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Testing the Umbrella Species Approach in Riparian Forests of Northern Portugal

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“All we have to decide is what to do with the time that is given us.”

J.R.R. Tolkien (1954) *“The Fellowship of the Ring”*

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Summary

Increasing biodiversity loss requires application of effective measures. The Umbrella species approach is one of such, where conservation of one or more species provides a protective Umbrella to several other species. This concept holds potential to design priority areas for conservation and guide habitat restoration. However, there is a need for testing this concept before application and identifying specific habitat features by which species benefit from conservation.

The riparian forest is generally a critical landscape feature that benefits a variety of aquatic, semiaquatic, as well as terrestrial species.

Here, we assessed the potential to guide riparian forest restoration in the Sabor River Basin (Northern Portugal) using the Iberian desman, a threatened semiaquatic mammal endemic of the Iberian Peninsula, as Umbrella species. Present day (2014) and historical (1995) data were used to assess relationships between riparian forest integrity, estimated through photointerpretation, and Iberian desman scats' presence. Effectiveness of the Iberian desman as *Umbrella* species, was tested based on relationships between number of species, abundance and presence of amphibian, reptile and fish and Iberian desman presence and scats' number. We used constrained ordination to evaluate relationships between assemblage structure of fish and amphibians and reptiles and riparian forest integrity. Finally, we evaluated variation in integrity of riparian forest among the two study years, using data obtained through photointerpretation.

Positive relationships were identified between Iberian desman presence and scats' presence and riparian forest integrity. Comparisons between current and historical data revealed that such relationship is presently stronger than it was twenty years ago. Also, the riparian forest integrity of the Sabor River Basin seems to have slightly increased in the past twenty years.

The number of endemic species of amphibians and reptiles was positively associated with Iberian desman scats' number, and so were two endemic species, the Iberian frog and the Iberian emerald lizard, with the Iberian frog being also strongly associated with Iberian desman presence. On the contrary, fish abundance, total number of species and number of native species decreased with Iberian desman presence and scats' number. Eventually, positive relationships were identified between riparian forest integrity and amphibian and reptile and fish assemblages.

Our study supports the vision that the Iberian desman has potential as an *Umbrella* species to guide habitat restoration and select priority areas of riparian forest for conservation. The riparian forest in the Sabor River Basin seems to be well-preserved and in a better ecological state than twenty years ago. Nonetheless, considering its importance for the whole riparian ecosystem and

the increasing human threats it is facing, we advocate a precautionary approach, through its restoration. This may favour the recovery of the Iberian desman and the persistence of several co-occurring species, including endemic ones.

Key-words: *Galemys pyrenaicus*, amphibians, reptiles, fish, habitat restoration.

Resumo

A atividade humana desempenha um papel importante na determinação dos padrões de biodiversidade que observamos hoje e vai continuar a fazê-lo num futuro próximo com o aumento da perturbação e modificação dos ecossistemas. A crescente perda de biodiversidade requer a avaliação e aplicação de medidas de gestão eficazes e financeiramente sustentáveis, tendo em conta a falta de fundos para investigação e conservação a nível global. O conceito de espécie *Umbrella*, em que a conservação de uma ou de um grupo de espécies pretende proteger as comunidades no global, tem sido debatido pela sua aplicação sem testes prévios à sua eficácia e pela necessidade de melhor definição de critérios de seleção da espécie *Umbrella*. Porém, torna-se cada vez mais claro que este conceito possui interesse, requerendo avaliação detalhada que fundamente a sua aplicação. O conceito parece ter potencial para projetar ações de gestão, tais como o desenho e seleção de áreas e habitats prioritários para conservação, e para guiar ações de restauração de habitat. Mas avaliações recentes salientam a necessidade de testar o conceito antes da sua aplicação e de identificar mecanismos através dos quais a espécie *Umbrella* e espécies coocorrentes beneficiam da conservação, tais como componentes de habitat e requisitos ecológicos específicos. Para além disto, parece relevante que as espécies coocorrentes e a espécie *Umbrella* respondam de forma semelhante ao stress ou recuperação ambiental.

As florestas ripícolas são um habitat perturbado pelo Homem e um componente essencial na manutenção da integridade e biodiversidade dos ecossistemas de água doce. Diversas espécies utilizam esta área devido às condições físicas que lhe estão associadas, bem como a disponibilidade de recursos alimentares e interações subjacentes. Em particular, a floresta ripícola é uma componente importante que parece beneficiar a toupeira-de-água (*Galemy pyrenaicus* - Sain-Hilaire, 1811), uma espécie sensível e ameaçada, e muitas outras espécies de vários taxa. Porém, fatores como poluição doméstica e industrial, extração de inertes, pastoreio intensivo, regulação de canais e construção de barragens estão a causar impactos neste habitat, com danos na floresta ripícola e tornando imperativa a sua preservação. Muitos destes fatores provocam mudanças na quantidade e qualidade da água, bem como modificam a estrutura das margens e rios que fornecem abrigo, alimento e proteção à toupeira-de-água, potencialmente afetando-a. De facto, estudos recentes e a decorrer apontam para uma drástica diminuição da presença de toupeira-de-água nos últimos anos, estando a sua presença limitada a locais com alta qualidade ambiental e necessita de avaliação de novas medidas de gestão que tenham em vista a sua conservação. Em adição, tem sido sugerido que espécies especialistas com requisitos específicos poderão ser melhores candidatos a espécie *Umbrella* que outras espécies generalistas. A toupeira-de-água, sendo relativamente rara e sensível à perturbação humana e ocorrendo em habitats específicos,

pode ser considerada uma espécie especialista, preenchendo importantes critérios chave utilizados para a seleção da espécie *Umbrella*.

Neste estudo, pretendeu-se avaliar o potencial da toupeira-de-água como espécie *Umbrella* para guiar a restauração da floresta ripícola, utilizando como caso de estudo a Bacia hidrográfica do Rio Sabor. As análises foram efetuadas com base em dados de prospeção de dejetos de toupeira-de-água recolhidos em 2014, e dados históricos de presença/ausência de toupeira-de-água recolhidos em 1995. Especificamente, testámos através de regressão logística, a diferentes escalas espaciais, as relações entre a presença de dejetos de toupeira-de-água e variáveis de integridade da floresta ripícola. Estas variáveis foram obtidas através de fotointerpretação de imagens aéreas dos dois anos, permitindo, através de testes de diferenças, comparar a floresta ripícola entre 1995 e 2014. As variáveis de integridade da floresta ripícola foram selecionadas com base em evidências anteriores da sua importância e pela sua facilidade de amostragem e incluem a conectividade longitudinal, a continuidade e a largura da mancha de floresta ripícola, bem como a presença de arbustos e copa. A fotointerpretação foi realizada à escala de 50 metros, com posterior aglomeração a escalas mais abrangentes, de 100, 200, 500 metros e total do local amostrado (88-978 metros). Para testar a eficácia da toupeira-de-água como espécie *Umbrella*, utilizámos regressão logística para avaliar as relações entre a presença de toupeira-de-água e número de dejetos da mesma e o número de espécies, abundância e presença de grupos de espécies coocorrentes aquáticas - peixes – e semiaquáticas – anfíbios e répteis. Esta análise foi realizada à escala total dos locais amostrados, avaliando apenas os locais comuns entre os grupos. As espécies coocorrentes foram caracterizadas em termos de abundância no caso dos peixes, com base em dados de 2012, e presença no caso dos anfíbios e répteis, com base em dados de 2014. Para avaliar se cada um destes grupos coocorrentes globalmente partilha a floresta ripícola como requisito ambiental com a toupeira-de-água e portanto poderia também beneficiar da sua restauração, foram realizadas análises de redundância para determinar a relação entre a estrutura das respetivas comunidades e as variáveis de integridade ripícola.

Foram identificadas relações positivas e significativas entre as variáveis de integridade ripícola e toupeira-de-água. A comparação da situação atual com os dados históricos revelou uma relação efetiva entre as variáveis de integridade ripícola e a presença toupeira-de-água mais forte em 2014, com a maioria das variáveis testadas a apresentar relações significativas e positivas, enquanto em 1995, a conectividade longitudinal apresentou uma relação significativa com a presença de toupeira-de-água. Apesar de esta relação ter sido detetada a todas as escalas, foi menos evidente à escala de 50 metros do que às restantes, demonstrando a importância da conservação da vegetação a larga escala para beneficiar a toupeira-de-água. O número de espécies endémicas de anfíbios e répteis parece apresentar uma relação com o número de dejetos de toupeira-de-água.

Para além disto, duas espécies endémicas – a rã ibérica e o lagarto-de-água - tiveram uma relação positiva com o número de dejetos de toupeira-de-água, sendo que a rã ibérica apresentou também uma forte relação com a presença de toupeira-de-água. Foram encontradas relações negativas entre a abundância, número de espécies e número de espécies nativas de peixes, e presença e número de dejetos de toupeira-de-água. Especificamente, as variáveis de integridade ripícola apresentaram uma relação positiva com o total de espécies e número de espécies nativas de peixes. O mesmo sucedeu com número de espécies endémicas de anfíbios e répteis.

Detetámos um ligeiro aumento de integridade da floresta ripícola em 2014, não tendo sido porém identificadas diferenças na Largura da floresta ripícola, refletindo possivelmente o declive do vale do Sabor, que mantém o crescimento da floresta ripícola limitado a uma estreita faixa nas margens do rio. Não foram detetadas diferenças entre os dois anos à escala total do local amostrado, revelando que as alterações ainda não são significativas a uma escala mais larga.

Este estudo demonstra que a toupeira-de-água possui potencial como espécie *Umbrella* para guiar a restauração de habitat e selecionar áreas de floresta ripícola prioritárias para conservação da biodiversidade no futuro, tendo em conta o crescente número de ameaças impostas pelo Homem. Porém, as relações encontradas entre toupeira-de-água e os grupos coocorrentes de peixes, anfíbios e répteis evidencia que a utilização da primeira como espécie *Umbrella* pode requerer estudos complementares, que avaliem diferentes requisitos ecológicos e factores de ameaça comuns entre espécies. A floresta ripícola da bacia do Rio Sabor parece revelar uma maior integridade hoje e a sua relação com a toupeira-de-água parece ser mais forte, mas a presença de toupeira-de-água parece continuar a diminuir em praticamente toda a sua área de distribuição. Mesmo que a floresta ripícola apresente uma melhoria ligeira na qualidade a nível global, existem áreas em pior estado que deverão ser recuperadas, nomeadamente no âmbito de medidas de mitigação e de minimização de impactes resultantes da construção do projeto de Aproveitamento Hidroelétrico do Baixo Sabor. Em particular, a restauração das condições de conectividade e presença de copa, poderá beneficiar fortemente a toupeira-de-água e outras espécies coocorrentes. Em termos gerais, tendo em conta o bom estado geral de conservação floresta ripícola na Bacia hidrográfica do Rio Sabor e, paralelamente, as crescentes ameaças que a própria enfrenta, sugerimos uma abordagem precaucionaria, que contemple a sua preservação e recuperação para favorecer a recuperação da toupeira-de-água e a persistência de várias espécies coocorrentes, incluindo espécies endémicas.

Palavras-chave: *Galemys pyrenaicus*, répteis, anfíbios, peixes, restauração de habitat.

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1. General Introduction

Human actions play an important role in the biodiversity patterns we see today (Ewers *et al.* 2013) and probably will remain to do so in the future due to the increase of human disturbance and modification of ecosystems. For this reason, appropriate planning for biodiversity conservation must become an integral part of any resource management (Suter *et al.* 2002). Identifying land and species to be managed should be based on detailed surveys and knowledge of the affected species' life histories, distributions and interactions with other species and the physical environment (Favreau *et al.* 2006). However, quantifying biodiversity, defining conservation goals, monitoring and assessing conservation measures is time consuming and requires the use of limited research-oriented funds. Consequently, conservation managers are forced to look for strategies – conservation shortcuts based on a small number of species as surrogates (Caro & O'Doherty 1999) that, hopefully, will allow the management of communities with minimal expenditures (Bifulchi & Lodé 2005). These shortcuts may include the application of different concepts such as flagship, keystone, indicator, umbrella or focal species (Favreau *et al.* 2006).

1.1. The Umbrella Species Concept

Umbrella species can be defined as species whose conservation confers a protective umbrella to numerous co-occurring species (Fleishman *et al.* 2001). This concept uses species' habitat and ecological resource requirements to guide ecosystem management (Roberge & Angelstam 2004). Thus, the needs of usually a single species, must encompass the needs of viable populations of co-occurring species (Sergio *et al.* 2008).

The general criteria used to identify a potential *Umbrella* species include well-known natural history and ecology, spatial overlap with co-occurring species of concern, moderate to negative response to disturbance, and relative ease of sampling (e.g., Caro & O'Doherty 1999; Seddon & Leech 2008). Therefore, an *Umbrella* species with large expanses of habitat can be used as a surrogate for many biodiversity components that have similar habitat requirements but less extensive spatial requirements (Suter *et al.* 2002). Also, it has been suggested that species with specialized resource requirements (e.g., dead wood, old-growth forest, riparian areas) may be better *Umbrellas* than generalist species (Branton & Richardson 2011). This way, by directing management efforts toward the requirements of most exigent species, one is likely to address the requirements of many cohabitants that use the same habitat (Roberge & Angelstam 2004). Other criteria include rarity of the species, relative sensitivity to human disturbance and population viability (Fleishman *et al.* 2000).

The *Umbrella* species approach has gained popularity and is often applied in management yet rarely tested (Suter *et al.* 2002). Indeed, there is considerable debate about its actual role in

conservation planning, lack of empirical evidence about its usefulness (e.g., Roberge & Angelstam 2004), efficiency (e.g., Branton & Richardson 2011; Favreau *et al.* 2006; Sergio *et al.* 2008) and criteria used for selecting the *Umbrella* species. Caro & O'Doherty (1999) recognized that this concept may help define the size, shape or habitat type of areas for conservation, if it is planned on the basis of the exigent area requirements. Also, the *Umbrella* species approach holds potential for habitat restoration planning, requiring similarity in the response of *Umbrella* and co-occurring species to environmental stress or recovery, as well as the identification of mechanisms by which co-occurring species benefit from conservation of an *Umbrella* species, such as specific resource requirements (Branton & Richardson 2014; Lambeck 1997).

1.2. The Riparian Forests

Riparian forests are considered a unique element of terrestrial ecosystems and a biologically important area for diversity, productivity and complexity (Naiman & Décamps 1997), providing a number of ecosystem services. Regionally, the riparian forest functions as a connector across landscapes and represents linear islands that are internally dynamic (Knopf & Samson 1994). Indeed, these areas play an important role in the character and morphology of streams (Beschta & Ripple 2012). Its biogeochemical and hydrological processes regulate, for example, the pollutant movement to receiving waters by filtration and often mitigate the impact of upland sources of contaminants on water quality (Vidon *et al.* 2010). Also, the riparian forest is the source of great organic matter inputs to the river (Ceneviva-Bastos & Casatti 2014), it modifies the microclimate of the stream and surrounding area (Naiman & Décamps 1997), and increases the systems' complexity and substratum (Studinski *et al.* 2012). Disturbance may affect how aquatic ecosystems function and deliver services to humans (Tanentzap *et al.* 2014).

Historically, riparian forests have had a high cultural and economic importance, with the exploitation of its natural functions defining the development of human settlements and urban development (Groffman *et al.* 2003). Specifically, Mediterranean regions are set to become a priority for conservation as we watch the long-term effects of human use of riparian areas (Aguar & Ferreira 2005). Here, as in many other regions, the future increase of human population will likely lead to further degradation of riparian areas, intensification of the hydrological cycle, increase in the discharge of pollutants and further proliferation of species invasions, which elevates the importance of these areas as strategic global resources (Tockner & Stanford 2002).

The riparian forests are also essential to the conservation and management of semiaquatic species (Semlitsch & Bodie 2003). In fact, they provide a distinctive aquatic and terrestrial habitat for wildlife (Hannon *et al.* 2002), given its physical conditions, prey resources and food web interactions (Richardson *et al.* 2005). Moreover, the riparian forest acts as an important corridor

for dispersal and foraging for most European mammal species (O'Connell *et al.* 1993) and is critical for the persistence of riparian obligate species (Olson *et al.* 2007). Species restricted to riparian areas are typically described as obligates, requiring streams or riparian areas for at least some phase of their life cycle (Richardson *et al.* 2005) and are as instrumental as riparian forests in maintaining the ecological functions through their activities (e.g., predation, damming, burrowing) (Stoffyn-Egli & Willison 2011).

1.3. The Iberian Desman

The Iberian desman *Galemys pyrenaicus* (Sain-Hilaire, 1811), can be considered a specialist (Igea *et al.* 2013), dependent on the structural and biological aspects that the riparian forest provides. Thus, the Iberian desman may be a good candidate to guide conservation, restoration and management of the riparian forest. It is a small semi-aquatic insectivore mammal, considered an Iberian endemism, occurring in the Pyrenees and northern Iberian Peninsula (Nores 2012). It is currently classified as "Vulnerable" in the IUCN Red List of Threatened Species (<http://www.iucnredlist.org/>), being strictly protected and listed in Annexes II and IV of the European Habitats Directive (92/43/ECC) and Appendix II of the Bern Convention. It is generally associated to cold and well preserved mountain rivers, with permanent water regime, strong or moderate current and sufficient water quality to maintain good populations of benthonic macro invertebrates, which are the basis of its specialised feeding habitats (Igea *et al.* 2013; Nores 2012). Indeed, this riparian obligate species has high and strict ecological requirements, being considered an excellent bio indicator of well-preserved freshwater ecosystems (Santamarina 1993; Aymerich & Gosálbez 2002). A major element of its habitat is the existence of suitable shelter, usually crevice or holes among stones and roots along stream banks (Palmeirim & Hoffman 1983). According to Melero *et al.* (2012), along with water quality, the availability of these resting sites should also be taken into account as the main factors affecting Iberian desman's conservation and be considered in management guidelines for habitat suitability, with a focus on riparian shorelines. Indeed, destruction of natural vegetation has been considered one of the main factors threatening Iberian desman populations, affecting resting sites, elevating stream temperatures and disturbing prey resources (Nores 2012).

During the last decades the distribution area of the Iberian desman has declined, and the species is in a state of regression in practically all of its geographic range (Igea *et al.* 2013). The situation has worsened during the last few years, particularly in the most southern populations, in Mediterranean climate regions (Igea *et al.* 2013). This reduction in its historical distribution area may be a phenomenon closely linked to changes in the water quality or quantity and in the morphological structure of banks and beds (Queiroz *et al.* 1996). In particular, habitat loss due to the destruction of riversides and river regulation, through the construction of hydroelectric power

stations and river contamination, have been shown to have a dramatic impact on Iberian desman populations (Némoz *et al.* 2011; Cabria *et al.* 2006).

In Portugal, the main study and national survey of the Iberian desman was conducted between 1995 and 1996 by the currently named Instituto da Conservação da Natureza e das Florestas (ICNF), from which resulted a reference document for the species (Queiroz *et al.* 1998). This study fulfilled important gaps on the knowledge about the desman's distribution and habitats, but no further national surveys have been conducted since then. Likewise, the last regional and local monitoring programs date from 2000/2001, when the Portuguese population was classified as in a state of regression (Quaresma 2001).

Evaluating the Iberian desman as an *Umbrella* species may be a priority for preserving the integrity of Mediterranean freshwater ecosystems. Firstly, the species is one of the most widely used indicators for assessing the condition of ecosystems and their biodiversity, becoming an increasingly important tool in conservation planning (Temple & Terry 2009). Secondly, small and threatened populations are currently the main focus of conservation biology, due to their vulnerability to extinction (Melero *et al.* 2012). Thirdly, climate change is already affecting the distribution of many species and the Iberian desman is likely to be a special concern (Morueta-Holme *et al.* 2010). Fourthly, animals with legal protection are often used as *Umbrella* species and these selections are overwhelmingly vertebrates (Rubinoff 2001). Finally, the Iberian Desman is considered a flagship species for biodiversity and evolution (Melero *et al.* 2014).

1.4. Study Aims and Approach

Here, we test the *Umbrella* species approach, with the Iberian desman as candidate, in the Sabor River Basin in the Northeast of Portugal. This is an important region for conservation of the fauna associated to aquatic systems, showing an overall good water quality and ecological status (Silva 2010). It is also renowned for its local biodiversity with high levels of plant endemism, several endangered bird species, and richness in many other taxa (Paterson *et al.* 2008). However, factors as domestic and industrial pollution, extraction of sediments, intensive farming and flow regulation (Silva 2010) are increasingly impacting the basin and damaging the associated riparian forest. Therefore, there is an urgent need for an overall protection of riparian areas in the region. Historical data collected in the Sabor River basin in 1995 through scat prospection revealed that the Iberian desman was present in 60% of the sites surveyed (Queiroz *et al.* 1998), but data from 2014 revisiting the same sites suggests that the species is currently present in merely 20% of the sites (Annexe 2; Quaglietta & Beja unpublished data). This indicates that the Iberian desman may enter a worse status of conservation in a near future if the limiting factors keep aggravating.

In this study, we evaluated the riparian forest as a specialized ecological requirement of the Iberian desman and tested the possibility of using the *Umbrella* species approach. We assessed the relationship between riparian forest integrity and Iberian desman occurrence and scats' number, as well as between some co-occurring aquatic and semiaquatic vertebrate species – fish, amphibians and reptiles - and riparian forest integrity and Iberian desman occurrence and scats' number. We also looked for potential changes in riparian forest integrity between 1995 and 2014.

1.5. Thesis outline

This thesis is organized in three chapters and 3 annexes. Chapter 1, this general introduction, defines the context and significance of the main issues addressed in this thesis. Chapter 2 corresponds to a manuscript prepared for submission to an international, peer-reviewed scientific journal (Journal of Applied Ecology), focusing on the testing of *Umbrella* species approach in Northern Portugal riparian forests, including a detailed description of the methods and results of our study, as well as its main findings and management implications. Chapter 3 synthesizes the main conclusions of work and our interpretation of the findings in a Mediterranean context and presents suggestions for management actions and future work to improve testing of *Umbrella* species approach in Portugal freshwater ecosystems and Mediterranean regions.

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2. Testing the Umbrella Species Approach in Riparian Forests of Northern Portugal

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Summary

1. Increasing biodiversity loss requires application of effective measures. The *Umbrella* species approach is one of such, where conservation of one or more species provides a protective *Umbrella* to several other species. This concept holds potential to design priority areas for conservation and guide habitat restoration. However, there is a need for testing this concept before application and identifying specific habitat features by which species benefit from conservation.

2. The riparian forest is generally a critical landscape feature that benefits a variety of aquatic, semiaquatic, as well as terrestrial species.

3. Here, we assessed the potential to guide riparian forest restoration in the Sabor River Basin (Northern Portugal) using the Iberian desman, a threatened semiaquatic mammal endemic of the Iberian Peninsula, as *Umbrella* species. Present day (2014) and historical (1995) data were used to assess relationships between riparian forest integrity, estimated through photointerpretation, and Iberian desman scats' presence. Effectiveness of the Iberian desman as *Umbrella* species, was tested based on relationships between number of species, abundance and presence of amphibian, reptile and fish and Iberian desman presence and scats' number. We used constrained ordination to evaluate relationships between assemblage structure of fish and amphibians and reptiles and riparian forest integrity. Finally, we evaluated variation in integrity of riparian forest among the two study years, using data obtained through photointerpretation.

4. Positive relationships were identified between Iberian desman presence and scats' presence and riparian forest integrity. Comparisons between current and historical data revealed that such relationship is presently stronger than it was twenty years ago. Also, the riparian forest integrity of the Sabor River Basin seems to have slightly increased in the past twenty years. The number of endemic species of amphibians and reptiles was positively associated with Iberian desman scats' number, and so were two endemic species, the Iberian frog and the Iberian emerald lizard, with the Iberian frog being also strongly associated with Iberian desman presence. On the contrary, fish abundance, total number of species and number of native species decreased with Iberian desman presence and scats' number. Eventually, positive relationships were identified between riparian forest integrity and amphibian and reptile and fish assemblages.

5. *Synthesis and applications.* Our study supports the vision that the Iberian desman has potential as an *Umbrella* species to guide habitat restoration and select priority areas of riparian forest for conservation. The riparian forest in the Sabor River Basin seems to be well-preserved and in a

better ecological state than twenty years ago. Nonetheless, considering its importance for the whole riparian ecosystem and the increasing human threats it is facing, we advocate a precautionary approach, through its restoration. This may favour the recovery of the Iberian desman and the persistence of several co-occurring species, including endemic ones.

Key-words: *Galemys pyrenaicus*, amphibians, reptiles, fish, habitat restoration.

2.1. Introduction

Human actions play an important role in the biodiversity patterns we see today (Ewers *et al.* 2013) and probably will remain to do so in the future with the increase of human disturbance and modification of ecosystems. As the list of endangered species grows longer, additional approaches are needed to conserve biological diversity, with more lands and waters in need of conservation management (Groves *et al.* 2002).

Today, given the insufficient research-oriented funds worldwide, approaches used for designing priority areas for conservation and habitat restoration may be an effective tool. The *Umbrella* species approach, in which the conservation of usually a single species confers a protective *Umbrella* to numerous co-occurring species (Fleishman *et al.* 2001), is recognized to help define size and habitat type of conservation areas (Caro & O'Doherty 1999; Branton & Richardson 2011). This concept, that uses species' ecological resource requirements to guide ecosystem management (Roberge & Angelstam 2004), has also been considered to hold potential for habitat restoration planning, requiring similarity in the response of *Umbrella* and co-occurring species to environmental stress or recovery and the identification of specific habitat features by which species benefit from conservation (Branton & Richardson 2014; Lambeck 1997). It has indeed been suggested that species with specialized resource requirements may be better *Umbrellas* than generalist species (Branton & Richardson 2011). This way, by directing management efforts toward the requirements of most exigent species, one is likely to address the requirements of many cohabitants (Roberge & Angelstam 2004). Other criteria for selecting *Umbrella* species include species rarity and relative sensitivity to human disturbance (Fleishman *et al.* 2000). It has been properly emphasized that to reliably apply this concept, we must test its effectiveness (Caro & O'Doherty 1999), determining when and where it can be an effective conservation tool and whether the species should be selected (Favreau *et al.* 2006).

The riparian forest is a unique element of terrestrial ecosystems and a biologically important area for its diversity, productivity and complexity (Naiman & Décamps 1997). Present and further degradation of riparian areas will lead to intensification of the hydrological cycle, increase in the discharge of pollutants and increasing proliferation of species invasions, which elevates the importance of these areas as strategic global resources (Tockner & Stanford 2002) and makes them

a priority for conservation. Riparian habitat is not only essential for water resources protection (Semlitsch & Bodie 2003), but also provides a distinctive aquatic and terrestrial habitat for wildlife (Hannon *et al.*, 2002), including species of conservation interest such as the Iberian desman *Galemys pyrenaicus* (Sain-Hilaire, 1811).

The Iberian desman is a small semi-aquatic insectivore mammal, endemic to the northern half of the Iberian Peninsula that is currently classified as Vulnerable under the IUCN Red List of Threatened Species (<http://www.iucnredlist.org/>). This species has high and strict ecological requirements, being considered an excellent bio indicator of well-preserved freshwater ecosystems (Aymerich & Gosálbez 2002; Santamarina 1993). During the last decades the distribution area of the Iberian desman has declined and it is in a state of regression in practically all of its geographic range (Igea *et al.* 2013). Changes in the water quality or quantity and in the morphological structure of banks and beds may be affecting the Iberian desman (Queiroz *et al.* 1996). The riparian forest and the Iberian desman are generally positively associated to water quality and are suffering with increasing human threats (e.g., Kominoski *et al.* 2013; Nores 2012; Santamarina 1993). The Iberian desman can be considered a habitat specialist (Igea *et al.* 2013) and may be a good candidate to guide restoration and management of the riparian forest, benefiting other co-occurring species.

Here, we test the *Umbrella* species approach, with the Iberian desman as candidate, in the Sabor River Basin in the Northeast of Portugal. This is an important region for conservation of fauna associated to the aquatic system, showing an overall good water quality and ecological status (Silva 2010). The Sabor River Basin is characterized by a topographically heterogeneous valley, renowned for its local biodiversity with high levels of plant endemism, several endangered bird species, and richness in many other taxa (Paterson *et al.* 2008). However, factors as domestic and industrial pollution, extraction of sediments, intensive farming and flow regulation (Silva 2010) are impacting this habitat and damaging the riparian forest.

Historical data collected in the Sabor River basin in 1995 through scats survey (Queiroz *et al.* 1998) revealed that the Iberian desman was present in 60% of the sites prospected. Yet, in 2014 the percentage of occupancy lowered to 20% (Annex 2; Quaglietta, L. & Beja, P. unpublished data). In this study, we evaluated the riparian forest as a specialized ecological requirement of the Iberian desman and tested the *Umbrella* species approach. Specifically, we assessed the relationship between Iberian desman presence and scats' presence and riparian forest integrity variables in 1995 and 2014. We analysed whether number of species, presence and abundance of fishes, amphibians and reptiles varied in a pattern similar to Iberian desman presence and number of scats. We evaluated whether variation in co-occurring species' abundance and presence could be

explained by riparian forest integrity variables. Finally, we evaluated potential changes in riparian forest integrity between 1995 and 2014.

2.2. Materials and Methods

2.2.1. Study Area

The study area (Fig. 1) comprised streams and riparian forest in the Sabor River basin in the Northeast of Portugal. This basin covers the eastern half of the Protected Area Montesinho Natural Park. The Sabor River, with headwaters in Spain, has a drainage area of approximately 3868 km² in national territory, extending for 131 km - with dominant guidance NE-SW, and discharging into the Douro River. The study area was included in Special Protection Areas and Sites of Community Importance of Montesinho/Nogueira and Rivers Sabor and Maças (<http://www.icnf.pt/>).

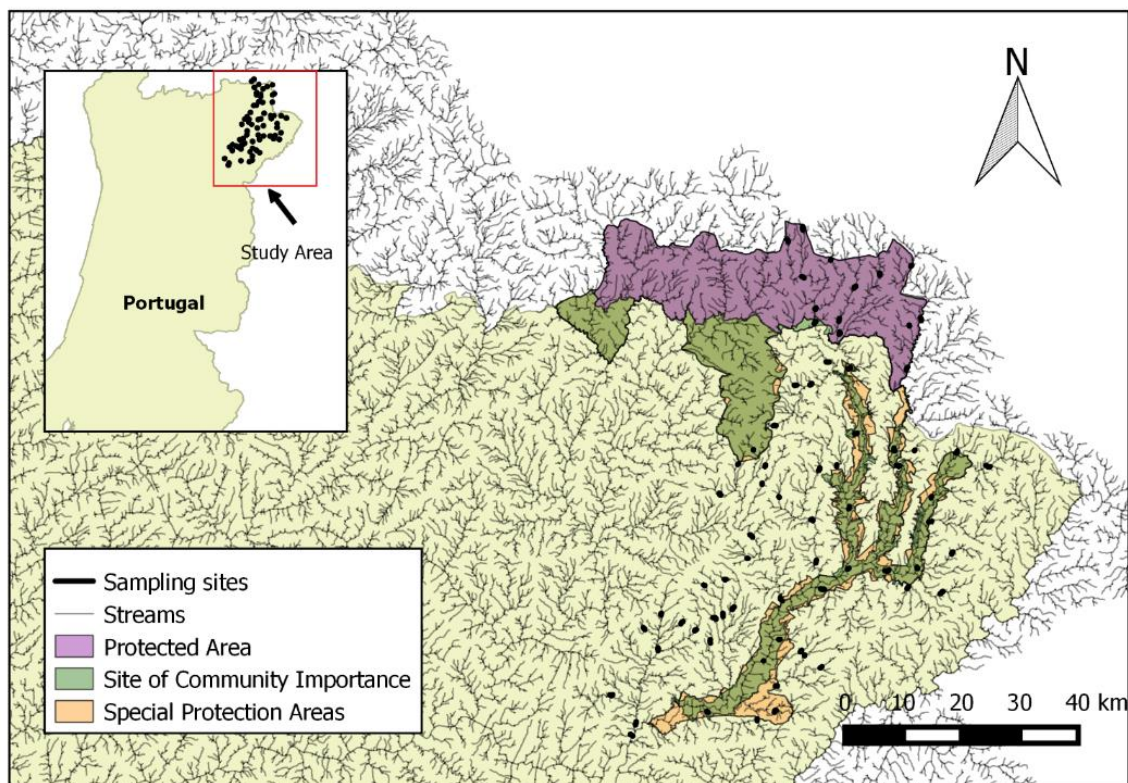


FIGURE 1: LOCATION OF STUDY AREA IN NORTHEAST PORTUGAL.

2.2.2. Iberian Desman Sampling

Due to the inherent sampling scheme, the selection of the sampling reaches was not aleatory, but directed primarily towards revisiting the same sites (n=40) in the Sabor River basin that were sampled in 1995 by Queiroz *et al.* (1998). To these sites, we added 31 new sites, selected considering river order and spatial distribution, in an attempt to obtain a good coverage of the

whole river network – while maintaining a minimum average distance of 1 km to avoid spatial dependence (Fig. 1).

The survey of Iberian desman took place between May and September of 2014. Detection of Iberian desman was performed by scat survey and validated by non-invasive genetics, after field collection (cf. Charbonnel *et al.* 2014; Quaglietta, L. & Beja, P. unpublished data). At each site, we covered a distance of maximum 600 m, following Queiroz *et al.* (1998). Accordingly to Nores *et al.* (1992), this is the distance with 95% probability of finding clues, in case of species presence. Stream bed and banksides (in a radius of 50 cm from the water) were sampled on foot, searching for Iberian desman scats, following the methodology used by Queiroz *et al.* 1998 and several other authors (e.g., Fernández-González *et al.* 2014; Nores *et al.* 1992). Iberian desman presence was assessed at site scale. Similarly, 1995 data only included presence at site scale. For each site, we also determined, through spatial location, Iberian desman scats' presence and number at different scales (50-200m and site scale). As only a subset of all scats were genetically validated, due to its inherent costs, we assumed that the scats to which we gave in the field the highest probability of belonging to the species, following used criteria as smell and general shape and size (Queiroz *et al.* 1998), were indeed of the Iberian desman. We then determined scats' number to obtain a quantitative expression of Iberian desman presence (Nores 1992).

2.2.3. Co-occurring Species Sampling

Relationships between co-occurring species and the Iberian desman were assessed at site scale, evaluating merely the sites that had sampling performed simultaneously for the Iberian desman and co-occurring species. For most co-occurring species, we used available data from previous studies.

Namely, for fish, we used available data from a companion study (Ferreira *et al.* 2013 in press.), collected between June and September of 2012, at 23 sites. Three to five 5-min sampling periods were carried at each site, using a single anode electrofishing gear (350-750 V, 3–5A, DC) and fish were identified to species, counted and then returned to the stream. For amphibians and reptiles, we used available data collected between April and June of 2014, at 51 sites. At each site, 200 m were sampled with a net, recording the presence of larvae or adults and identifying to species level. Amphibian and reptile data sets were improved with presences opportunistically registered during Iberian desman sampling.

2.2.4. Riparian Forest Integrity Assessment

Riparian forests' integrity was assessed based on photointerpretation performed on 1995 and 2014 aerial photographs of the study area. The total length of each Iberian desman sampling site was

divided in portions of 50 m, and the structure of the riparian forest was visually examined at 1:2500 scale and assigned to each 50 m portion using Qgis® software.

The degree of riparian integrity was assessed from 8 Riparian Integrity Variables - henceforth referred as RIVS - previously used to describe riparian forest integrity or evaluate its conservation status (e.g., Magdaleno & Martinez 2014; Salinas *et al.* 2000). A table with description of RIVs definition and assessment is presented in Annex 1.1. Riparian Category is relative to riparian continuity. Longitudinal Connectivity indicates the percentage of the bank length covered by riparian forest and was assessed separately for right and left margins. The Riparian Vegetation Width was recorded and averaged from several measurements taken randomly along each 50m sample, assessing right and left margins separately. Total Width of the riparian forest (including the river channel) was assessed from left to right margin. We also assessed the Presence of Shrub and Canopy, separately, in each margin.

After mapping the vegetation at 50 m portions' scale, we averaged the values of each variable at 100m, 200m, 500m and total length of sampled streams scales.

2.3. Data Analyses

We evaluated the relationship between the Iberian desman presence and scats' presence and RIVs with data from both 2014 (50-200m and site scale) and 1995 (at site scale) surveys [see par. 2.3.1.]. Two separate sets of analysis were conducted for fish (n= 12) and reptiles and amphibians (n= 12). We created a new category for fish, the abundance and number of Portugal native species (that are also Iberian endemisms) (Annexe 3.2). We excluded *Chondrostoma arcasii* (Steindachner, 1866) from our analyses, because of its endangered status (<http://www.iucnredlist.org/>), as the *Umbrella* species approach is intended to provide conservation and delineate suitable habitat for species that are less sensitive than the *Umbrella* candidate (Seddon & Leech 2008). For amphibians and reptiles we considered a new category - the number of Iberian endemism (Annexe 3.1). We evaluated relationships between number of species, presence, abundance, native or endemic species of co-occurring vertebrates and Iberian desman presence and scats' number at site scale [2.3.2]. We evaluated relationships between number of species, presence, abundance, native or endemic species of co-occurring vertebrates and riparian forest integrity variables [2.3.3.]. We assessed whether riparian forest integrity varied between 1995 and 2014, at the different sampled scales [2.3.4.].

2.3.1. Iberian Desman and Riparian Forest

Here, photointerpretation area was restricted to the length of stream where Iberian desman was prospected. We evaluated relationships between Iberian desman presence and RIVs for the two

study years (2014 and 1995) at site scale using logistic regression and Iberian desman presence as dependent variable. Since we had available information from 2014 data on the spatial location of Iberian desman scats' presence, we also built generalized linear mixed-effects models using such variable as dependent variable, and did so from 50 to 200 meters scales. For each model, Iberian desman scats' presence was used as dependent variable and site was used as random effect. To build these models, Riparian Category, Presence of Canopy, Longitudinal Connectivity, Width Sum and Total Width were the explanatory variables, which were scaled. Tests were considered significant at $\alpha = 0.05$.

2.3.2. Co-occurring Species and Iberian Desman

We used Iberian desman scats' number and presence and scaled these variables. We averaged fish abundance by site, dividing the total number of individuals captured at each site by the total number of sampling periods performed in that site. Relationships were assessed through generalized linear modelling, with Iberian desman presence and scats' number as explanatory variables and total number of species, number of native species, abundance and abundance of native species as dependent variables. Relationships between Iberian desman and amphibians and reptiles were assessed through generalized linear modelling, with Iberian desman presence and scats' number as explanatory variables and total number of species and number of endemic species as dependent variables. Tests were considered significant at $\alpha = 0.1$ to reduce the likelihood of a type II error which would be more likely with $\alpha = 0.05$ because of small sample size (Bryant 2004).

2.3.3. Co-occurring Species and Riparian forest

We used redundancy analyses to evaluate the variation of co-occurring species that can be explained by RIVs (explanatory variables) with R[®] (R Core Team 2015) and vegan package (Oksanen *et al.* 2015). Here, we evaluated sites only with the co-occurring species - fish (n=15) and amphibian and reptile (n=51). As the original species data included many zeros and rare species in abundance data, we applied the Hellinger transformation to both groups (Legendre & Gallagher 2001). Given the correlation between RIVs, we used a forward selection function to determine the subset of explanatory variables for constrained ordination. We based our selection on this process and the global significance of the model, with significance being defined at the 10% level ($P = 0.1$). The significance of the relationship between species variables and RIVs, as well as axes significance was evaluated through the application of a global Montecarlo permutation test (999 permutations). Correlations between each axis and RIVs were considered significant ($P < 0.1$) at a critical value of $r = 0.23$ for amphibians and reptile data and $r = 0.44$ for fish data.

2.3.4. Variation in Riparian Forest Integrity between 1995 and 2014

Variation in RIVs between 1995 and 2014 was evaluated in the 40 sites sampled in both study years. For this temporal evaluation, photointerpretation area was larger, built from two 500 meters circular buffers placed at the beginning and ending point of each stream sampled in the field, taking into account the Iberian desman's average home range of $523 \pm 50,85$ meters of river (Nores 2012). As RIVs were moderately correlated, we joined values from both margins that were assessed separately, in order to reduce and simplify the subset of variables to test. For Longitudinal Connectivity both margins were joined through mean. Presence of Shrub and Presence of Canopy, were categorized with 0 = absence on both sides; 1 = presence on one side; and 2 = presence on both sides. Riparian Vegetation Width (hereafter Width Sum) was joined through the sum of both margins' values. Given that the two samples of 1995 and 2014 did not meet the assumptions of normality and homogeneity of variance, differences were assessed with non-parametric Wilcoxon Signed-Rank Test. Tests were considered significant at $\alpha = 0.05$.

2.4. Results

2.4.1. Iberian Desman and Riparian Forest

Iberian desman presence in the 2014 at site scale was associated to Riparian Category, Longitudinal Connectivity, Presence of Canopy, and Width Sum, with the first three having the strongest relationships (Table 1). Longitudinal Connectivity and Iberian desman presence in the 1995 were also positively correlated (Table 1). Fine-scale Iberian desman scats' presence (2014) was positively related to Riparian Category, Presence of Canopy, Longitudinal Connectivity and Width Sum (Table 2). These relationships were found from 100 to 200 meters scale, with Riparian Category and Longitudinal Connectivity having stronger relationships. At 50 meters scale, only Width Sum presented significant relationships. There were no relationships between Total Width and Iberian desman presence and scats' presence at any scale.

TABLE 1: RELATIONSHIPS ASSESSED THROUGH LOGISTIC REGRESSION BETWEEN DEPENDENT VARIABLE IBERIAN DESMAN PRESENCE AND EXPLANATORY VARIABLES RIVS AT SITE SCALE AND REFERRED TO THE TWO SAMPLING SEASONS (1995 AND 2014). SIGNIFICANT RESULTS AT ALPHA = 0.05 IN BOLD

	1995			2014		
	<u>Iberian Desman Presence</u> (df = 39)			<u>Iberian Desman Presence</u> (df = 70)		
Riparian Category	P = 0.18	Estimate = 0.46	Variance = 23.84	P < 0.001	Estimate = 1.26	Variance = 15.59
Canopy Presence	P = 0.51	Estimate = 0.23	Variance = 2.16	P < 0.001	Estimate = 1.28	Variance = 44.30
Longitudinal Connectivity	P < 0.05	Estimate = 0.78	Variance = 62.75	P < 0.005	Estimate = 1.17	Variance = 17.08
Width Sum	P = 0.44	Estimate = 0.39	Variance = 5.48	P < 0.005	Estimate = 0.77	Variance = 17.19
Total Width	P = 0.27	Estimate = 0.38	Variance = 5.77	P = 0.53	Estimate = 0.19	Variance = 5.84

TABLE 2: RELATIONSHIPS ASSESSED THROUGH LOGISTIC REGRESSION BETWEEN DEPENDENT VARIABLE IBERIAN DESMAN SCATS PRESENCE AND EXPLANATORY VARIABLES RIVS AT 50-200 M SCALE AND REFERRED TO 2014 SAMPLING SEASON. SIGNIFICANT RESULTS AT ALPHA = 0.05 IN BOLD

	<u>Scale (meters)</u>		<u>Scats' Presence</u>
Riparian Category	<u>50</u>	P = 0.06	Estimate = 0.96
	<u>100</u>	P < 0.01	Estimate = 1.66
	<u>200</u>	P < 0.005	Estimate = 1.63
Presence of Canopy	<u>50</u>	P = 0.61	Estimate = 0.28
	<u>100</u>	P < 0.05	Estimate = 1.36
	<u>200</u>	P = 0.005	Estimate = 1.35
Longitudinal Connectivity	<u>50</u>	P = 0.34	Estimate = 0.40
	<u>100</u>	P < 0.005	Estimate = 1.55
	<u>200</u>	P < 0.005	Estimate = 1.75
Total Width	<u>50</u>	P = 0.16	Estimate = 0.43
	<u>100</u>	P = 0.19	Estimate = 0.51
	<u>200</u>	P = 0.27	Estimate = 0.70
Width Sum	<u>50</u>	P < 0.001	Estimate = 0.85
	<u>100</u>	P < 0.001	Estimate = 1.52
	<u>200</u>	P < 0.001	Estimate = 1.54

2.4.2. Co-occurring Species and Iberian Desman

The number of species and native species of fish were negatively associated to Iberian desman scats' number (Table 3). Abundance of native species and abundance of species of fish and Iberian desman presence and scats' number were also negatively related. Number of endemism of amphibian and reptile species and Iberian desman scats' number were positively associated (Table 4). Close analyses at a species level revealed positive relationships between presence of Iberian frog presence and Iberian desman presence and scats' number and between Iberian emerald lizard presence and Iberian desman scats' number (Table 4).

TABLE 3: RELATIONSHIPS ASSESSED THROUGH LOGISTIC REGRESSION BETWEEN DEPENDENT FISH VARIABLES AND EXPLANATORY VARIABLES OF THE IBERIAN DESMAN, THE *UMBRELLA* SPECIES CANDIDATE, AT SITE SCALE. SIGNIFICANT RESULTS AT ALPHA = 0.1 IN BOLD

<u>Fish</u>		<u>Scats' number</u>			<u>Iberian desman Presence</u>
Number of Native Species	P < 0.05	Estimate = -4.58	Variance =	P = 1.00	Estimate = -9.85 Variance =
		24.82			75.18
Number of Species	P < 0.05	Estimate = -4.77	Variance =	P = 1.00	Estimate = -9.92 Variance =
		24.91			75.09
Abundance	P < 0.1	Estimate = -12.57	Variance =	P < 0.01	Estimate = -17.37 Variance
		26.21			= 73.79
Abundance of Native Species	P < 0.01	Estimate = -10.59	Variance =	P < 0.05	Estimate = -14.63 Variance
		26.20			= 73.80

TABLE 4: RELATIONSHIPS ASSESSED THROUGH LOGISTIC REGRESSION BETWEEN DEPENDENT AMPHIBIANS AND REPTILES VARIABLES AND EXPLANATORY VARIABLES OF THE IBERIAN DESMAN, THE *UMBRELLA* SPECIES CANDIDATE, AT SITE SCALE. SIGNIFICANT RESULTS AT ALPHA = 0.1 IN BOLD

<u>Amphibians and reptiles</u>		<u>Scats' number</u>			<u>Iberian desman Presence</u>
Number of Species	P = 0.62	Estimate = 0.04	Variance =	P = 0.93	Estimate = 0.01 Variance = 27.83
		72.17			
Endemic Number	P < 0.1	Estimate = 0.21	Variance =	P = 0.20	Estimate = 0.18 Variance = 29.31
		70.69			
Iberian frog Presence	P < 0.1	Estimate = 0.60	Variance =	P < 0.001	Estimate = 1.01 Variance =
		17.00			83.00
Iberian emerald lizard Presence	P < 0.1	Estimate = 0.64	Variance =	P = 0.18	Estimate = 0.60 Variance = 26.84
		73.16			

2.4.3. Co-occurring species and Riparian forest

Riparian forest variables explained 22.62 % of the variance in fish abundance, abundance and number of native species and number of species, using axis 1 alone. The cumulative variance explained by axes 1 and 2 was 32 % (Fig. 2). The relation between species and RIVs was significant for the first axis (eigenvalue = 0.07, $P = 0.008$) and for the global model ($F = 1.45$, $P = 0.067$). Width sum was almost correlated with axis 1 ($r = 0.35$); Longitudinal Connectivity was correlated with axis 2 ($r = 0.43$). In the ordination, abundance of native species and three native species were clustered on the negative side of axis 1, which was characterized by higher Total Width and lower Width Sum. Number of species clustered on the positive side of axis 1, characterized by higher Width Sum. *Squalius alburnoides* (Steindachner, 1866), which has a vulnerable conservation status (Annex 3.2; <http://www.iucnredlist.org/>), and number of native species clustered on the positive side of axis 2, characterized by higher Longitudinal Connectivity. In addition to significant correlations with environmental axes, several environmental variables were correlated. Presence of Shrub was correlated with Longitudinal Connectivity ($r = 0.55$) and the latter with Width Sum ($r = 0.44$). Presence of Canopy and Width Sum were positively correlated ($r = 0.48$).

Presence of Shrub, which was significantly correlated with axis 3. In addition to significant correlations with environmental axes, environmental variables were highly and positively correlated between them.

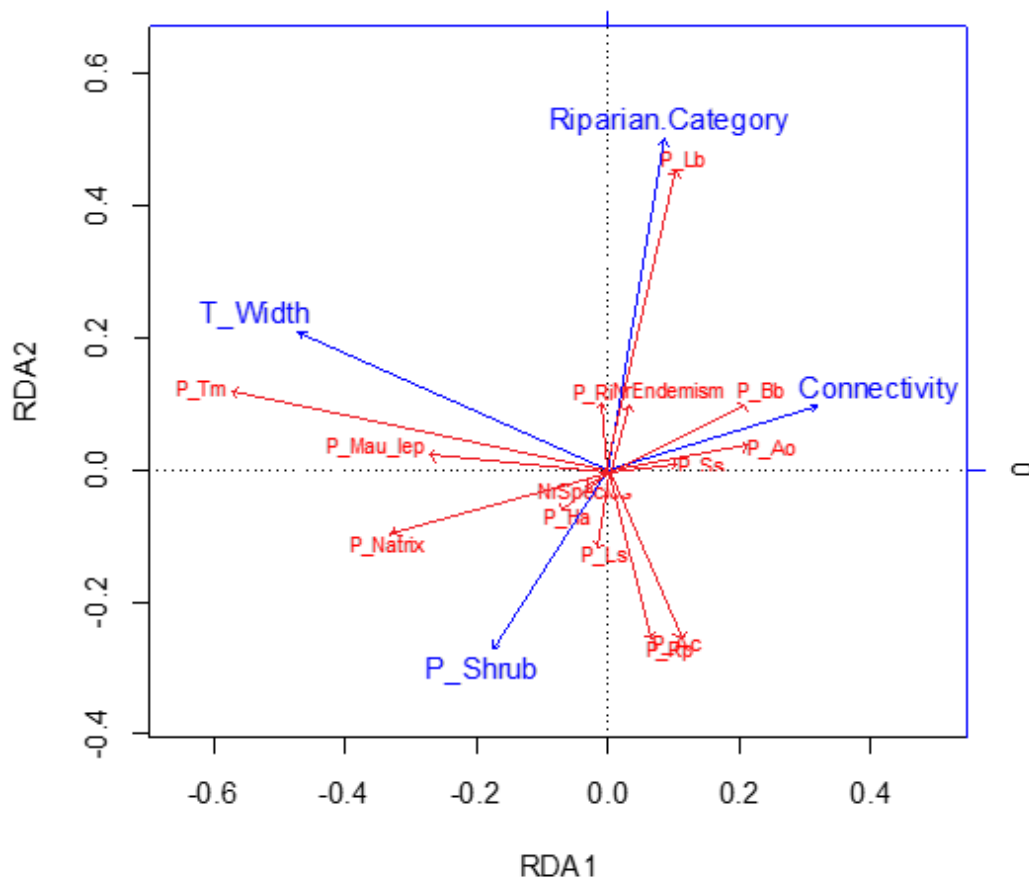


FIGURE 3: REDUNDANCY ANALYSES OF PRESENCE OF AMPHIBIAN AND REPTILE (RED) AND RIPARIAN INTEGRITY VARIABLES (BLUE). LONGITUDINAL CONNECTIVITY INCREASES TOWARDS 0.4 AND TOTAL WIDTH INCREASES TOWARDS -0.6. AXIS 2: RIPARIAN CATEGORY INCREASES TOWARDS 0.6. PRESENCE OF AMPHIBIAN: AC *ALYTES CISTERNASII*, LS *LISSOTRITON BOSCAI*, RI *RANA IBERICA*, HA *HYLA ARBOREA*, Ss *SALAMANDRA SALAMANDRA*, AO *ALYTES OBSTETRICANS*, Bb *BUFO BUFO*, Tm *TRITURUS MARMORATUS*, Rp *RANA PEREZII (PELOPHYLAX PEREZI)*. PRESENCE OF REPTILE: LS *LACERTA SCHREIBERI*, MAU LEP *MAUREMYS LEPROSA*, NATRIX *NATRIX SP.* RIPARIAN INTEGRITY VARIABLES: P_SHRUB PRESENCE OF SHRUB, CONNECTIVITY LONGITUDINAL CONNECTIVITY, T_WIDTH TOTAL WIDTH, RIPARIAN CATEGORY.

2.4.4. Variation in Riparian Forest Integrity between 1995 and 2014

Riparian Category, Longitudinal Connectivity, Presence of Canopy and Shrub were significantly higher in 2014 than in 1995 (Table 5, Figs. 4-6). This difference was detected at 50, 100, 200 and 500 meters scales, but not for the site scale, with the exception of Riparian Category (Table 1). Total Width and Width Sum did not vary between 1995 and 2014, with the exception of Width Sum

at 50 meters scale (Table 5). Between 50-200 meters scales, Presence of Canopy was the variable with strongest differences among the two sampling years, while at 500 m scale the larger difference concerned the Riparian Category (Table 5). A table with variation of RIVs at a site scale between both years is present in Annexe 1.2.

TABLE 5: ANALYSIS OF POPULATION DIFFERENCES ON RIVS BETWEEN 1995 AND 2014 AT DIFFERENT SCALES USING WILCOXON SIGNED-RANK TEST. SIGNIFICANT RESULTS AT ALPHA = 0.05 IN BOLD.

<u>Scale</u> <u>(meters)</u>	<u>Riparian Category</u>	<u>Longitudinal</u> <u>Connectivity</u>	<u>Presence of Canopy</u>	<u>Presence of Shrub</u>	<u>Width Sum</u>	<u>Total Width</u>						
Total (site)	P < 0.05	V = 49.5	P = 0.06	V = 103.5	P = 0.09	V = 10	P = 0.09	V = 136	P = 1	V = 0	P = 0.15	V = 6
500	P < 0.001	V = 8322	P < 0.001	V = 13206	P < 0.001	V = 2998.5	P < 0.01	V = 1254	P = 0.45	V = 414.5	P = 0.73	V = 1406
200	P < 0.001	V = 27543	P < 0.001	V = 49058	P < 0.001	V = 18904	P < 0.01	V = 2683	P = 0.93	V = 5696.5	P = 0.51	V = 5510
100	P < 0.001	V = 116120	P < 0.001	V = 181220	P < 0.001	V = 74794	P < 0.001	V = 5417.5	P = 0.81	V = 26205	P = 0.33	V = 17082
50	P < 0.001	V = 292520	P < 0.001	V = 523190	P < 0.001	V = 297260	P < 0.01	V = 4021.5	P < 0.05	V = 125890	P = 0.78	V = 48066

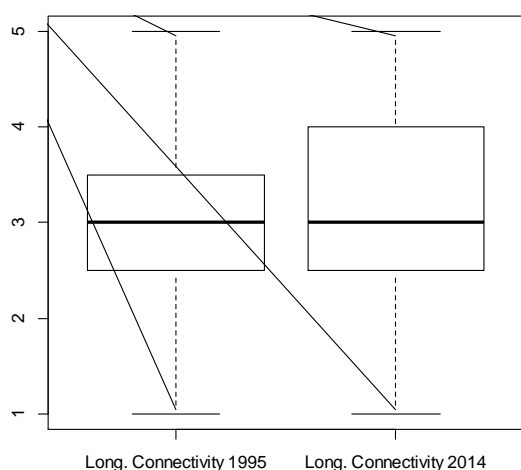


FIGURE 4: BOXPLOTS OF THE MEDIAN PRESENCE OF LONGITUDINAL CONNECTIVITY VALUES IN THE TWO SAMPLING YEARS, THE 1995 (LEFT) AND 2014 (RIGHT) AT 50 METERS SCALE. THE LOWER LIMIT OF THE BOX REPRESENTS THE 1ST QUARTILE AND THE UPPER LIMIT THE 3RD QUARTILE. WHISKERS REPRESENT THE MINIMUM AND MAXIMUM VALUE OF LONGITUDINAL CONNECTIVITY.

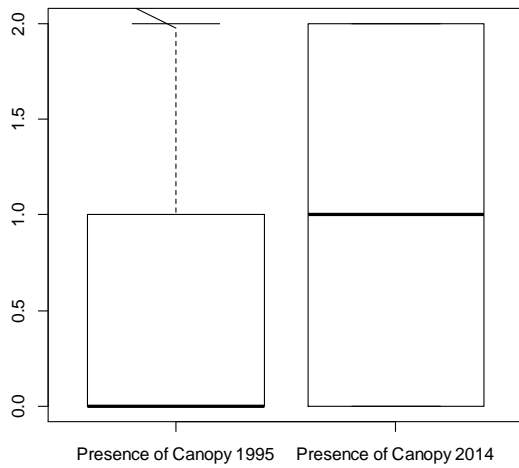


FIGURE 5: BOXPLOTS OF THE MEDIAN PRESENCE OF CANOPY VALUES IN THE TWO SAMPLING YEARS, THE 1995 (LEFT) AND 2014 (RIGHT) AT 100 METERS SCALE. THE LOWER LIMIT OF THE BOX REPRESENTS THE 1ST QUARTILE AND THE UPPER LIMIT THE 3RD QUARTILE. WHISKERS REPRESENT THE MINIMUM AND MAXIMUM VALUE OF PRESENCE OF CANOPY.

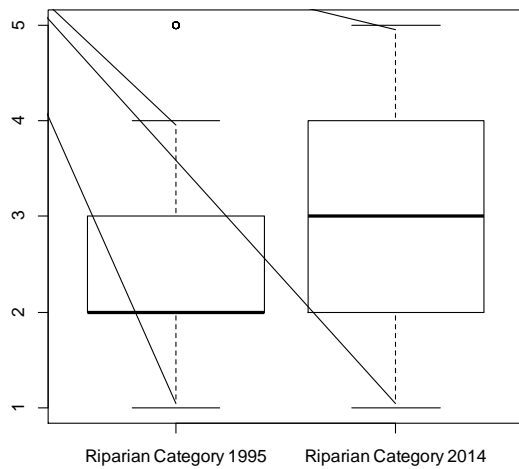


FIGURE 6: BOXPLOTS OF THE MEDIAN RIPARIAN CATEGORY VALUES IN THE TWO SAMPLING YEARS, THE 1995 (LEFT) AND 2014 (RIGHT) AT 500 METERS SCALE. THE LOWER LIMIT OF THE BOX REPRESENTS THE 1ST QUARTILE AND THE UPPER LIMIT THE 3RD QUARTILE. WHISKERS REPRESENT THE MINIMUM AND MAXIMUM VALUE OF RIPARIAN CATEGORY. THE DOT REPRESENTS AN OUTLIER.

2.5. Discussion

The *Umbrella* species concept was defined a long time ago, but there's still debate on its actual efficiency (e.g., Branton & Richardson 2011; Favreau *et al.* 2006; Sergio *et al.* 2008). The applicability of the *Umbrella* species concept on habitat restoration has been rarely tested, with assessments generally happening only after restoration was performed (Branton & Richardson 2014). Here, we tested the riparian forest as a specialized ecological requirement of the Iberian desman with encouraging results, since the Iberian desman seemed to present a strong relationship with most RIVs. Also, at a fine-scale most RIVs did not have any relationship with the Iberian desman, suggesting the importance of preserving riparian vegetation integrity at large scales. Higher values of RIVs at large scale, likely reflecting enhanced riparian forest integrity, may represent for the Iberian desman the continuous presence of water (Stella *et al.* 2012), lower water temperatures, more food resources and availability of marking and resting sites (Melero *et al.* 2012; Nores 2012).

The relationship between Iberian desman presence and riparian forest integrity was stronger in 2014 than in 1995, with more RIVs being associated to the Iberian desman presence in the former study period. In 1995 the Longitudinal Connectivity was the only variable with relationship found with the Iberian desman. Although it is likely that several other factors are affecting the decrease in Iberian desman populations, we argue that, while the human threats are increasing and the populations are getting smaller, the species seems to presently occur in habitats with better conditions and well-preserved riparian forest, whereas in 1995, the populations were spread across more heterogeneous areas.

Focusing on a particular species is not sufficient to maintain biodiversity and other ecosystem-scale attributes for the long-term (Naiman & Rogers 1997). Based on this assumption, we tested relationships between *Umbrella* and co-occurring species, attempting to obtain useful outcomes for the whole riparian ecosystem. While negative relationships were found between fish and Iberian desman presence and scats' number, positive relationships between the latter and number of endemic species of amphibians and reptiles were found and a closer look into single species data showed significant relationships with two endemic species – the Iberian frog and the Iberian emerald lizard (Annex 3.1). These species are often sympatric and share some habitat requirements with the Iberian desman, inhabiting places with abundant riparian forest and suffering similar threats, such as riparian forest cuts and alterations, dam construction and pollution (Loureiro *et al.* 2008). This appears to confirm that taxonomic similarity does not seem to be as important to the effectiveness of this concept as similar habitat features or habitat requirements (Seddon & Leech 2008). The negative relationship between Iberian desman and fish can reflect at least partially some variation in the distribution of both groups, as the Iberian desman is usually confined to small

mountain streams (Pedroso & Chora 2014) with small depth (Nores 2012), while fish have, in general, a wider distribution and great adaptability to different ecological conditions (Gonçalo 2014). In addition, with the exception of *Salmo trutta* and *Chondrostoma duriense*, most fish present in our data can be found in more downstream portions of the river (Gonçalo 2014).

When analysing relationships between co-occurring species and Iberian desman, we found that some relationships were significant with number of scats and not with presence of Iberian desman, and that abundance detected relationships better than number of species. This indicates that more detailed metrics may provide a stronger understanding of the relationships between species. Indeed, Branton & Richardson (2014) already showed that more sensitive metrics increase resolution on the efficiency assessment of the *Umbrella* species approach.

We tested if these co-occurring species also benefited of riparian forest in a well preserved state. RIVs explained some variation in fish data, with number of native species and the threatened *Squalius alburnoides* appearing to be associated with Longitudinal Connectivity. *Squalius alburnoides* has a vulnerable status and has been recognized to be less frequent in highly degraded rivers (Gonçalo 2014). It seems that three native species - *Squalius alburnoides*, *Chondrostoma duriensis* and *Barbus bocagei* were related to riparian Total width, which includes the river width. This can be related with the three being considered flexible species in terms of environmental conditions and with *Chondrostoma duriensis* and *Barbus bocagei* being generally found in medium to final portions of rivers, but also in reservoirs (Gonçalo 2014). The total number of fish species was associated with Total Riparian Width, indicating that we find more fish species where riparian forest is larger. Thus, fish and Iberian desman appear to share management needs concerning the riparian forest width. The riparian forest is known to enhance the physical heterogeneity that supports many groups of fish (Coelho *et al.* 2014) and previous studies showed that removal of trees and increase in deforested patches were associated with decreased fish abundance (Dale Jones III *et al.* 1999).

RIVs explained low variation in amphibians and reptile data. Therefore, our study provided a mere indication that these co-occurring species might have a positive relationship with RIVs, with number of endemic species being associated with Continuity of the riparian forest. Presence of several species of amphibian and reptile seemed to have relationship with Longitudinal Connectivity, Presence of Shrub and Total Riparian Width, evidencing the possible importance of different RIVs. Indeed, this seems consistent with the knowledge that amphibians use both freshwater and forest components of riparian habitats, with different uses between different members of assemblages (Hannon *et al.* 2002). In addition, reptiles often live and forage in aquatic habitats most of the year but migrate to upland habitats to nest or overwinter (Semlitsch & Bodie 2003).

Notably, we found differences in the riparian forest over the last 20 years. The results of the analyses comparing the RIVs distributions in the two sampling years suggested that vegetation is in a better state in 2014 than it was in 1995. This may be partly due to low population density (Silva 2010) and decreasing agricultural activity in rural areas (Stella *et al.* 2012). At a site scale, this difference was only detected in Continuity of the riparian forest, indicating that this difference and improvement in riparian forest integrity is still restricted to small areas, comparing with River basin scale. With the exception of Total Riparian Width at 50 m scale, we did not detect changes in Width related variables, which seems to be consistent with the extent and deepness of the valley where the Sabor River basin is mostly placed (Asensi *et al.* 2011). Indeed, the high slope and its heterogeneous topography are likely to naturally maintain the riparian forest in contained strips.

2.5.1. Management Implications

This study was a novelty as regard to Portugal freshwater ecosystems, having important implications for the conservation and management of the riparian forest, which provides a number of services and overall habitat diversity (Dale Jones III *et al.* 1999) to the Iberian desman and many other species. We provided new insights on the efficiency of the Iberian desman as an *Umbrella* species, indicating an association between this species and its habitat with listed and native species of fish and endemic species of amphibians and reptiles. However, focusing only on the Iberian desman may not provide conservation of all species, this being especially true for fish, highlighting the need for a suite of complementary studies of conservations planning scenarios. We call for future studies to assess more co-occurring species, such as macroinvertebrates - commonly used as bio indicators (Colin *et al.* 2015) and Iberian desman's main food resource (Santamarina 1993) - and evaluate different environmental variables between groups, such as degrees of disturbance or water quality.

The Sabor River basin, mainly in its northern region, presents a good ecological status (Portela 2014). Our study reveals consistent results when assessing riparian forest integrity. It provided interesting insights, indicating that the Iberian desman may hold potential to guide restoration planning, which could be necessary in a near future, considering the expectable increase in human pressure on the riparian ecosystems of the Sabor River Basin due to the construction and long-term impacts of the Lower Sabor Hydroelectric Plant. Particularly, we advocate precautionary actions targeted to the restoration of the connectivity and canopy cover of the riparian forest. In this regard, our findings could be used for future management planning to select priority areas for conservation in Mediterranean regions, preserving the riparian forest as a mechanism by which the *Umbrella* species benefits co-occurring species. Also, the natural riparian forest width must be preserved and a buffer of land should be designed, as previously pointed out by others (e.g., Stoffyn-Egli & Willison 2011), to limit human activities, such as agriculture, around the riparian

forest. This type of effective integrative approach is important to protect freshwater ecosystems and should be evaluated in other parts of Mediterranean regions, pursuing regional conservation and restoration planning.

2.6. Acknowledgements

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3. Final Remarks

The *Umbrella* species concept was defined a long time ago, but its actual efficiency is still under debate (e.g., Branton & Richardson, 2011; Favreau *et al.* 2006; Sergio *et al.* 2008). Here, we tested the riparian forest as a specialized ecological requirement of the Iberian desman with encouraging results. We documented an association between Iberian desman occurrence and riparian forest integrity and between these two and listed and native species of fish and endemic species of amphibians and reptiles, supporting the efficiency of the Iberian desman as an *Umbrella* species in the Sabor River basin. This study was a novelty as regard to Portugal freshwater ecosystems, and may have important implications for the conservation and management of the riparian forest, which provides a number of services and overall habitat diversity (Dale Jones III *et al.* 1999) to the Iberian desman and many other species.

3.1. Iberian Desman and Riparian Forest

We found a stronger relationship between the Iberian desman and riparian forest in 2014 than in 1995. Although it is likely that several other factors are affecting the decrease in Iberian desman populations, we argue that, while the threats are increasing and the populations are getting smaller, the species occurs in habitats with better conditions and well-preserved riparian forest, whereas in 1995, the populations were spread across more heterogeneous areas. Riparian forest at large scale may represent for the Iberian desman the continuous presence of water (Stella *et al.* 2012), lower water temperatures, more food resources and availability of marking and resting sites (Melero *et al.* 2012; Nores 2012). At a fine-scale, relationships between riparian forest and the Iberian desman were less evident, suggesting that future restoration and selection of priority areas for conservation of riparian forest, intended to protect this species, should be performed at large scale.

3.2. Co-occurring Species and Iberian Desman

Positive relationships between the Iberian desman and amphibians and reptiles were found and a closer look into single species data showed significant relationships with two endemic species – the Iberian frog and the Iberian emerald lizard. These species are often sympatric and share some habitat requirements with the Iberian desman, inhabiting places with abundant riparian forest and suffering similar threats, such as riparian forest cuts and alterations, dam construction and pollution (Loureiro *et al.* 2008). On the other side, negative relationships were found between fish and Iberian desman, which can reflect, at least partially, some variation in the distribution of both groups, as the Iberian desman is usually confined to small mountain streams (Pedroso & Chora 2014) with small depth (Nores 2012), while fish have, in general, a wider distribution and great adaptability to different ecological conditions (Gonçalo 2014). Considering that fish data were from

a different sampling year and restricted to few common sites, however, further studies are needed to better assess the nature of this relationship. Amphibian and reptile data presented less limitations (with the exception of the small percentage of presence of the Iberian emerald lizard - Annex 3.1), but further studies are required also in this case to confirm and better understand the relationship of these groups with the Iberian desman.

Our results seem to confirm findings from a previous study from Branton & Richardson (2014), indicating that more sensitive and detailed metrics may provide a stronger understanding of relationships between species and increase resolution on the efficiency assessment of the *Umbrella* species approach. This should be reflected in future studies, which should include sampling and calculation of biomass and abundance whenever possible.

3.3. Co-occurring Species and Riparian forest

Number of native species and a threatened species of fish appeared to be associated with riparian forest integrity, with more species of fish found where the riparian forest has well-preserved riparian forest width. Fish and Iberian desman seem to share management needs concerning the riparian forest. Indeed, the riparian forest is known to enhance the physical heterogeneity that supports many groups of fish (Coelho *et al.* 2014) and previous studies showed that removal of trees and increase in deforested patches were associated with decreased fish abundance (Dale Jones III *et al.* 1999).

RIVs explained low variation in amphibians and reptile data. Therefore, our study provides a slight indication that these co-occurring species, and particularly endemic species, might have a relationship with riparian forest integrity. This seems consistent with the knowledge that amphibians use both freshwater and forest components of riparian habitats, with different uses between different members of assemblages (Hannon *et al.* 2002). Reptiles often live and forage in aquatic habitats most of the year but migrate to upland habitats to nest or overwinter (Semlitsch & Bodie 2003).

3.4. Variation in Riparian Forest Integrity Between 1995 and 2014

Notably, we found variation in the riparian forest integrity of the Sabor River basin over the last 20 years. Vegetation appeared to be in a better state in 2014 than it was in 1995, though this improvement does not seem to be evident at large scale. Further studies are required to better assess these changes, particularly land use and cover changes and its influence at a large scale. The lack of changes in Width related variables, seems to be consistent with the extent and deepness of the valley of the Sabor River. Indeed, the high slope and its heterogeneous topography are likely to naturally maintain the riparian forest in contained strips. Future management actions must take

this into account, restoring the continuity and connectivity longitudinally and maintaining the natural width of the riparian forest. We suggest a future close monitoring of the riparian forest communities, including field sampling to assess structure, quality and composition of present riparian forest. This detailed sampling will allow more informed land management decisions in the future.

Our study suggests that the riparian forest of the Sabor River basin is in a well-preserved state, which is consistent with what has been reported elsewhere for the same river basin (Portela 2014). This is partly due to the low population density (Silva 2010) and decreasing agricultural activity in rural areas (Stella *et al.* 2012). This study allowed us to discover features about the integrity and dynamics of riparian forest that may have implications for stream and watershed management. Our findings could be used for future management planning to select priority areas for conservation, preserving the riparian forest as a mechanism by which the *Umbrella* species benefits co-occurring species. Considering that some of the analysed areas of the Sabor River basin revealed a contrasting pattern, with a more degraded riparian vegetation, we advocate precautionary actions targeted to the restoration of the connectivity and canopy cover of the riparian forest. Particularly, the natural riparian forest width must be preserved and a buffer of land should be designed (e.g., Stoffyn-Egli & Willison 2011) to keep human activities, such as agriculture, limited around the riparian forest. Noteworthy, our sampling of Riparian Integrity Variables was used as a first indication for selection of areas with less riparian integrity in the Sabor River basin, with the ultimate intention to restore the riparian forest within mitigation and minimization measures resulting from the construction of the Lower Sabor Hydroelectric Plant.

3.5. Management Implications

The presence of Iberian desman seems to be decreasing in practically all of its geographic range (Igea *et al.* 2013). The range of this species is strongly related to climate and climate change will most likely constitute a major threat (Morueta-Holme *et al.* 2010). This way, the threatened status of the Iberian desman make it a good candidate as an *Umbrella* species, but it is important that we increase our efforts to understand more about this species populations, causes of decline and ecological requirements.

Considering the expectable increase in human pressure, and to limit more degradation of riparian forests, our study provided valuable insights as the Iberian desman may hold potential to guide restoration planning. However, focusing only on the Iberian desman may not provide conservation of all considered species, including fish species, which highlights the need for a suite of complementary studies of conservations planning scenarios. We highlight the need of future studies assessing more co-occurring species, such as macroinvertebrates - commonly used as a bio

indicator (Colin *et al.* 2015) and Iberian desman's main food resource (Santamarina 1993) - and evaluate different environmental variables between groups, such as degree of disturbance or water quality.

This type of effective integrative approach is important to protect freshwater ecosystems and should be evaluated in other parts of Mediterranean regions. Besides, these findings, despite its limitations, may help design studies to evaluate new management practices to retain riparian habitats and its biodiversity in other parts of the Mediterranean. The riparian forest in Mediterranean regions is considered a habitat of high value for conservation, but it is vulnerable to the cumulative and increasing human impacts, such as agriculture, streamflow regulation, nutrient enrichment, fire, grazing and climate change (Stella *et al.* 2012). Moreover, current policies for the maintenance of riparian buffers in Mediterranean countries mostly fail in preserving enough terrestrial habitat for riparian obligate animal species (Stoffyn-Egli & Willison 2011). Thus, there's a pressing need for an overall protection of riparian ecological functions and biodiversity.

3.6. References

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4. Annexes

Annex 1 - Integrity of Riparian Forest

Annex 1.1 - Definition of Riparian Integrity Variables

TABLE 1: CHARACTERIZATION OF RIPARIAN INTEGRITY VARIABLES, THROUGH A SUMMARY DESCRIPTION OF THE SAMPLING PROCEDURES AND ASSIGNED VALUES

<u>Riparian Integrity Variables</u>	<u>Value</u>	<u>Description</u>
<u>(RIVs)</u>		
Riparian Category	Riparian continuity and area with vegetation	
	1	Absent or sparse, with vegetation reduced to isolated trees or shrubs
	2	Semi-continuous on one margin and absent or sparse on the other, with vegetation appearing in small patches and significantly fragmented
	3	Semi-continuous on both margins, with vegetation corridor moderately fragmented
	4	Continuous on one margin and semi-continuous on the other, with vegetation covering the full length of the segment but slightly fragmented
	5	Continuous on both margins, no visible fragmentation of vegetation
Riparian Vegetation Width	Recorded and averaged from several measurements taken randomly along each 50m sample, assessing right and left margins separately	

1	Width < 10 m
2	Width between 10 and 30m
3	Width > 30 m

Total Width (including the river channel)	Assessed from left to right margin
1	Width < 10 m
2	Width between 10 and 30m
3	Width > 30 m

Presence of Canopy	Presence of Canopy, assessing right and left margins separately
0	Absent
1	Present

Presence of Shrub	Presence of Shrub, assessing right and left margins separately
0	Absent
1	Present

Longitudinal Connectivity	Percentage of bank length covered by riparian forest, assessing right and left margins separately
1	< 30% (Bad)
2	30-50% (Deficient)
3	50-70% (Moderate)
4	70-90% (Good)

5 > 90% (Very Good)

Annex 1.2 - Variation in Riparian Integrity Variables between 1995 and 2014

TABLE 2: MEAN AND STANDARD DEVIATION OF RIPARIAN INTEGRITY VARIABLES AT A SITE SCALE, IN 1995 AND 2014

<u>Riparian Integrity Variables (RIVs)</u>	<u>1995</u>	<u>1995</u>	<u>2014</u>	<u>2014</u>
	<u>Mean</u>	<u>Standard</u>	<u>Mean</u>	<u>Standard</u>
		<u>Deviation</u>		<u>Deviation</u>
Riparian Category	2,75	0,95	3,21	1,03
Riparian Vegetation Width	2,15	0,53	2,35	0,68
Total Width (including the river channel)	1,93	0,53	1,85	0,55
Presence of Canopy	0,25	0,49	0,62	0,80
Presence of Shrub	1,65	0,74	1,93	0,26
Longitudinal Connectivity	2,61	0,65	3,04	0,76

Annex 2 - Variation in Iberian desman Presence between 1995 and 2014

TABLE 3: PERCENTAGE OF IBERIAN DESMAN PRESENCE IN 1995 AND 2014 IN, RESPECTIVELY, 40 AND 71 SITES OF THE SABOR RIVER BASIN (NORTHERN PORTUGAL)

<u>Iberian desman</u>	<u>Presence (%)</u>
1995	60,00
2014	19,72

Annex 3 - Definition of Co-occurring Species

Annex 3.1 - Characterization of Amphibian and Reptile Species

TABLE 4: PERCENTAGE OF PRESENCE AND STANDARD DEVIATION OF AMPHIBIAN AND REPTILE SPECIES OCCURRING IN THE SAME SITES (RIVERS AND STREAMS OF THE SABOR RIVER BASIN) OF THE IBERIAN DESMAN, WITH REFERENCE TO CONSERVATION STATUS (ACCORDING TO IUCN RED LIST) AND TYPE OF OCCURRENCE (ACCORDING TO ICNF)

<u>Amphibian and Reptile Species</u>	<u>Presence</u> (%)	<u>Standard</u> <u>Deviation</u>	<u>Conservation Status</u>	<u>Type of Occurrence</u>
<i>Alytes cisternasii</i> (Boscá, 1879)	17,65	0,39	Near Threatened	Iberian endemism
<i>Alytes obstetricans</i> (Laurenti, 1768)	15,69	0,37	Least Concern	Resident
<i>Bufo bufo</i> (Linnaeus, 1758)	35,29	0,48	Least Concern	Resident
<i>Hyla arborea</i> (Linnaeus, 1758)	3,92	0,20	Least Concern	Resident
<i>Lissotriton boscai</i> (Lataste, 1879)	17,65	0,39	Least Concern	Iberian endemism
<i>Rana iberica</i> (Boulenger, 1879)	33,80	0,48	Near Threatened	Iberian endemism
<i>Pelophylax perezi</i> (Seone, 1885)	86,27	0,35	Least Concern	Resident
<i>Salamandra salamandra</i> (Linnaeus, 1758)	7,84	0,27	Least Concern	Resident
<i>Triturus marmoratus</i> (Latreille, 1800)	25,49	0,44	Least Concern	Resident
<i>Natrix sp.</i>	23,53	0,43	Least Concern	Resident
<i>Mauremys leprosa</i>	19,61	0,40	Not Assessed	(Least Resident)

(Schweigger, 1812)			Concern in Portugal)
<i>Lacerta schreiberi</i> (Bedriaga, 1878)	7,84	0,27	Not Assessed (Least Iberian endemism Concern in Portugal)

Annex 3.2 - Characterization of Fish Species

TABLE 5: ABUNDANCE MEAN AND STANDARD DEVIATION OF FISH SPECIES OCCURRING IN THE SAME SITES (RIVERS AND STREAMS OF THE SABOR RIVER BASIN) OF THE IBERIAN DESMAN, WITH REFERENCE TO CONSERVATION STATUS (ACCORDING TO IUCN RED LIST) AND TYPE OF OCCURRENCE (ACCORDING TO ICNF)

<u>Fish Species</u>	<u>Abundance</u>	<u>Standard</u>	<u>Conservation Status</u>	<u>Type of Occurrence</u>
	<u>Mean</u>	<u>Deviation</u>		
<i>Squalius alburdoides</i> (Steindachner, 1866)	5,79	14,92	Vulnerable	Native/Iberian endemism
<i>Barbus bocagei</i> (Steindachner, 1864)	4,81	9,67	Least Concern	Native/Iberian endemism
<i>Chondrostoma duriense</i> (Coelho, 1985)	5,06	10,51	Least Concern	Native/Iberian endemism
<i>Squalius carolitertii</i> (Doadrio, 1987)	3,64	7,77	Least Concern	Native/Iberian endemism
<i>Cobitis paludica</i> (de Buen, 1930)	0,12	0,58	Least Concern	Native/Iberian endemism
<i>Salmo trutta</i> (Linnaeus, 1758)	0,17	0,47	Least Concern	Native
<i>Alburnus alburnus</i>	0,60	2,87	Least Concern	Non-indigenous
<i>Gambusia holbrooki</i> (Girard, 1859)	0,24	1,09	Least Concern	Non-indigenous

<i>Gobio gobio</i>	0,12	0,41	Least Concern	Non-indigenous
<i>Lepomis gibbosus (Linnaeus, 1758)</i>	0,44	1,14	Least Concern	Non-indigenous
Non identified cyprinids	2,27	5,96	-	-
