Discrete Sites" as absence points, AIC-based model selection found two statistically best models (Table 25). The null model was not among the best models (ΔAIC=1007.77). The variables bank or bed (Figure 16, a)), exposed (Figure 16, b)) and speed (Figure 16, d)) showed high importance in explaining the scats' abundance (RI of all the predictors=1), unlike the variable musk (Figure 16, c)); RI=0.57). Effect sizes of the model averaged predictors indicated that riverbed (bank or bed 2) and exposed negatively affected the scats' abundance (p=<0.001 for both) while speed (p=<0.001) and presence of musk (p= 0.107) are positively associated with it (Table 26).

For the data using "Random Sites" as absence points, AIC-based model selection found one statistically best model (Table 28) also for predicting the Pyrenean desman scats' abundance ("Nspraints") with the null model not being as part of the best models (ΔAIC=1997.12). All the variables included in the best model showed high importance: exposed (Figure 17, b); RI=1), speed (Figure 17, d); RI=1) bank or bed (Figure 17, a); RI=1) and musk (Figure 17, c); RI=1). Exposed (p=<0.001) and riverbed (bank or bed 2; p=<0.001) had a negative effect on scats' abundance whereas speed and presence of musk presented a positive effect (p=<0.001, for both) (Table 29).

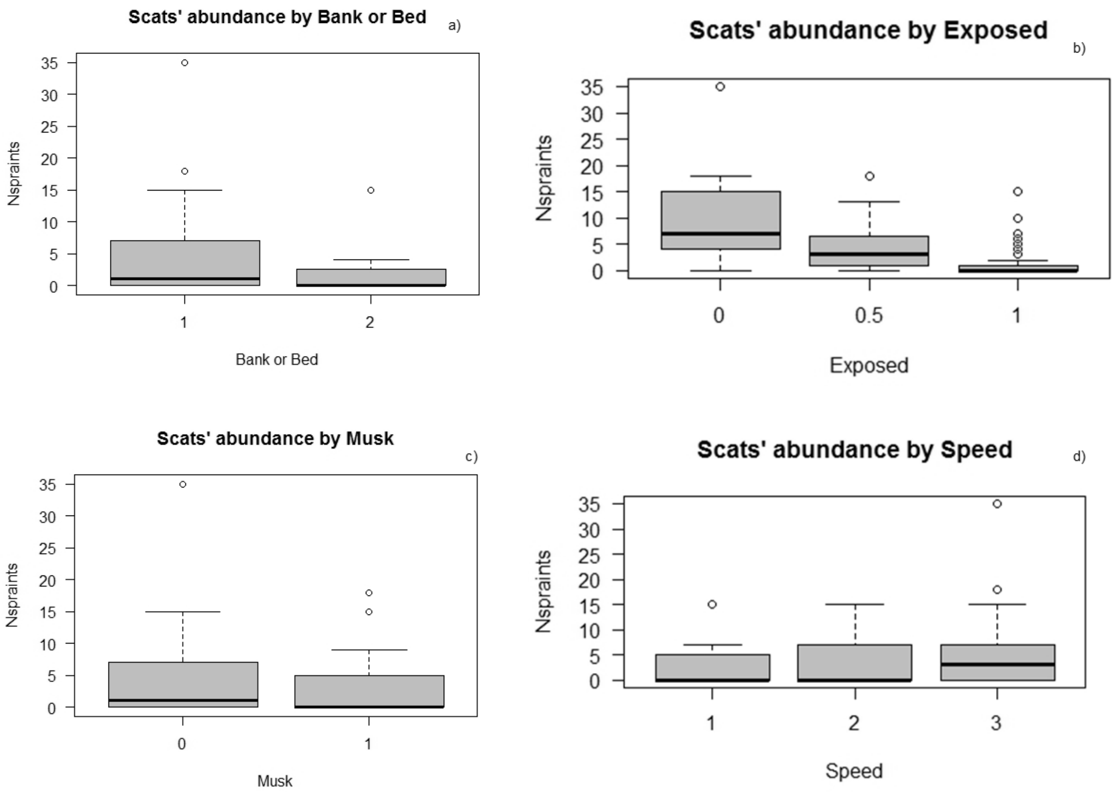


Figure 15 - Data exploration of the response variable: scats' abundance ("Nasprainst") in relation to the variables: Bank or Bed, Exposed, Musk and Speed, integrated in the model using 2014+2015 data with "Discrete Sites" as absence points. a) Variation of the abundance of scats according to the place where it is located(1- bank; 2- riverbed); b) Variation of the abundance of scats in relation to the exposed categories (0- non-exposed; 0.5- partially exposed; 1 – exposed; c) Variation of the abundance of scats in relation to the presence (1) or absence (0) of musk; d) Variation of the abundance of scats in relation to the different categories of speed (1- null/almost null; 2- weak; 3- medium/strong).

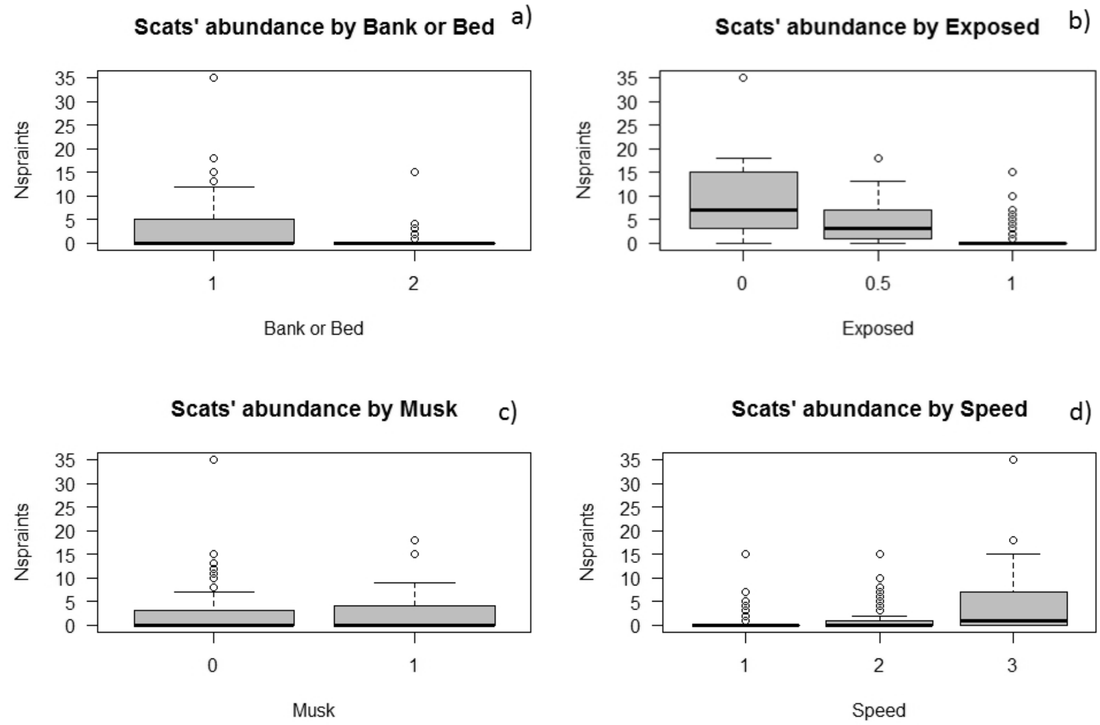


Figure 16 - Data exploration of the response variable: scats' abundance ("Naspraints") in relation to the variables: Bank or Bed, Exposed, Musk and Speed, integrated in the model using 2014+2015 data with "Random Sites" as absence points. a) Variation of the abundance of scats according to the place where it is located(1- bank; 2- riverbed); b) Variation of the abundance of scats in relation to the exposed categories (0- non-exposed; 0.5- partially exposed; 1 – exposed; c) Variation of the abundance of scats in relation to the presence (1) or absence (0) of musk; d) Variation of the abundance of scats in relation to the different categories of speed (1- null/almost null; 2- weak; 3- medium/strong).

Table 25 - Best models (ΔAIC < 2) for prediction of the Pyrenean desman scats' abundance using 2014+2015 data with "Discrete Sites" as absence points obtained after the AIC-based model selection. Best models are in bold and underlined.

Component models:					
	Df	**logLik**	**AIC**	**ΔAIC**	**Weight**
Bank or Bed + Musk + Exposed + Speed	**8**	**-836.69**	**1689.37**	**0.00**	**0.57**
Bank or Bed + Exposed + Speed	**7**	**-837.96**	**1689.92**	**0.54**	**0.43**
Exposed + Speed	6	-859.89	1731.77	42.40	0.00
Musk + Exposed + Speed	7	-859.54	1733.07	43.70	0.00
Bank or Bed + Musk + Exposed	7	-930.19	1874.39	185.01	0.00
Bank or Bed + Exposed	6	-933.15	1878.30	188.93	0.00
Musk + Exposed	6	-949.17	1910.33	220.96	0.00
Exposed	5	-950.48	1910.96	221.58	0.00
Bank or Bed + Musk + Speed	7	-1125.36	2264.72	575.35	0.00
Bank or Bed + Speed	6	-1126.59	2265.18	575.80	0.00
Musk + Speed	6	-1222.00	2456.01	766.63	0.00
Speed	5	-1231.13	2472.26	782.88	0.00
Bank or Bed	5	-1264.92	2539.84	850.47	0.00
Bank or Bed + Musk	6	-1264.89	2541.79	852.41	0.00
Musk	5	-1340.03	2690.06	1000.69	0.00
(Null)	4	-1344.57	2697.14	1007.77	0.00

Table 26 - Output for the average model of the best model resultant of the GLMM for scats' abundance using 2014+2015 data with "Discrete Sites" as absence points. Significant results in bold.

Model-averaged coefficients					
	Estimate	**Std. Error**	**Adjusted SE**	**z value**	**Pr (> |z|)**
(Intercept)	-0.4043	0.3335	0.3344	1.209	0.227
Exposed	-2.5473	0.1369	0.1372	18.564	**<0.001**
Speed	0.8882	0.1018	0.1021	8.700	**<0.001**
Musk 1	0.2355	0.1458	0.1462	1.610	0.107
Bank or Bed 2	-1.7895	0.3140	0.3149	5.683	**<0.001**

Table 27 - Relative importance (RI) from model-averaging of the GLMM for scats' abundance using 2014+2015 data with "Discrete Sites" as absence points.

Relative variable importance				
	Exposed	**Speed**	**Bank or Bed**	**Musk**
Importance	1	1	1	0.57

Table 28 - Best models (ΔAIC < 2) for prediction of the Pyrenean desman scats' abundance using 2014+2015 data with "Random Sites" as absence points obtained after the AIC-based model selection. Best models are in bold and underlined.

Component models:					
	Df	**logLik**	**AIC**	**ΔAIC**	**Weight**
<u>Bank or Bed + Musk + Exposed + Speed</u>	**<u>7</u>**	**<u>-1113.22</u>**	**<u>2240.45</u>**	**<u>0.00</u>**	**<u>1</u>**
Bank or Bed + Exposed + Speed	6	-1127.03	2266.05	25.60	0.00
Exposed + Speed	6	-1136.24	2284.48	44.04	0.00
Musk + Exposed + Speed	5	-1158.84	2327.69	87.24	0.00
Bank or Bed + Musk + Exposed	6	-1218.31	2448.61	208.17	0.00
Bank or Bed + Exposed	5	-1239.18	2488.37	247.92	0.00
Musk + Exposed	5	-1253.10	2516.20	275.76	0.00
Exposed	4	-1279.74	2567.48	327.03	0.00
Bank or Bed + Musk + Speed	6	-1700.79	3413.58	1173.13	0.00
Bank or Bed + Speed	5	-1751.12	3512.25	1271.80	0.00
Musk + Speed	5	-1794.71	3599.43	1358.98	0.00
Speed	4	-1817.75	3643.49	1403.05	0.00
Bank or Bed	5	-2026.83	4063.66	1823.21	0.00
Bank or Bed + Musk	4	-2037.53	4083.06	1842.61	0.00
Musk	4	-2110.99	4229.98	1989.54	0.00
(Null)	3	-2115.78	4237.57	1997.12	0.00

Table 29 - Output for the average model of the best models resultant of the GLMM for scats' abundance using 2014+2015 data with "Random Sites" as absence points. Significant results in bold.

Model-averaged coefficients					
	Estimate	**Std. Error**	**Adjusted SE**	**z value**	**Pr (> |z|)**
(Intercept)	-0.04718	0.39529	0.39595	0.119	0.905
Musk 1	0.54474	0.10446	0.10464	5.206	**<0.001**
Exposed	-2.44949	0.08465	0.08480	28.887	**<0.001**
Bank or Bed 2	-0.81753	0.12513	0.12534	6.522	**<0.001**
Speed	0.63567	0.06534	0.06545	9.712	**<0.001**

Table 30 - Relative importance (RI) from model-averaging of the GLMM for scats' abundance using 2014+2015 data with "Random Sites" as absence points.

Relative variable importance				
	Exposed	**Speed**	**Bank or Bed**	**Musk**
Importance	1	1	1	1

3.3 General Habitat Characterization

The effect of the explanatory variables in the KAI, used to understand which variables determine higher scats' abundance per km of transect resulted in three best models after the AIC-based model selection (Table 31), with the null model not being among the best models (ΔAIC=29.36). The variables: %spraintability (Figure 18, b)); RI=1), %pool (Figure 18, e); RI=0.88), %coverage (Figure 18, a); RI=0.8) and mwidth (Figure 18, d); RI=0.8) were all present in all of the best models with only %spraintability showing high importance in explaining scats' abundance per km of transect. The second and third best models included the variables speed (Figure 18, c); RI=0.41) and %riffle (Figure 18, f); RI=0.38) which showed the lowest importance.

Effect sizes of the model averaged predictors showed that only high spraintability (spraintability 5) had an almost significant effect in the response variable (p=0.0618) showing a positive effect (Table 38).

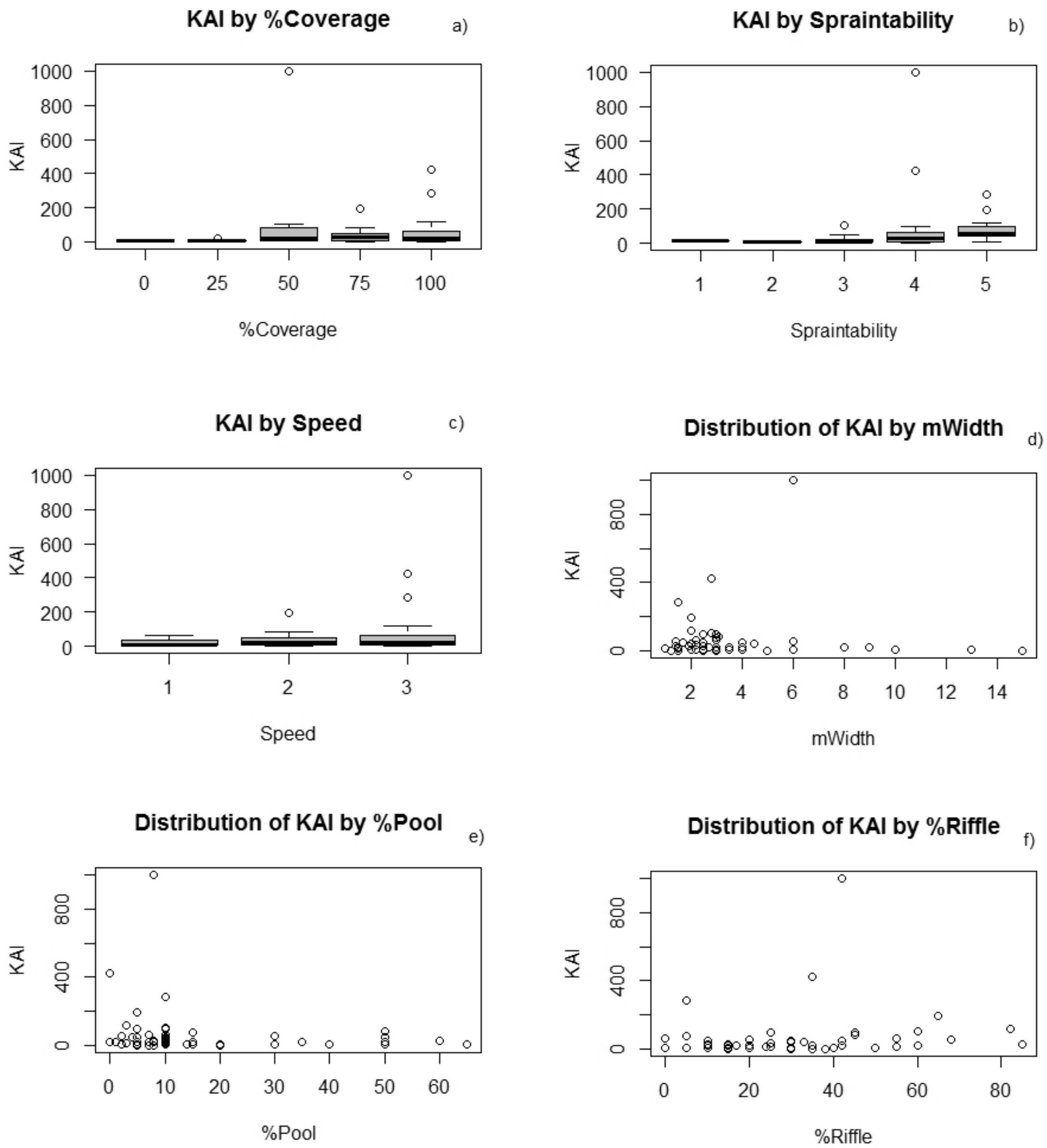


Figure 17 - Data exploration of the response variable: kilometric abundance index (KAI) in relation to the variables:%coverage, spraintability, speed, mwidth, %pool and %riffle integrated in the model used to predict the abundance of Pyrenean desman scats per km of transect. Graphic a) represents the boxplot with the KAI in relation to % of coverage (0%; 25%; 50%; 75%; 100%); Graphic b) represents the boxplot with the KAI in relation to spraintability (1: <5%; 2: 5%-19%; 3: 20%-39%; 4: 40%-69%; 5: 70%-100%); Graphic c) represents boxplot with the KAI in relation to the different categories of speed (1- null/almost null; 2- weak; 3- medium/strong); Graphic d) represents a scatterplot with the KAI in relation to the numeric variable mWidth; Graphics e) and f) represents a scatterplot with the KAI in relation to the percentages attributed to the variables pool and riffle.

Table 31 - Best models (ΔAIC < 2) for prediction of the Pyrenean desman KAI obtained after AIC-based model selection. All the models considered for model selection are in Apendix 1, Table 38.

Component models:					
	Df	**logLik**	**AIC**	**ΔAIC**	**Weight**
%Coverage + Spraintability + mWidth + %Pool	12	-49.77	123.55	0.00	0.26
%Coverage + Speed + Spraintability + mWidth + %Pool	14	-48.03	124.07	0.52	0.2
%Coverage + Spraintability + mWidth + %Pool + %Riffle	13	-49.56	125.13	1.58	0.12

Table 32 - Output for the average model of the best models resultant of the LM for the KAI. Almost significant results underlined.

Model-averaged coefficients					
	Estimate	**Std. Error**	**Adjusted SE**	**z value**	**Pr (> |z|)**
(Intercept)	0.806316	1.301493	1.325812	0.608	0.5431
mWidth	-0.40265	0.042683	0.043627	0.923	0.3560
Spraintability2	-0.050686	0.837625	0.860631	0.059	0.9530
Spraintability3	0.295776	0.768896	0.789900	0.374	0.7081
Spraintability4	1.028074	0.794614	0.815734	1.260	0.2076
Spraintability5	1.555030	0.811526	0.832729	1.867	<u>0.0618</u>
%Pool	-0.012259	0.007792	0.007941	1.544	0.1226
Coverage 25%	1.314898	1.022773	1.037928	1.267	0.2052
Coverage 50%	1.474132	1.006924	1.019470	1.446	0.1482
Coverage 75%	1.123617	0.883668	0.897651	1.252	0.2107
Coverage 100%	0.936704	0.833715	0.848990	1.103	0.2699
Speed 2	-0.011603	0.370980	0.381291	0.030	0.9757
Speed 2	0.085889	0.379899	0.389921	0.220	0.8257
%Riffle	0.001562	0.004501	0.004590	0.340	0.7337

Table 33 - Relative importance (RI) from model-averaging of the LM for for the KAI data.

Relative variable importance						
	Spraintability	**%Pool**	**mWidth**	**%Coverage**	**Speed**	**%Riffle**
Importance	1	0.88	0.80	0.80	0.41	0.38

4 Discussion

4.1 General discussion

In this study we verified that at a small-site scale, Pyrenean desman selected as habitat requirements non-exposed sites, preferably at riverbanks near locations of high river flow. Alder presence showed high importance in explaining the scat deposition despite the non-significance results we obtained which contradicted the expected. Presence of musk covering the marking site revealed inconsistent importance and significance making it difficult to determine if the variable is in fact important for Pyrenean desman selection.

The negative selection of exposed sites supports the theories that sheltered places constitute a key resource for Pyrenean desman, not just for individual protection from predators and for resting behaviour but also for indirect communication of resources availability between the species (Melero et al., 2012). They also support the lower probability of scats' detection expected for exposed sites due to the fast degradation caused by the atmospheric agents (i.e. sun, rain, variation in water flow) (Queiroz et al., 1998).

Permanent fast flowing water is referred as one of the main important habitat characteristics required for Pyrenean desman's presence (Marcos, 2004; Queiroz et al., 1998). That is consistent with our results for speed, which indicate that Pyrenean scats' presence increases with water speed (medium/strong). The preference for high water speed is likely related to the abundance of aquatic macroinvertebrates (main prey of *Galemys pyrenaicus*). Presence of aquatic macroinvertebrates is greater in these fast-flowing stretches due to a low degree of sedimentation (Biffi et al., 2016; Charbonnel et al., 2015) which provides high levels of dissolved oxygen and food particles. The presence of scats near these places possibly signals the availability of food resources.

Queiroz et al., 1998 studies on Pyrenean desman's habitat selection for scat deposition reported high number of scats detected in the riverbed rather than in the bankside. On the other hand, more recent studies: Biffi et al., 2016; Pedroso & Chora, 2014 referred a preferable selection by Pyrenean desman of the substrate in the riverbanks. Our study contradicted the results obtained by Queiroz et al., 1998 and supported the recent ones indicating a significantly lower scat deposition in the riverbed. Differences between our results and the obtained by Queiroz et al., 1998 were probably associated with the variation on scats' detectability between the two studies due to contrasting weather conditions, habitat structure of the places sampled and observer's bias (which can influence the facility of scat's detection) (Charbonnel et al., 2015).

Lower selection of the substrate in the riverbed for scats' deposition could be explained by the probability of finding less abundant emergent items and variability in types of substrate in comparison to the riverbanks. The substrate variety in the riverbanks allows high availability of crevices and cavities which Pyrenean desmans uses as resting sites instead of digging (Melero et al., 2012). Banks also present high density of riparian vegetation with some exposed roots creating natural cavities also considered as good shelters. Since shelters are considered as minimum requirement for the species presence (ICN, 2005) it seems possible that the low variability of the emergent substrate in the riverbed decreases the selection in favour of the riverbanks.

Alder presence is referred as very important to *Galemys pyrenaicus* because apart from providing shelter it is a deciduous tree very common among the riparian vegetation which leaves create a heavy layer on the soil retaining the rainwater and preventing changes in the soil and in the water quality (Ramalhinho & Tavares, 1989). The aquatic macroinvertebrates are very sensitive to changes in water conditions and since Pyrenean desman depends on them to feed it seems that the existence of a significant positive relation between the two is possible. Our results indicate a high importance of the variable in determining the scat deposition, confirming the expected, despite the non-significant effect we obtained.

As referred above, results for musk presence are inconsistent. Based on the field observations and on descriptive statistics, we expected a negative effect of the variable musk on scats' presence/abundance which was contradicted by the significantly positive effects obtained. Musk is mostly found covering exposed substrate where it is frequent to find scats from *Cinclus cinclus* (which can be very similar to Pyrenean desmans' scats). Since not all the scats were genetically confirmed until the present data, it is possible that some are false presences.

For the larger scale evaluation of the habitat preferences only spraintability seemed to have values closer to the significance indicating that high of spraintability explained the higher abundance of scats' found per kilometre of transect. In other words, transects with high substrate heterogeneity are possibly highly selected by Pyrenean desman. This is consistent with the habitat selection studies developed by (Biffi et al., 2016; Charbonnel et al., 2014, 2015; Queiroz et al., 1998).

The analyses considered in this study tried to describe Pyrenean desman habitat preferences using two types of scale: a small-site scale – describing the differences for Pyrenean desman occurrence at a small-site characterization (~0.5 m^2) and a larger scale – describing differences at the transect level. This was of great importance to account given the fact that there is an urgent need to improve the studies on Pyrenean desmans' habitat descriptors avoiding finer habitat associations based on grid cells with coarse resolution that cannot account for the spatial structure of the stream network and the species' scale resolution (Biffi et al., 2016) .

4.2 Results from Scats' Presence

Considering the results for scat deposition in 2015, it is clear that Pyrenean desman was significantly more present in less exposed substrate. This indicates that exposed sites are less selected by *Galemys pyrenaicus* for leaving their marks. However, when using "Random Sites" two other variables were included in the model: bank or bed and stream speed which showed an effect on scats' presence. Riverbed (bank or bed 2) had a negative effect on scats' presence indicating that Pyrenean desman avoid the riverbed, selecting the bankside for scat deposition. Scats' presence was also associated with higher stream speed near the substrate. Musk and bank or bed variables were excluded from the model with "Discrete Sites" due to the lack of convergence obtained when these variables were included.

Alder showed high importance in determining the presence of scat deposition although we obtained no significant effect when the variable was included in both analyses for 2015 data using the two different types of absence points. We were not expecting the non-significance of this variable but the fact that it was included in the best model with high importance value indicates that alder explain the presence of scat deposition.

There were some differences in the variables selected by the models depending on the type of absence data used: "Discrete Sites" or "Random Sites". This is probably related to the variation that each method captures from the sites. "Discrete Sites" were selected closer to the "True Sites" (where the scat was present), and may present a lower variability. Also, we cannot exclude that some of the places selected as absence points are actually suitable for scats' deposition, but were not chosen just by chance – certainly Desmans will not deposit scats in all possible available places. Additionally, a possible spatial autocorrelation could also be explaining these results; however we did not test this hypothesis. "Random Sites", on the other hand, were randomly collected along the river, being less affected by the possibility of similarity with "True Sites". Also, the methodology applied for assessing this type of absence points allowed to cover a more extensive area of the river, making it possible to represent more precisely the river heterogeneity and to detect more differences in habitat availability.

In order to increase the sample size – even at the cost of using fewer variables - we assessed the data from both years (2014 and 2015). Here, the variables considered were identical for the models using "Discrete Sites" or "Random Sites" as absence points: musk, exposed and speed. Bank or bed was excluded for both models due to lack of convergence. In this particular case, model non-convergence is probably related to lack of variance in the explanatory variable with one of the predictors perfectly describing the criterion in study. Exposed showed similar importance and significance as in the 2015 only analysis. Although variable speed was included in both models referring to the different year's datasets, we found differences for the significance value (non-significant for 2015 data and positively significant when added the 2014 data). These differences could be explained by the increase in the sampled number which enhanced the variability of the predictors within the variable. With respect to "Random Sites" data and similarly to the results obtained for 2015 it seemed consensual that Pyrenean desman preferably selected places of high water speed and avoid exposed sites.

4.3 Results from Scats' Abundance

Considering the data from 2015, the results indicate that Pyrenean desman scats' appeared particularly associated with less exposed sites and with higher water speed, and in a similar way for the two types of absent points considered. Musk was not included in the "Discrete Sites" model, but it was included in "Random Sites" models, with high desman scats' abundance in the substrate covered with musk. This model also indicates that lower scats' abundance was found in riverbed rather than riverbanks. Alder presence showed again non-significant effect on the scats' abundance, however the high importance value of the variables suggested again, that alder has some relevance for scat deposition behaviour in desman species.

Due to the lack of convergence, alder and bank or bed variables were also excluded from the model with "Discrete Sites". Differences obtained in the variables affecting scats' abundance depending on the type of absence data used are related to the method applied for the data collection, again. However, there are notable minor differences between methodologies using abundance as response variable rather than the dichotomous.

For the years 2014 +2015 there were also no differences between models using "Discrete Sites" or "Random Sites". The variables considered in the models of both methods were: musk, exposed, speed and bank or bed. There was only one difference between 2014+2015 and 2015 only for the "Discrete Sites" analysis, because 'bank or bed' had to be excluded from the later analysis due to lack of convergence. As in the analysis for the chosen/not chosen, Pyrenean desmans avoid exposed substrates and preferably select high water speed. Riverbed (bank or bed 2) was also avoided for scat deposition, indicating a preferable selection for riverbanks as referred in more recent studies (Biffi et al., 2016; Pedroso & Chora, 2014).

In general, we can say that Pyrenean desman scats were found preferably in non-exposed substrate, near locations of high river flow, in riverbanks and revealed some preference for substrate covered with musk.

4.4 General habitat characterization

This analysis adjusted to a larger scale was used to check what general habitat variables have an effect on the abundance of Pyrenean desman scats' found per km of transect prospected between the sites where the presence of Pyrenean desman was detected. The most important variables considered in the model were: the percentage of coverage, spraintability, riverbed width and the percentage of pool, but from these variables only spraintability seemed to have an importance in determining more scats' abundance indicating that transects with high substrate heterogeneity are preferred by Pyrenean desman. These results are in accordance with studies from Biffi et al., 2016 where it is referred that the species seemed to occupy sites with abundant emergent items and high heterogeneity of river substrates and shelters used as resting sites. Non-significant results for the other variables are probably justified by the low variety found between the sites considered. These sites were all confirmed as sites of Pyrenean desman's presence and because of that, all the variables tested presented many similarities between them.

4.5 Data limitations

We outline here some of the limitations of the study. One of the most limiting problems was the high number of missing values which compromised the consideration of some variables for the models. This leaves aside the possibility of finding other important variables affecting the habitat selection. It is important to improve the methodology applied in field considering the hypothesis of using more efficient and practical forms.

Also, we needed to considered scats which identification was only based on the morphology and in the percentage of certainty of being *Galemys* attributed in field by the most experienced observer. Despite the reasonable degree of confidence we need to ensure the genetic confirmation of all the scats considered in order to avoid false positives that could overestimate the number of presences.

Another limiting factor to refer was the lack of convergence verified for some models when some variables were included. This lack of convergence means that the coefficients are not meaningful because the iterative process was unable to find solutions and it did not allow me to consider the same number of starting variables to all the models. In order to avoid these problems in future the use of more quantitative variables should be considered rather than categorical to increase variance between the variable predictors and avoid undefined results due to zero cell counts when applying many dummy variables to models with binomial distribution. The number of zeros used in Poisson distribution should also be controlled.

4.6 Conclusion

At a larger scale, the use of local habitat by the Pyrenean desman appears to be driven by higher spraintability with transects with abundant emergent items and greater percentage of substrate heterogeneity preferably selected.

At a small-site scale the species seemed to select non-exposed sites, preferably at riverbanks near locations of high river flow.

Higher heterogeneity of the river substrate allowed the high availability of crevices and cavities which Pyrenean desmans uses as resting sites instead of digging (Melero et al., 2012). These resting sites are usually formed by emergent rocks or exposed roots from the riparian vegetation. They are non-exposed and are mainly located along the riverbanks (where usually more substrate variety is found). The possibility to escape from predators as well as the possible important role of resting sites for the social organization of Pyrenean desman seem to justify this habitat selection for scat deposition.

The preference for high water speed is likely related to the abundance of aquatic macroinvertebrates (main prey of *Galemys pyrenaicus*). Presence of aquatic macroinvertebrates is greater in this fast-flowing stretches due to lack of sedimentation (Biffi et al., 2016; Charbonnel et al., 2015) which provides high levels of dissolved oxygen and food particles.

Our results indicate a general, common behavioural pattern for habitat selection by Pyrenean desman individuals with resting sites, and food availability signalled as key resources for the presence of the study species. In the future, the development of home range occupancy studies and daily activity patterns on the species will increase our knowledge on the individuals' socio-spatial organization and behaviour and will allow identifying other key habitat parameters for the Pyrenean desman species which will contribute to improve the design of future conservation actions.

For now, it is of great importance to introduce Pyrenean desman to the general public and highlight the need for more conservation actions focused on the quality of aquatic ecosystems and the riparian vegetation. Only by understanding how Pyrenean desman uses the available habitat resources and how the species behaves it will be possible to define more specific conservation measures that promote habitat quality and suitability.

5 References

Ajo, R. S., & Cosío, Á. S. (2009). El Desmán Ibérico (*Galemys pyrenaicus*) en Cantabria. *Locustella, Anuario de La Naturaleza de Cantabria*, *6*, 80–88.

Almeida, D., Barrientos, R., Merino-Aguirre, R., & Angeler, D. G. (2012). The role of prey abundance and flow regulation in the marking behaviour of Eurasian otters in a Mediterranean catchment. *Animal Behaviour*, *84*(6), 1475–1482.

Aymerich, P., Fernández, Á., & Gonsálbez, J. (2013). Conservar al desmán ibérico : un reto pendiente. *Quercus*, *329*, 26–34.

Barton, K. (2014). MuMIn: Multi-model inference. R package version 1.10.0. Available at: http://r-forge.r-project.org/projects/mumin/.

Bates, D., Maechler, M., Bolker, B., & Walker, S. (2014). lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1-7. Available at: http://lme4.r-forge.r-project.org/.

Beira, E. (2014). Preservação patrimonial do vale do Tua. *Dissertação de Mestrado de Engenharia Civil.* Universidade do Minho.

Biffi, M., Charbonnel, A., Buisson, L., Blanc, F., Némoz, M., & Laffaille, P. (2016). Spatial differences across the French Pyrenees in the use of local habitat by the endangered semi-aquatic Pyrenean desman (*Galemys pyrenaicus*). *Aquatic Conservation: Marine and Freshwater Ecosystems*, *26*(4), 761–774.

Cabria, M. T., Rubines, J., Gómez-Moliner, B., & Zardoya, R. (2006). On the phylogenetic position of a rare Iberian endemic mammal, the Pyrenean desman (*Galemys pyrenaicus*). *Gene*, *375*, 1–13.

Caracterização Física | Rota da Terra Fria. (2016). Viagem à Natureza: Rota da Terra

Fria Transmontana. Retrieved 30 May 2016, from http://www.rotaterrafria.com/pages/88.

Castién, E., & Gonsálbez, J. (1995). Diet of *Galemys pyrenaicus* (Geoffroy, 1811) in the North Of the Iberian Peninsula. *Netherlands Journal of Zoology*, *45*(3), 422–430.

Charbonnel, A., Buisson, L., Biffi, M., D'Amico, F., Besnard, A., Aulagnier, S., … Laffaille, P. (2015). Integrating hydrological features and genetically validated occurrence data in occupancy modelling of an endemic and endangered semi-aquatic mammal, *Galemys pyrenaicus*, in a Pyrenean catchment. *Biological Conservation*, *184*, 182–192.

Charbonnel, A., D'Amico, F., Besnard, A., Blanc, F., Buisson, L., Némoz, M., & Laffaille, P. (2014). Spatial replicates as an alternative to temporal replicates for occupancy modelling when surveys are based on linear features of the landscape. *Journal of Applied Ecology*, *51*, 1425–1433.

Chora, S. C., & Quaresma, C. M. (2001). Toupeira-de-água, *Galemys pyrenaicus*: Padrões de ocupação espacial e capacidade de transposição da minihídirca de Fráguas (Rio Paiva, Bacia do Douro). *Congr. Nac. Conser. da Nat. (ICN)*, Lisboa.

Esteban, J. G., & Iglesias, P. A. (2012). Inventario de las poblaciones de *Galemys pyrenaicus* en la cuenca del río Ulla y caracterización de su hábitat. *Xunta de Galicia-Consellería Do Medio Rural E Do Mar*, Galicia.

Fernandes, M., Herrero, J., Aulagnier, S., & Amori, G. (2008). *Galemys pyrenaicus* (Pyrenean Desman). *The IUCN Red List of Threatened Species 2008*. Downloaded on 23 December 2014.

Fernández-López, J., Fernández-González, Á., & Fernández-Menéndez, D. (2014). Confirmación de la depredación de nutria paleártica *Lutra lutra* (Linnaeus, 1758) sobre desmán ibérico *Galemys pyrenaicus* (E. Geoffroy Saint-Hilaire, 1811) mediante el empleo de técnicas moleculares. *Galemys, Spanish Journal of Mammalogy*, *26*(1), 1–4.

Ferreira, M., Filipe, A. F., Bardos, D. C., Magalhães, M. F., & Beja, P. (2016). Modeling stream fish distributions using interval-censored detection times. *Ecology and Evolution*, *6*(15), 5530–5541.

Ferreiro, N. (2007). Caracterização da Qualidade Ecológica do Rio Tua. *Tese de Mestrado Em Hidrobiolgia.* Faculdade de Ciências Da Universidade Do Porto.

Fox, J., Weisberg, S., Adler, D., Bates, D., Baud-Bovy, G., Ellison, S., Firth, D. et al. (2016). car: Companion to Applied Regression. R package version 2.1-3. Available at: https://r-forge.r-project.org/projects/car/.

Gillet, F., Cabria, M. T., Némoz, M., Blanc, F., Fournier-Chambrillon, C., Sourp, E., … Michaux, J. R. (2014). PCR-RFLP identification of the endangered Pyrenean desman, *Galemys pyrenaicus* (Soricomorpha, Talpidae), based on faecal DNA. *Mammalia, 184*, 182–192.

González-Esteban, J., Castién, E., & Gosálbez, J. (1999). Morphological and colour variation in the Pyrenean desman *Galemys pyrenaicus* (Geoffroy, 1811). *Zeitschrift Fur Saugetierkunde*, *64*(1), 1–11.

González-Esteban, J., Villate, I., & Castién, E. (2003). Sexual identification of *Galemys pyrenaicus*. *Acta Theriologica, 48*(4), 571–573.

González-Esteban, J., Villate, I., Castién, E., Rey, I., & Gosálbez, J. (2002). Age determination of *Galemys pyrenaicus*. *Acta Theriologica*, *47*, 107–112.

ICN. (2005). Plano Sectorial da Rede Natura 2000 - Fichas de Caracterização e Gestão das espécies d Mamíferos: *Galemys pyrenaicus* (Toupeira-de-água). *Anexo II da Directiva de Habitats*. *Instituto de Conservação Da Natureza*, Lisboa.

ICN. (2014a). Plano Sectorial da Rede Natura 2000. Lista Nacional de Sítios - SIC Continente: Rio Paiva. *Instituto de Conservação Da Natureza*, Lisboa.

ICN. (2014b). Plano Sectorial da Rede Natura 2000. Lista Nacional de Sítios - SIC Continente: Rios Sabor e Maçãs. *Instituto de Conservação da Natureza*, Lisboa.

Marcos, A. F. A. (2004). Effects of River Impoundment on the Presence of the Pyrenean desman (*Galemys pyrenaicus*). *MSc Environmental Biology*. University of Wales, Swansea.

Melero, Y., Aymerich, P., Luque-Larena, J. J., & Gosàlbez, J. (2012). New insights into social and space use behaviour of the endangered Pyrenean desman (*Galemys pyrenaicus*). *European Journal of Wildlife Research*, *58*(1), 185–193.

Melero, Y., Aymerich, P., Santulli, G., & Gosàlbez, J. (2014). Activity and space patterns of Pyrenean desman (*Galemys pyrenaicus*) suggest non-aggressive and non-territorial behaviour. *European Journal of Wildlife Research*, *60*(4), 707–715.

Mendes, C. A. S. (2005). Clima | Geoparque Terras de Cavaleiros. Retrieved 30 July 2016, from http://www.geoparkterrasdecavaleiros.com/pt-pt/content/clima.

Moreira, G. (2013). Fluvial System Restoration – Case Study : River Tua. *Dissertação de Mestrado Em Engenharia Do Ambiente, Perfil de Engenharia de Sistemas Ambientais.* Faculdadade de Ciências e Tecnologia Da Universidade Nova de

Lisboa.

Nores, C., Ojeda, F., Ruano, A., Villate, I., Gonzalez, J., Cano, J. M., … Gonza, J. (1998). Estimating the population density of *Galemys pyrenaicus* in four Spanish rivers. *Journal of Zoology*, *246*, 454–457.

Nunes, A. (2015). Recursos Hídricos Na Bacia Do Rio Sabor : Evolução Recente E Relações Com Mudanças Sócio-Ambientais. *Actas Do 6º Congresso Ibérico Sobre Gestão E Planificação Da Água, 10.*

Nunes, S. (2003). Monumentos sob tumulus e meio físico no território entre o Corgo e o Tua (Trás-os-Montes) : aproximação à questão. *Dissertação de Mestrado Em Arqueologia.* Faculdade de Letras Da Universidade Do Porto, *2*, 1–299.

Parque Natural de Montesinho (2016). Instituto de Conservação da Natureza e das Florestas, Lisboa. Retrieved 28 May 2016, from http://www.icnf.pt/portal/ap/p-nat/pnm/estat-cons.

Parque Natural Regional do Vale do Tua (2013). Adaptado do Regulamento nº 364-A/2013. Instituto de Conservação da Natureza e das Florestas, Lisboa. Retrieved 30 May 2016, from http://www.icnf.pt/portal/ap/amb-reg-loc/pnrv-tua.

Pedroso, N. M., & Chora, S. (2014). The Iberian desman *Galemys pyrenaicus* (E. Geoffroy Saint-Hilaire, 1811) in Portugal: status and conservation. *Conservation and Management of Semi-Aquatic Mammals in Southwestern Europe*, *3*, 13–18.

Pinto, R. (2013). A paisagem do Sítio de Interesse Comunitário Rio Paiva : caracterização e avaliação do estado ecológico. *Dissertação de Mestrado Em Ecologia, Ambiente E Território.* Faculdade de Ciências Da Universidade Do Porto.

Queiroz A. I. & Almada V., (1993). Some observations of Pyrenean desman (*Galemys pyrenaicus* Geoffroy) specimens in short captivity. *Proceedings of the Meeting on the Pyrenean Desman*, Lisbon, 33-39.

Queiroz, A.I., (1996). Sobre o comportamento das toupeiras-de-água, *Galemys pyrenaicus* Geoffroy e *Desmana moschata* L.. *Dissertação de Mestrado em Etologia*. Instituto Superior de Psicologia Aplicada, Lisboa.

Queiroz, A. I., A. Bertrand, & Khakhin., G. (1996). Status and conservation of Desmaninae in Europe. *Council of Europe Nature And Environment Series*, 76.

Queiroz A. I., Quaresma C. M., Santos C. P., Barbosa A.J.& Carvalho H.M.,(1998). Bases para a Conservaçao da Toupeira-de-Água,*Galemys pyrenaicus*. *Estudos de Biologia e Conservação da Natureza, nº 27*. *Instituto de Conservação da Natureza*, Lisboa, 27, 1-118.

Queiroz, A. I., Alves, P. C., Barroso, I., Beja, P., Fernandes, M., Freitas, L., … Sequeira, M. (2005). *Galemys pyrenaicus*, Toupeira-de-água. *Livro Vermelho Dos Vertebrados de Portugal (Cabral, M.J. et Al., (Eds))*. *Instituto de Conservação da Natureza*, Lisboa, 435–436.

Ramalhinho, M. G., & Tavares, P. (1989). Distribution and Ecology of *Galemys pyrenaicus* (Geoffroy, 1811) (Insectivora, Talpidae) in the "Parque Natural de Montesinho." *Arquivos Do Museu Bocage*, *1*(27), 385–392.

Richard, P. B. (1985). The Sensorial World of the Pyrenean Desman, *Galemys pyrenaicus*. *Acta Zoologia Fenica*, *173*, 255–258.

Richard B. & Michaud C., (1975). Le carrefour trachéen dans l'adaptation du Desman des Pyrénées (*Galemys pyrenaicus*) à la vie dulçaquicole. *Mammalia* 39 (3): 467-

477

Rio Paiva, o mais belo rio de Portugal (2010). Infopédia, Porto. Retrieved 1 June 2016, from http://www.riopaiva.org/rio-paiva/ .

Sabor: Trás-os-Montes (2012). Long Term Ecological Researh (Rede LTER - Portugal), Lisboa. Retrieved 28 May 2016, from http://www.lterportugal.net/sabor.

Sillero-Zubiri, C., & Macdonald, D. W. (1998). Scent-marking and territorial behaviour of Ethiopian wolves *Canis simensis*. *Journal of Zoology*, *245*(3), 351–361.

Silva, E. (2001). Estudo da capacidade de transposição de obstáculos do rio Ardena pela toupeira-de-água (*Galemys pyrenaicus*). *Relatório de Estágio Para Obtenção de Licenciatura Em Biologia.* Faculdade de Ciências Da Universidade Do Porto.

Stone, R. D. (1985). Home Range Movements of the Pyrenean Desman (*Galemys pyrenaicus*) (Insectivora: Talpidae). *Zeitschrift Fur Angewanate Zoologie*, 25–36.

Stone, R. D. (1987a). The activity patterns of the Pyrenean desman (*Galemys pyrenaicus*) (Insectivora : Talpidae), as determined under natural conditions. *Journal of Zoology, 213*, 95–106.

Stone, R. D. (1987b). The social ecology of the Pyrenean desman (*Galemys pyrenaicus*) (Insectivora : Talpidae), as revealed by radiotelemetry. *Journal of Zoology*, *212*, 117–129.

Stone, R. D., & Gorman, M. L. (1985). Social organization of the European mole (Talpa europaea) and the Pyrenean desman (*Galemys pyrenaicus*). *Mammal Review*, *15*(1), 35–42.

Vidal, O., Perez-Serra, A., & Pla, C. (2010). A sex determination protocol for the

Iberian desman (*Galemys pyrenaicus*) based on a three primer amplification of DBX and DBY fragments with non-invasive samples. *Conserv. Genet.*, *11*(3), 1185.

6 Appendix

6.1 Appendix 1

Table 34 - Transects of repeated visits and number of sampling repetitions divided per year.

Repetitive Samples		Coordinates		Year	
Sabor	**Watercourse**	**X**	**Y**	**2014**	**2015**
Angueira-2	Ribª de Angueira	708399	4604880	1	1
Azibeiro-1	Ribª das Veigas	674845	4609266	1	1
Chacim-2	Rio Azibo	678841	4593791	1	1
Fervença-1	Ribª do Pernacal	689943	4627445	1	1
G-207	Ribª do Medal	683208	4567022	1	2
G-353	Rio Maçãs	703134	4644486	1	1
G-368	Rio de Onor	691367	4632265	1	2
G-378	Rio Igrejas	691193	4634426	1	2
G-380	Ribª Baçal	689491	4645062	1	1
G-391	Rio Sabor	687142	4636318	1	1
G-43	Rio Sabor	687017	4633663	1	1
G-439	-	-	-	1	1
G-45	Rio Azibo	678097	4596735	2	1
G-46	Ribª do Teixedo	677586	4611628	1	2
G-49	Ribª de Salsas	679319	4605854	1	2
G-50	Ribª de Viveiros	695270	4615460	1	1
G-51	-	687111	4623612	1	1
G-52	Rio Tua	646405	4579707	1	1
G-53	Confluência Rio Maçãs - Rio Sabor	694323	4591767	1	2
G-55	-	683720	4586077	2	
G-59	Rio Sabor	694372	4602335	1	1
G-61	-	689160	4592838	2	
G-85	-	663032	4582701	1	1
Tua	**Watercourse**	**X**	**Y**	**2014**	**2015**
G-292	Ribª São Mamede	629042	4571044	1	1
G-31	Rio Guadramil	702149	4641898	1	1
G-32	Rio Rabaçal	644499	4609683	1	1
G-426	Rio Rabaçal	653389	4643827	1	1
G-427	Rio Rabaçal	656023	4636348	1	1
G-433	Rio Mente	650628	4645965	1	1

Table 35 - Variables collected for Marking Site Characterization included in 2015 and 2014+2015 analyses which showed high correlation values. (**) means that correlation is significant at 0.01 (2 tails).

Correlation Matrix Marking site Characaterization			
2015 Data			
Variables		**TD**	**TR**
Shading	**Coverage**	0.814**	0.836**
Habitat	**Speed**	-0.733**	-0.737**
BankorBed	**BankSlope**	-0.856**	-0.881**
BankorBed	**cmHeightBank**	-0.573**	-0.635**
cmHeightBank	**BankSlope**	0.730**	0.768**
2014+2015 Data			
Variables		**TD**	**TR**
Habitat	**Speed**	-0.704**	-0.735**

Table 36 - Variables collected during both years for General Habitat Characterization which showed high correlation values. (**) means that correlation is significant at 0.01 (2 tails).

Correlation Matrix General Habitat Characterization		
Variables		
%Rockbank	**%Outcrop**	0.710**
%Pebble	**%Cobble**	0.607**
%Boulder	**%Outcrop**	0.555**
mWidth	**cmDepth**	0.574**
%Shading	**%Coverage**	0.742**
%Riffle	**%Run**	0.593**

Table 37 - Sites of Galemys presence for both years. X signals Galemys' presence, 0 indicates presence not detected and the blank space signal data absence (because the site was not sampled for that year).

Sites with *Galemys*' presence	Watercourse	Coordinates		Year	
		X	Y	2014	2015
1	Rio Paiva	606442	4521620	X	
14	Rio Touro	610679	4525777	X	
Chacim-2	Rio Azibo	677426	4593120	X	0
Fervença-1	Ribª do Pernacal	689408	4627390	X	0
G-292	Ribª São Mamede	629046	4571091	0	X
G-30	Rio de Curros	629393	4604037	X	
G-33	Rio de Curros	624572	4594948	X	
G-37	Rio Tinhela	633218	4579991		X
G-384	Rio Sabor	681857	4647991	X	
G-390	Rio Maçãs	684509	4641888	X	
G-395	Rio Baceiro	678286	4644548	X	
G-399	Rio Baceiro	676284	4636838		X
G-403	Rio Tuela	669687	4646376		X
G-412	Rio Tuela	671747	4637017		X
G-415	Rio Tuela	665716	4637504	X	
G-419	Rio Rabaçal	659811	4645714	X	
G-426	Rio Rabaçal	653308	4644000	0	X
G-43	Rio Sabor	687044	4633644	0	X
G-443	Ribª de Veigas	661932	4638275	X	
G-449	Ribª da Anta	667860	4646677		X
G-451	Rio Tuela	668455	4644366		X
G-453	Rio Tuela	668340	4642422		X
G-456	Rio Tuela	671486	4643947	X	
G-46	Ribª do Teixedo	677546	4611691	X	X
G-47	Ribª do Pecal	681275	4616118	X	
G-49	Ribª de Salsas	679315	4606090	X	X
G-50	Ribª de Viveiros	695025	4615337	X	0
G-75	Rio Torto	634513	4613937	X	
G-79	Ribª de São Cibrão	672115	4621752	X	X
G-81	Rio de Macedo	662991	4614420		X
Gebelim-1	Ribª Zacarias	673723	4589258	X	
Olga-1	Ribª de Vila Franca	679785	4609282	X	
PIMATP12	Rio Sabor	681079	4616133		X
Ribª Moinhos	Ribª Moinhos	671823	4566421	X	X
S10	Ribª Rabo do Burro	669682	4590279		X
S13	Ribª de Limãos	683980	4614905		X
S18	Ribª de Onor	696795	4646965		X
S22	Ribª do Teixedo	677905	4608927		X

S4	Rib ª do Medal	675999	4562957		X
Serzeda-1	Ribª de Serzeda	684451	4623194	X	
T10	Rio Tinhela	617341	4595659		X
T14	Rio Mousse	643947	4632753		X
T16	Afluente Rio Tinhela	636819	4588240		X
T5	Ribª de Mós	671436	4612771		X
TP26	Rio Azibo	683249	4587294		X
TP57	Rio Sabor	694390	4615831		X
TP76	Ribª Moinhos	672297	4564974		X
TP78	Ribª Zacarias	672086	4588591		X

Table 38 - All the models considered in model selection for prediction of the Pyrenean desman KAI. 1 - %Coverage; 2 – Speed; 3 – Spraintability; 4- mWidth; 5- %Pool; 6- %Riffle.

Component models:					
	Df	**logLik**	**AIC**	**ΔAIC**	**Weight**
12345	12	-49.77	123.55	0.00	0.26
13456	14	-48.03	124.07	0.52	0.2
123456	13	-49.56	125.13	1.58	0.12
1346	15	-48.03	126.05	2.51	0.07
135	12	-51.47	126.93	3.38	0.05
35	11	-52.82	127.64	4.09	0.03
2345	7	-56.83	127.67	4.12	0.03
345	10	-54.05	128.09	4.55	0.03
235	8	-56.09	128.18	4.63	0.03
1235	9	-55.11	128.22	4.67	0.02
12346	13	-51.12	128.25	4.70	0.02
356	14	-50.42	128.85	5.30	0.02
1356	8	-56.52	129.04	5.49	0.02
3456	12	-52.64	129.27	5.73	0.01
36	9	-55.78	129.55	6.01	0.01
346	7	-57.82	129.63	6.09	0.01
23456	8	-56.95	129.91	6.36	0.01
2356	11	-53.99	129.97	6.42	0.01
12356	10	-54.99	129.98	6.44	0.01
136	14	-51.11	130.22	6.68	0.01
2346	11	-54.27	130.54	6.99	0.01
236	10	-55.32	130.63	7.09	0.01
1236	9	-56.39	130.78	7.23	0.01
1234	13	-52.99	131.98	8.43	0.00
134	13	-54.31	134.63	11.08	0.00
234	11	-57.42	136.84	13.29	0.00
123	9	-59.59	137.17	13.63	0.00
23	12	-56.70	137.40	13.86	0.00
3	8	-60.92	137.85	14.30	0.00
34	6	-63.53	139.06	15.51	0.00
13	7	-62.66	139.32	15.77	0.00
146	10	-59.76	139.52	15.97	0.00
1246	8	-62.59	141.18	17.63	0.00
46	10	-61.00	142.00	18.46	0.00
246	4	-67.30	142.61	19.06	0.00
1456	6	-65.36	142.72	19.18	0.00
16	9	-62.58	143.16	19.62	0.00

6	7	-64.92	143.84	20.30	0.00
12456	3	-68.93	143.86	20.31	0.00
26	11	-61.00	143.99	20.44	0.00
126	5	-67.22	144.45	20.90	0.00
456	9	-63.28	144.55	21.00	0.00
2456	5	-67.30	144.59	21.04	0.00
245	7	-65.31	144.62	21.08	0.00
156	6	-66.87	145.75	22.20	0.00
56	8	-64.91	145.82	22.27	0.00
1245	4	-68.91	145.83	22.28	0.00
256	10	-63.01	146.03	22.48	0.00
1256	6	-67.17	146.34	22.79	0.00
145	10	-63.28	146.55	23.00	0.00
45	8	-65.28	146.56	23.02	0.00
5	4	-69.31	146.62	23.07	0.00
125	3	-71.06	148.11	24.57	0.00
25	9	-65.22	148.43	24.88	0.00
15	5	-69.24	148.47	24.93	0.00
124	7	-67.40	148.79	25.25	0.00
24	9	-65.74	149.47	25.93	0.00
4	5	-69.75	149.50	25.96	0.00
12	3	-72.80	151.61	28.06	0.00
14	8	-67.85	151.70	28.15	0.00
2	7	-69.01	152.01	28.47	0.00
(Null)	4	-72.22	152.43	28.89	0.00
1	2	-74.45	152.90	29.36	0.00