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Ecological impacts of changing riverine habitats on terrestrial species:

A case study with bats in a semi-arid region

Francisco Nicolau Loureiro de Amorim
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Francisco Nicolau Loureiro de Amorim
Biodiversidade, Genética e Evolução
Departamento de Biologia
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Orientador
Hugo Emanuel Vitorino Rebelo, Investigador Auxiliar, CIBIO-InBIO,
Faculdade de Ciências da Universidade do Porto

Coorientador
Stephen J. Rossiter, Professor and Head of Organismal Biology Department,
Queen Mary University of London

"I am, somehow, less interested in the weight and convolutions of Einstein’s brain than in the near certainty that people of equal talent have lived and died in cotton fields and sweatshops.“

Stephen J. Gould, The Panda's Thumb: More Reflections in Natural History

Nota Prévia

Na elaboração desta dissertação, e nos termos do número 2 do Artigo 4º do Regulamento Geral dos Terceiros Ciclos de Estudos da Universidade do Porto e do Artigo 31º do D.L. 74/2006, de 24 de Março, com a nova redação introduzida pelo D.L. 230/2009, de 14 de Setembro, foi efetuado o aproveitamento total de um conjunto coerente de trabalhos de investigação já publicados ou submetidos para publicação em revistas internacionais indexadas e com arbitragem científica, os quais integram alguns dos capítulos da presente tese. Tendo em conta que os referidos trabalhos foram realizados com a colaboração de outros autores, o candidato esclarece que, em todos eles, participou ativamente na sua conceção, na obtenção, análise e discussão de resultados, bem como na elaboração da sua forma publicada.

Lista de artigos

Capítulo 2 – Amorim F., Jorge I., Beja P., & Rebelo H. (2018) Following the water? Landscape-scale temporal changes in bat spatial distribution in relation to Mediterranean summer drought. *Ecology and Evolution*, 8(11), 5801–5814, doi: 10.1002/ece3.4119.

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Capítulo 4 – Amorim F., Pita R., Mata V.A., Beja P. & Rebelo H. (*submited*) Rapid changes in bat demography and social structure following large-scale habitat disturbance: the case of hydroelectric dam flooding.

Capítulo 5 – **Amorim F.**, Mata V.A., Beja P., & Rebelo H. (2015) Effects of a drought episode on the reproductive success of European free-tailed bats (*Tadarida teniotis*). *Mammalian Biology - Zeitschrift für Säugetierkunde*, 80(3), 228–236, doi: 10.1016/j.mambio.2015.01.005.

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To my daughter Amélia

To Marta

Abstract

Understanding the causes and consequences of multiple stressors affecting ecosystems worldwide is key to halt the present biodiversity crisis. This task is particularly urgent in ecosystems that are subject to multiple human activities, or those that can be severely affected by climate change. This thesis addressed the effects on biodiversity of changing riverine habitats, providing four compelling case studies focusing on the bat community of a Mediterranean river valley in North-Eastern Portugal, the valley of the Sabor River. Insectivorous bats are flying predators showing fast responses to disturbance and ecological changes, and are thus acknowledged as excellent indicators of environmental change. A special focus was given to the European free-tailed bat (*Tadarida teniotis*), a species which is strongly associated with the Mediterranean region and assumed to be adapted to cope with the harsh conditions observed therein. This model system was used to understand the ecological and demographic implications of changing riverine landscapes in semi-arid regions, while bringing significant outputs for conservation management. The specific goals were to i) understand how bats living in semi-arid regions explore different habitat features as a response to water availability; ii) understand the changes in the geographic range and population size of bats adapted to semi-arid regions to variations in ecological conditions mediated by large scale and long term climatic changes; iii) determine the demographic and social consequences of river impoundment on the population of a crevice-dwelling species; iv) determine how severe droughts may affect reproductive success of bat species adapted to semi-arid regions; v) develop a set of management guidelines that can promote the conservation of bats inhabiting semi-arid regions.

The value of water availability for biodiversity in semi-arid regions was thoroughly discussed, as well as, the implications of disturbance events such as severe droughts and the profound alteration of riverine habitats by hydroelectric infrastructures. The results revealed that the Sabor river and its' tributaries are of utmost importance for the overall bat community, especially during summer when water scarcity at the study area peaks. Furthermore, bats were able to track the spatiotemporal variation in water availability. Although the consequences of early seasonal drying of riverbeds as well as decreased soil moisture due to climate change are unknown, I provided evidences that *Tadarida teniotis* was able to skip reproduction during extremely dry years, a strategy that is expected to maximize adult survival. However, the cumulative effect of multiple dry years may result in recruitment failure, reducing subsequent breeding population. Consequently, population size may decrease and species geographic range can be severely restricted. A similar response was also detected on the results of the demographic history of the species before and after the Last Glacial Maximum, when its' range became restricted to two main Western Palearctic refugia, one in the Italian Peninsula and another further east in the Anatolian/Middle East region. However, despite the similar responses it is likely that the underlying mechanisms were different in the LGM from those we might observe in face of climate change (reproductive failure).

Despite the ecological importance of riverine habitats, the conflicts with multiple human uses, such as hydropower facilities, have led to their profound alteration. Results from this thesis provided evidence on the immediate consequences of the alteration caused by a hydroelectric dam on the social structure and demography of bats. Shortly after the deforestation and subsequent flooding of the Sabor River valley, *T. teniotis* population size increased substantially in roosts that were unaffected by landscape disturbance leading to changes in the social structure.

The results obtained showed how in five years *T. teniotis* populations living in the study area were subject to two disturbance events resulting from habitat alteration and extreme climatic events. The cumulative effects of such stressors can contribute to species declines occurring faster than otherwise predicted, even in common and abundant species. Overall, the case studies provided novel information on the effects of multiple threats to bats living in riverine habitats, and were used to establish a set of management guidelines to protect their populations in semi-arid regions worldwide.

Key words: capture-mark-recapture, climate change, demography, disturbance events, European free-tailed bat, habitat use, habitat loss, phylogeography, population structure, reproductive success, resource tracking, social structure, *Tadarida teniotis*, water scarcity

Resumo

Compreender as causas e as consequências das diferentes ameaças que afectam os ecossistemas, é a essencial para travar a actual crise de biodiversidade. Esta tarefa é particularmente urgente em ecossistemas sujeitos a múltiplas actividades humanas, ou naqueles que possam, ou são severamente afectados pela acção das alterações climáticas. Esta tese abordou os efeitos que a alteração de habitats ribeirinhos tem sobre a biodiversidade, usando um sistema modelo, com quatro casos de estudo, que se focaram na comunidade de morcegos de um vale Mediterrânico do Nordeste de Portugal, o vale do Rio Sabor. Os morcegos insectívoros são predadores voadores que demonstram uma rápida resposta a perturbações e alterações ecológicas, sendo por este motivo reconhecidos como excelentes indicadores de alterações ambientais. Foi dado um foco especial ao Morcego rabudo (*Tadarida teniotis*), uma espécie fortemente associada à região Mediterrânica e por isso presumivelmente adaptada às condições rigorosas observadas nesta região. Este sistema modelo, espécie-habitat, foi utilizado para perceber as implicações ecológicas e demográficas que a alteração de habitats ribeirinhos em regiões semiáridas acarreta para a biodiversidade, e ao mesmo tempo fornecer respostas para a sua gestão e conservação. Os objectivos específicos foram: i) perceber como os morcegos que habitam regiões semiáridas exploram a matriz de habitat em função da disponibilidade hídrica; ii) perceber quais as alterações na distribuição geográfica e tamanho populacional da espécie *T. teniotis*, em resposta a variações das condições ecológicas mediadas por eventos de alterações climáticas de larga escala e longa duração; iii) determinar os efeitos da construção de barragens e consequente inundação de vales sobre a demografia e estrutura social de morcegos fissurícolas; iv) determinar como secas severas podem afectar o sucesso reprodutivo de espécies de morcego adaptadas a regiões semiáridas; v) desenvolver um conjunto de linhas orientadores para a gestão e promoção da conservação de morcegos em regiões semiáridas.

A importância da disponibilidade hídrica para a biodiversidade em regiões semiáridas foi amplamente discutida, assim como as implicações de eventos de perturbação como, alteração de habitats ribeirinhos, como é o caso da construção de uma grande barragem e secas severas. Os resultados revelaram que o rio Sabor e os seus afluentes são de extrema importância para a generalidade da comunidade de morcegos, especialmente durante o verão, quando a escassez de água, nesta área de estudo atinge o seu máximo. Para além disso, ficou demonstrado que os morcegos conseguem seguir a variação espaciotemporal a disponibilidade de água. Apesar de se desconhecer as consequências da seca sazonal prematura nos leitos do rio, bem como na humidade do solo, em virtude das alterações climáticas, os resultados demonstraram que a espécie *T. teniotis* pode evitar a reprodução durante anos extremamente secos uma estratégia que poderá maximizar a sobrevivência dos adultos. No entanto, o efeito cumulativo de múltiplos anos com precipitação abaixo da média e falhas na reprodução, pode levar a um decréscimo no recrutamento de juvenis, reduzindo a população reprodutora. Assim, o efeito da alteração da dinâmica populacional poderá levar a uma diminuição do tamanho da população, e até mesmo da distribuição geográfica da espécie. O mesmo tipo de resposta foi aliás detectado no estudo da história demográfica da espécie durante e após o Último Máximo Glaciar, onde se demonstra que a espécie ficou restrita a dois refúgios principais no Paleártico Ocidental, um localizado na península Itálica, e outro mais a este na região da Anatólia/Médio Oriente. No entanto, apesar das respostas poderem ser similares, é provável que os mecanismos na sua origem sejam bastante distintos.

Apesar da importância ecológica dos habitats ribeirinhos, os conflitos com a utilização humana, tais como barragens hidroeléctricas, têm conduzido à sua profunda alteração. Os resultados desta tese apresentaram evidências das consequências imediatas produzidas pela alteração da paisagem causada por uma grande barragem, tanto ao nível demográfico como da estrutura social das populações de morcegos. Pouco tempo após a desflorestação e inundação do vale do Rio Sabor, o número de indivíduos da espécie *T. teniotis* aumentou consideravelmente em abrigos que não foram afectados pela alteração da paisagem, conduzindo a alterações da sua estrutura social.

Os resultados apresentados mostraram como em apenas cinco anos as populações de *T. teniotis* da área de estudo foram sujeitas a dois eventos de perturbação resultantes da alteração da paisagem provocada por uma barragem e por um evento climático extremo. O efeito cumulativo destas duas ameaças pode contribuir para que o declínio populacional ocorra mais rapidamente do que o previsto, do que quando as ameaças são consideradas isoladamente, mesmo em espécies comuns e abundantes. No geral, os casos de estudo e o modelo aqui apresentado, fornecem informação inovadora sobre os efeitos de múltiplas ameaças às populações de morcegos que dependam directa ou indirectamente de habitats ribeirinhos. Esta informação foi usada para estabelecer um conjunto de linhas orientadores com vista à protecção das populações de morcegos em regiões semiáridas.

Palavras chave: alterações climáticas, captura-marcação-recaptura, demografia, escassez hídrica, estrutura populacional, eventos disruptivos, filogeografia, Morcego rabudo, perda de habitat, sucesso reprodutivo, seguimento de recursos, *Tadarida teniotis*, uso do habitat

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List of Abbreviations

AHBS – Baixo Sabor Hydroelectric Facility

a.g.l. – above ground level

AIC – Akaike Inforgation Criterion

a.s.l. – above sea level

BCI – Body Condition Index

CBD – Convention on Biological Diversity

CMR – Capture Mark Recapture

GLM – Generalized Linear Models

HW – Hardy-Weinberg

IPCC – Intergovernmental Panel on Climate Change

IUCN – International Union for Conservation of Nature

LCL – Lower Confidence Limit

LGM – Last Glacial Maximum

NE – North Eastern

UCL – Upper Confidence Limit

USA – United States of America

WGS – World Geodetic System

WWTP – Waste Water Treatment Plant

Chapter 1

General Introduction

"We must, in fact, not divorce the stream from its valley in our thoughts at any time. If we do we lose touch with reality. The real lake is not a basin with two vertical sides as in the textbook."

Hynes (1975). The stream and its valley. *SIL Proceedings, 1922-2010*, 19(1), 1-15.

1.1 Biodiversity conservation

Biodiversity is a broad term that refers to the variability among living organisms, measured at the genetic, the species and the ecosystem level (Hawksworth, 1995). The first time that the term ‘Biodiversity’ appeared in a publication was by the entomologist E. O. Wilson in 1988 (Wilson & Harvard University, 1988). However, the term ‘Biological Diversity’ was previously used by a number of authors, including Wilcox (Wilcox, 1984), to define "the variety of life forms (…) at all levels of biological systems (i.e., molecular, organismic, population, species and ecosystem).”. More recently in the book “Biodiversity: an introduction” Gaston and Spicer (Gaston & Spicer, 2004) defined it as the "variation of life at all levels of biological organization". Overall, the term Biodiversity encompasses a multitude of concepts from the more commonly and long established species diversity and species richness to the more complex concepts of composition (the identity and variety of elements), structure (the physical organization and pattern of elements), and function (ecological and evolutionary processes) (Noss, 1990).

Interestingly, it was even before coining the term ‘Biodiversity’ that E. O. Wilson wrote his paper “The Biological Diversity Crisis: A Challenge to Science” (Wilson, 1985), acknowledging what is now called the ‘Biodiversity crisis’.

> “*The worldwide deterioration of natural environments, especially severe in the tropics, is causing the extinction of species at a rate considered by many ecologists to be without precedent in the history of the Earth. Yet the extent of biological diversity, and hence the magnitude of its current decline, has never been precisely measured*”

However, only 17 years later the importance of biodiversity and the need to protect it would be officially acknowledged with the creation of “The Convention on Biological Diversity” (CBD) which was signed by 168 nations. Despite all the goodwill, evidence that biodiversity and associated ecosystem services are in rapid decline as a direct and indirect consequence of human actions have accrued in the last decades (Macdonald & Service, 2007; Rands et al., 2010). In an attempt to halt this biodiversity crisis, the United Nations (UN) have declared the period of 2011-2020 to be the Decade on Biodiversity and the CBD outlined an ambitious vision: “...by 2050, biodiversity is valued, conserved, restored and wisely used, maintaining ecosystem services, sustaining a healthy planet and delivering benefits essential for all people.”. However, at the end of 2018 the World Wildlife Fund (WWF) released the Living Report 2018 (WWF, 2018) recognizing that “Earth is losing biodiversity at a rate seen only during mass extinctions”. To counter this trend the countries that are parties to the CDB met once again in November 2018 to work on a new plan for the period after 2020.

Since the beginning of the 20th century we have lost species at a rate 100 times higher than that observed in-between the five previous mass extinctions and if it was not for human activities most of these species would have taken between 800 and 10 000 years to disappear (Ceballos et al., 2015). Even when no extinction occurs, the dramatic reduction of species abundances is likely to shift the functional groups within a community leading to cascading effects onto ecosystem functioning and human well-being (e.g., Cardinale et al., 2006; Díaz et al., 2006; Hooper et al., 2012; Dirzo et al., 2014)

Human activities are the main driver of biodiversity loss (Dirzo et al., 2014; Ceballos et al., 2015), where the largest contribution comes from habitat loss and alteration, exploitation of plants and animals through harvesting, logging, hunting and fishing; climate change; pollution and the spread of invasive species (Tollefson, 2019). Typically, these stressors have been studied in isolation, although it is often their cumulative effects that threaten species. For instance, substantial changes in terrestrial species’ populations and distributions have already been detected world-wide in response to the combination of climate change and habitat loss (e.g. Mantyka-pringle et al., 2012; Selwood et al., 2015; Segan et al., 2016). Some regions can be particularly susceptible to these stressors, including those where decreased precipitation due to climate change will exacerbate the effects of habitat loss and fragmentation (Mantyka-Pringle et al., 2012).

Although Biodiversity loss and ecosystem degradation is a widespread phenomenon acting at a global scale (Tollefson, 2019), some ecosystems have been more seriously affected than others. This is the case of freshwater habitats, which figure among the most threatened habitats in the world (Sala et al., 2000; Dudgeon et al., 2006). Freshwater ecosystems are havens for a large number of species and despite representing less than 1 % of the wold’s surface (Gleick, 2014) a total of 126 000 of plant and animal species is estimated to be confined to such habitats (Balian et al., 2008). Freshwaters also represent most of the water on Earth that is directly usable by humans and more than half of the fresh and accessible runoff water is now used in human activities (Vitousek, 1997). Although much attention has been given to the perilous state of freshwater habitats and their biodiversity (Strayer & Dudgeon, 2010; Geist, 2011), species and ecosystems in the surrounding matrix are often neglected (Kingsford, 2000; Ward et al., 2002). Riverine landscapes may be critical for the survival of a large number of terrestrial species (McKinstry et al., 2004) and an effective conservation of riverine biodiversity will depend on our ability to understand the interplay between both freshwater habitat and its surrounding matrix.

1.2 Biodiversity in riverine landscapes

Given the complexity of riverine environments as well as the threats to the high biodiversity they support, considerable effort is required to understand and protect these systems. Riverine landscapes, also referred as river corridors, contain a variety of elements including surface waters (a gradient of lotic and lentic waterbodies), the fluvial stygoscape (alluvial aquifers), riparian systems (alluvial forests, marshes, meadows) and geomorphic features (islands, ridges and swales, levees and terraces, deltas, fringing floodplains, wood debris deposits and channel networks) (Ward et al., 2002). Notwithstanding, the definition of riverine landscapes is highly context dependent (e.g. local communities, state agency) and can even differ between research disciplines (Verry et al., 2004). The main feature of such landscapes is of course the main channel (Figure 1.1), and even though most authors agree on the inclusion of the contiguous floodplain area, the difficulty arises on the delimitation of the transition zone, which links the floodplains to the permanently dry uplands. Here I will use riparian area in the broader sense of Malanson (1993) to refer to the ecosystems adjacent to the river, including the floodplain and the transitional upland fringes (bluff). The latter can be either flat or nearly vertical (if valley is v-shaped) and may include hillslopes, ravines, forests, and prairies, often modified by land use (Figure 1.1) (Verry et al., 2004).

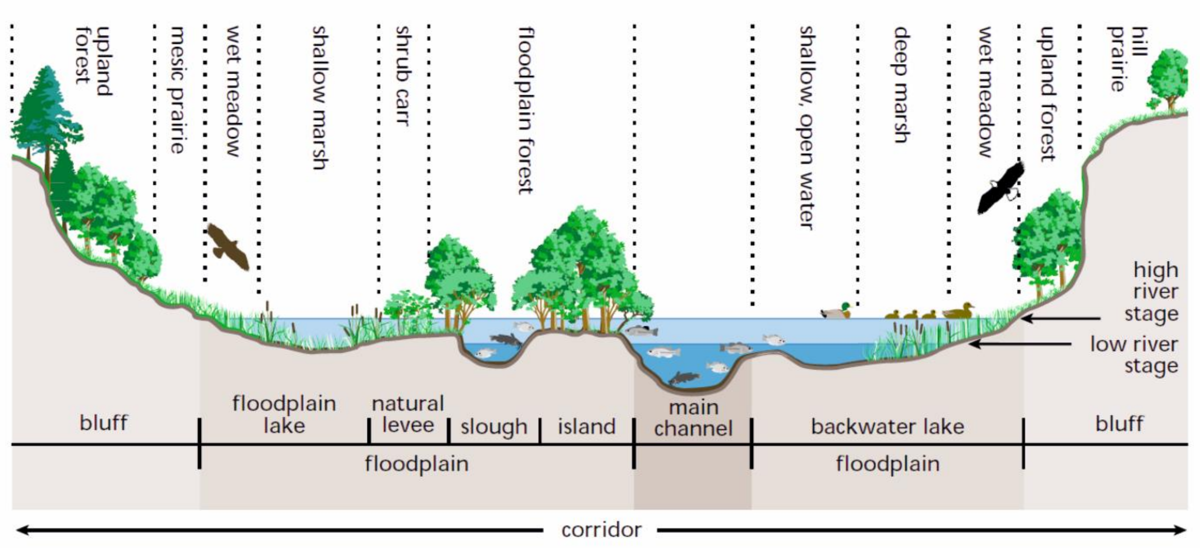

Figure 1.1 – Representative cross section of a riverine landscape. Vertical scale and channel width are greatly exaggerated. Source: FISRWG (1998). Stream Corridor Restoration: Principles, Processes, and Practices.

In riverine landscapes, the river itself is highly biodiverse supporting multiple taxa from fish to invertebrates, fungi and microorganisms, which greatly contribute to the functional biodiversity of these ecosystems (Geist, 2011). The value of riverine landscapes is further exacerbated by the role of riparian areas that serve as interface between terrestrial and aquatic ecosystems. These habitats tend to be structurally more complex and more productive in plant and animal biomass than adjacent upland areas (Naiman et al., 2005) but their importance is often neglected (Kingsford, 2000; Ward et al., 2002). Numerous species are restricted to riparian habitats throughout their life cycles (e.g., some amphibians, reptiles, birds, and mammals) while the seasonal use of these habitats may be critical for the survival of many others (McKinstry et al., 2004). The reason for this high biological diversity is that riparian habitats supply food, cover, and water, while also serving as migratory routes and connectors between habitats for a variety of wildlife (McKinstry et al., 2004), rendering them particularly relevant for conservation (Hughes & Rood, 2003). Riparian habitats are acknowledged to hold higher bird assemblages richness, abundance and diversity (Palmer & Bennett, 2006; Schneider & Griesser, 2009) while the plant cover provides food resources, protection from predators and microclimate to small mammals (Hamilton et al., 2015). The algal succession promotes insect emergence, which is the base of multi-level trophic interactions between floodplains and upland taxa, like birds, amphibians or mammals (Power et al., 2004, 2009).

Riverine ecosystems also provide goods and services of critical importance to human societies (e.g. drinking and irrigation water, power production, harvest of plants, fish, game, and sites for homes, farms, and industries) in addition to ecosystem services (Strayer & Dudgeon, 2010; Geist, 2011). Despite their undeniable value for biodiversity conservation, the human uses of these habitats have increased so greatly that they produce large scale negative impacts placing them among the most threatened habitats in the world (Dudgeon et al., 2006; Geist, 2011).

1.2.1 **Threats to riverine habitats**

The main threats to riverine habitats are roughly those acting directly on freshwater, and can be grouped as follow: overexploitation; flow modification; destruction or degradation of habitat; and invasion by exotic species (e.g. Naiman et al., 1995; Naiman & Turner, 2000; Jackson et al., 2001; Malmqvist & Rundle, 2002; Rahel, 2002; Postel & Richter, 2003; Revenga et al., 2005). Overexploitation mainly affects fishes, reptiles and some amphibians, but according to the trophic cascade theory, changes in the abundance of top predators can lead to ecosystem disruption through a cascade of trophic interactions regulating zooplankton, algal dynamics and nutrient cycles in aquatic ecosystems (Reid et al., 2000). Pollution of riverine habitats is a consequence of multiple human activities, including discharge of industrial, agricultural and domestic effluents and excessive nutrient enrichment (e.g. Stauffer, 1998; Smith, 2003). Habitat destruction or degradation could be either direct (e.g. sand extractions) or indirect. Among the indirect effects, conversion of land use within the drainage basin leading to shore erosion, higher river sediment loads and changes in surface runoff are likely to be the most relevant (Dudgeon et al., 2006). Habitat degradation can be highly influenced by other threats such as pollution or flow modification. The latter is widespread across the globe (e.g. Dynesius & Nilsson, 1994; Vörösmarty et al., 2000; Nilsson et al., 2005) and is often linked to deforestation, stream channelization, increased grazing, and nutrient pollution (Patten, 1998; Brinson & Malvárez, 2002). Riverine habitats also show high levels of exotic species either through widespread invasion or deliberate introduction with dramatic effects over the native species (Dudgeon et al., 2006).

Adding to the previously identified stressors, global scale challenges such as climate change will most likely act over all the threat categories through increasing temperatures and shifts in precipitation patterns (Dudgeon et al., 2006). These changes have direct local effects on plant and animal survival, growth and phenology, biotic interactions, and soil processes (Figure 1.2) (Rustad et al., 2001; de Graaff et al., 2006; Parmesan, 2006). Indirect effects, such as watershed-scale effects on hydrology, water management, and fluvial geomorphology, will also influence riverine ecosystems (Figure 1.2). Notwithstanding, increased frequency and severity of floods and droughts in face of climate change leads to an overriding need for flood protection or water storage (Dudgeon et al., 2006), thus promoting water-engineering responses (Vörösmarty et al., 2000). Apart from impoundments directly targeting flow regulation, the number of hydropower dams as also increased worldwide in an attempt to meet the growing energy demands while trying to tackle climate change (REN21, 2017).

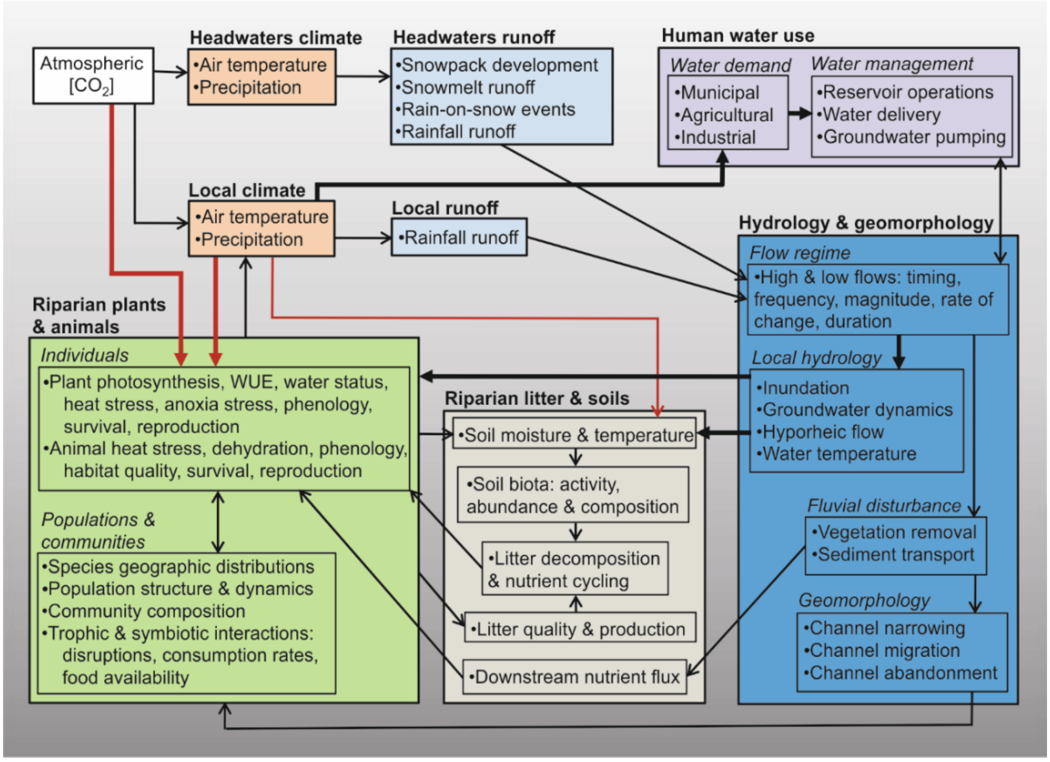

Figure 1.2 – Synthesis of the linkages between [CO_2] and climate change and components of riparian ecosystems in semiarid and arid regions. Direct effects of [CO_2] and climate change on riparian plants, animals, and soil are shown as red arrows. Linkages in need of further clarification are shown as heavy arrows. Not all potential effects shown in the picture are discussed in the text. Source: Perry et al. (2012). Glob Change Biol, vol. 18(3): 821-842.

Large-scale water abstraction and impoundment by dams (Nilsson & Berggren, 2000) further aggravates flow modification and loss of riverine habitats and even some of the largest rivers now run dry for part of the year (Postel & Richter, 2003). The impacts of dams on freshwater biodiversity has been long documented, and in 1985 Cushman reviewed the ecological effects of varying flows downstream from hydroelectric facilities, remarking on their negative impacts on the abundance, diversity, and productivity of downstream freshwater species. Moreover, the barrier effects promoted by damns will prevent downriver sediment flow and block the migration of animals upstream or downstream (Pelicice et al., 2015; Lees et al., 2016). Despite the large body of literature focusing on the impact of dams over freshwater species, few studies have assessed their impacts on terrestrial vertebrates (Gibson et al., 2017) and those who do so are mostly focused on reservoir land-bridge islands (for a comprehensive review see Jones et al., 2016). Dams lead to a sudden and extensive habitat loss due to flooding of upstream terrestrial and riparian areas that are used by countless species (Naiman & Decamps, 1997), thus severely affecting species composition and abundance at a local or even regional scale (Nilsson & Berggren, 2000). By forcing populations to move to areas already occupied it may also increase resource competition and species movements within the matrix until populations reach a new equilibrium (Santos et al., 2008). Downstream aquatic insect abundance and diversity will also decrease due to the loss of egg-laying sites (Figure 1.3, Kennedy et al., 2016). Because aquatic insects are a cornerstone of river and riparian food webs, this will have negative effects on a large number of insectivorous species such as fish, birds and bats (Kennedy et al., 2016; Poff & Schmidt, 2016).

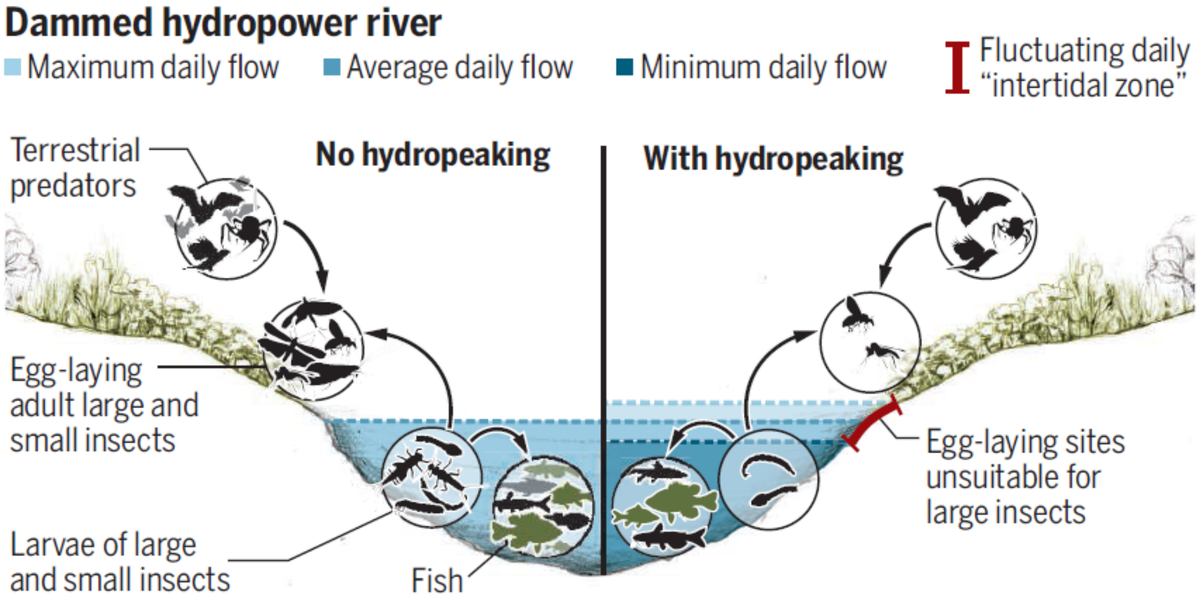

Figure 1.3 – Ecologically important insect groups lay their eggs along river-edge habitats, which will be washed out due to daily fluctuation of the artificial intertidal zone, making them especially sensitive to dam water management practices such as on-peak hydroelectricity generation. Source: Poff and Schmidt (2016). Science, vol. 353(6304): 1099-1100.

Threats to riverine landscapes may be aggravated in arid and semi-arid landscapes facing highly variable flow regimes due to seasonal water scarcity. Here climate change will strongly affect riparian hydrology and its communities (Serrat-Capdevila et al., 2007; Barnett et al., 2008; Nunes et al., 2008), while water demand for multiple human uses will increase the pressure over the already vulnerable riparian ecosystems. Thus, a comprehensive understanding of the effects of multiple threats on arid and semi-arid riverine landscapes and their biota becomes a basic requirement to forecast the future and to support conservation and management actions.

1.2.2 Riverine landscapes in arid and semi-arid regions

Freshwater and riparian habitats may be particularly important in arid and semi-arid regions, where there is a natural seasonal shortage of water. Although riverine habitats represent less than 1 % of these landscapes (Patten, 1998) they provide well developed riparian corridors along streams and form narrow linear structures with high vegetation productivity contrasting with the sparsely vegetated upland matrix (Hamilton et al., 2015). During the dry season, water becomes a limiting resource and these habitats become crucial for the survival of many taxa (Schwinning et al., 2004). Thus, species persistence will depend on the ability to exploit the spatiotemporal heterogeneity shaped by seasonal droughts. As the resources and their spatial distribution change over time, it is highly likely that species' distributions change accordingly to track them (Benton et al., 2003). Resource tracking has been described for a large number of species, from birds to mammals (e.g. Aikens et al., 2017; Thorup et al., 2017). A classic example of resource tracking is the Green Wave Hypothesis (van der Graaf et al., 2006) which predicts that migrating herbivores should track or 'surf' the leading edge of spring green-up, where forage quality is the highest (Aikens et al., 2017). Similar, in arid and semi-arid African ecosystems, animals such as buffalos adjust their movements to the seasonal shifts in surface-water availability (Funston et al., 1994). In fact, riverbeds in arid and semi-arid Africa are among the most used habitats by terrestrial vertebrates, with large numbers of ungulates migrating herein according to food availability (Mills & Retief, 1984) and offering a unique feeding opportunity for their predators (Geffen et al., 1992). Species conservation in arid and semi-arid regions thus depend on our understanding of these patterns.

To inform and implement effective conservation and management strategies for riverine biodiversity we will need to assess the threats and environmental conditions of riverine landscapes. However, because measuring everything of potential interest within a changing ecosystem is unfeasible, there is a long-standing tradition of managers and researchers to focus on indicator species (e.g. Niemi & McDonald, 2004; De Cáceres et al., 2013). Insectivorous bats [bats] are flying predators with high mobility showing fast responses to disturbance and ecological changes, and are thus acknowledged as excellent indicators of environmental change (Jones et al., 2009; Bader et al., 2015). Moreover, bats show high biological diversity and ecological significance (e.g. Whittaker & Jones, 1994; Altringham, 1996; Cleveland et al., 2006; Boyles et al., 2011) but they have also experienced a global decline in their populations over the last century, with almost 16% of species within this group being highly threatened or extinct (IUCN, 2019). Finally, because they rely heavily on riverine landscapes (see section 1.3) bats are an excellent indicator group to track how changes therein affect the whole species community.

1.3 Bats and riverine ecosystems

Riverine ecosystems are of utmost importance for bats since they provide drinking water and foraging habitat (Russo & Jones, 2003; Rainho, 2007). Additionally, water availability can have strong effects on the condition and reproductive output of individuals (Adams & Hayes, 2008; Adams, 2010), further stressing its' importance for the survival and persistence of bat populations. This close relationship has been reported for a number of species, including the soprano pipistrelle, *Pipistrellus pygmaeus* (Nicholls & A. Racey, 2006), Nathusius' pipistrelle, *Pipistrellus nathusii* (Flaquer et al., 2009), and other *Pipistrellus* spp. (Scott et al., 2010), Schreiber's bat, *Miniopterus schreibersii* (Serra-Cobo et al., 2000) and noctules, *Nyctalus* spp. (Rachwald, 1992; Vaughan et al., 1997; Racey, 1998). Rivers and freshwater habitats are also important migratory paths and stopover sites for migrating bats (Flaquer et al., 2009), and are perceived as landmarks used for commuting (Serra-Cobo et al., 2000; Russo et al., 2002).

Various studies have reported high levels of bat activity over water bodies and even small springs, ephemeral pools, waterholes or artificial water bodies, such as farm dams, may be beneficial for bats (Racey, 1998; Tuttle et al., 2006; Razgour et al., 2010; Sirami et al., 2013). Often, water bodies attract species that are otherwise widespread across the landscape (Lisón & Calvo, 2011; Hintze et al., 2016) acting as hotspots of bat diversity. Using ecological niche modelling Lisón & Calvo (2013) showed that pipistrelle species have a strong preference for water sites, while a telemetry study by Salsamendi et al. (2012) concluded that *Rhinolophus mehelyi* foraged close to water bodies, where it was judged to have access to drinking water and higher insect abundances. Bats need to drink water regularly since they can lose significant amounts of water through either respiratory system or wing membranes (Herreid & Schmidt-Nielsen, 1966; Muñoz-Garcia et al., 2016), yet very few studies have specifically assessed the use of water bodies as drinking areas (but see Seibold et al., 2013; Russo et al., 2010, 2012).

The link between riverine habitats and bats is even more relevant in the context of reproduction. During pregnancy and lactation females experience greater metabolic requirements, and thus have a high demand for water and food (Kunz et al., 1995; Mclean & Speakman, 1999; Adams & Hayes, 2008; Frick et al., 2010). Under such circumstances, water bodies and riparian habitats can offer unique foraging and drinking opportunities. Moreover, pregnant females tend to roost in sites with relatively high ambient temperature allowing accelerated gestation (Racey, 1982), but this will in turn increase water loss (Webb et al., 1995). The need for water intake is even higher during lactation, when water loss by females peaks (Kurta et al., 1990). Focusing on lactating and non-reproductive females of fringed myotis (*Myotis thysanodes*) Adams & Hayes (2008) found that lactating females visited water bodies to drink more often than non-reproductive females. This visits consistently happened every night regardless of climatic conditions, whereas non-reproductive females would often visit this sites when temperatures were high and relative humidity low (Adams & Hayes, 2008).

In another study on the same species, Adams (2010) found that reproduction could drop as low as 50 % during dry years. This close relationship between bats and water often leads to roosts being located near water habitats (Jones et al., 1998; Korine et al., 2013) and in some species even sex segregation on roost location has been found, with female nursery roosts being closer to water bodies than male roosts (Encarnação et al., 2005).

Associations between bats and water seem to be particularly strong in arid and semi-arid environments, including for instance the Mediterranean, Middle East, Africa, Australia and parts of North America (Russo & Jones, 2003; Rebelo & Brito, 2007; Monadjem & Reside, 2008; Razgour et al., 2010; Hagen & Sabo, 2014; Korine et al., 2016). Here, water sites and riparian habitats are among the most used habitat by bats, supporting a large number of endangered or vulnerable species (Russo & Jones, 2003). During natural dry periods, when water availability is lower, the association between bats and freshwater habitats is expected to be even stronger (Rainho, 2007). In such circumstances, permanent, and even temporary artificial water sites, can determine species distribution, abundance and persistence (Lisón & Calvo, 2011) highlighting the importance of these structures for bat conservation in arid environments (Korine & Pinshow, 2004; Razgour et al., 2010).

Given the importance of riverine habitats in supporting bat communities, understanding how the main threats to such systems affect them is paramount for the conservation and management of bat populations. Water pollution, destruction or degradation of riverine landscapes (especially from damming), and at a broader scale climate change can all have implications for bat conservation. Large-scale habitat changes stemmed from damming are known to potentially affect the survival of bat populations through the permanent loss of roosts and foraging areas (Cosson et al., 1999). For instance, the construction of a large dam in southern Portugal resulted in a decline in bat activity over the submerged area and an increase on the activity of disturbance-resistant species in the surrounding area of the reservoir (Rebelo & Rainho, 2009). Nevertheless, the driving processes affecting local bat communities have been seldom assessed. Additionally, although roost loss has been previously identified as one of the major effects of dams on bats, to my best knowledge no study as specifically addressed this issue.

As previously discussed, climate change pose a major threat to riverine landscapes, and although it can affect bats in multiple ways (Sherwin et al., 2013) for the purpose of the present thesis I will cover aspects directly related to water availability and species persistence under droughts. In semi-arid regions, water habitats are mainly restricted to larger rivers during the seasonal natural drought, but in face of climate change, this effect is likely to extend to the remaining seasons. The spatiotemporal variation in resource availability may force bats to travel longer distances to find drinking water and foraging habitats that when combined with higher temperatures may lead to greater energetic demands and evaporative water loss. Even though few studies have addressed the impacts of climate change on demographic processes, it is expected to have negative effects on both survival and reproduction (Adams & Hayes, 2008; Sherwin et al., 2013). Ultimately, the effect of climate change will depend on a species adaptive potential (Hoffmann & Sgró, 2011) or the ability to shift its range to new unoccupied areas (Rebelo et al., 2010). To better understand how species cope with such dramatic changes it is possible to investigate how they persisted during past climate changes and the last glacial period provides an excellent opportunity to understand how bats have survived during the extreme climatic conditions of that epoch. At present, there are already evidences of northward movements among European bats. For example, the Mediterranean species Kuhl's pipistrelle (*Pipistrellus kuhlii*) has expanded its range in response to rising temperatures (Sachanowicz et al., 2006; Ancillotto et al., 2016) while climate change might also be responsible for the expansion of the Nathusius' pipistrelle in the UK (Lundy et al., 2010). The extent to which bat species are affected by climate change will depend on their biogeographic affinities (Rebelo et al., 2010). Increasing our knowledge on the biogeography and the long-term demographic history of bat populations will allow us to understand the present patterns of species distribution and to predict how they might react to future climatic events.

1.4 The model system

1.4.1 **The biological context**

This thesis focused on bats mainly to understand how changing riverine landscapes affect biodiversity in the semi-arid region of the Mediterranean. Here, water availability is usually high from autumn to early spring, either through precipitation (Mariotti et al., 2002; Magalhães et al., 2007) or soil moisture (Miller & Hajek, 1981), which in turn contributes to high levels of photosynthetic activity (Peñuelas et al., 1998) and primary productivity (Melillo et al., 1993). During these seasons, water also flows in both temporary and permanent streams, although this is followed by a declining flow in late spring and subsequent summer drying of watercourses that ends with the first rains of the fall (Gasith & Resh, 1999). Late spring and summer are characterized by dry and hot periods (Blondel, 2006) when soil moisture is at its lowest (Miller & Hajek, 1981) and surface water is frequently restricted to the main tributaries, weirs, and dams. Summer droughts lead to seasonal limitation of plant growth and yield (Galmés et al., 2007; Flexas et al., 2014) and may have consequences for the fine-scale distributions of both invertebrates and vertebrates, particularly flying insectivorous vertebrates, such as bats, that may easily track this seasonal spatiotemporal variations (Bailey et al., 2004; Baxter et al., 2005).

Species living in Mediterranean region have adapted to the temporal variations of resource availability, in some cases even synchronizing their life cycles to match those variations (Ruiz-Olmo et al., 2002; Hinks et al., 2015). Overall, temporal match between vegetation phenology and insect emergence (Forrest & Thomson, 2011) should lead to a bottom-up effect, in which insectivorous vertebrates should synchronize their life cycles to the insect blossom (Gray, 1993; Fukui et al., 2006), while triggering a response from their predators. However, studies on spatiotemporal resource tracking showing how species actively move across the landscape matrix and change their habitat associations in response to Mediterranean summer drought are lacking (but see Rueda et al., 2008). Bats represent an excellent model to test this effect, and understanding how their populations respond to natural changes in water availability is especially relevant to forecast the consequences of increased frequency and severity of droughts faced by the Mediterranean region as a consequence of climate change (Dai, 2011). In this regard, phylogeographic approaches allow understanding how bat populations coped with past variations in ecological conditions mediated by large scale and long term climatic changes. In the specific context, glacial periods can be especially relevant since water availability is much lower than during inter glacial periods like the one we are living in.

As described earlier (sections 1.2.1), riverine landscapes worldwide (but especially in arid and semi-arid regions) have been subject to large-scale water abstraction and impoundment by dams. The construction of the Baixo Sabor Hydroelectric Infrastructure (AHBS), and the subsequent flooding of the Sabor river valley in our study area, represented a rare opportunity to test *in situ* the immediate effects of dams over bat populations. To understand the ultimate causes of population changes we need information on demographic processes, especially when targeting vertebrate species (Henry et al., 2008). Due to the unfeasibility of doing this to the entire bat community, I have narrowed the study of demography to the European free-tailed bat (*Tadarida teniotis*). *T. teniotis* is a medium-large crevice-dweller that mainly occurs in the Palearctic (Dietz et al., 2009; Benda & Piraccini, 2016), where it is strongly associated with the Mediterranean region (Horáček et al., 2000; Rebelo et al., 2010) and thus assumed to be adapted to cope with the harsh conditions observed therein. Previous work also suggested this was a locally abundant species which could be easily captured (Amorim et al., 2013), and it was thus considered a good model species to study the demographic implications of the droughts and changes in riverine ecosystems. Notwithstanding, I also investigated how *T. teniotis* responded to past climate changes from the glacial to the interglacial period and how this species coped with such dramatic events and low water availability.

1.4.2 **Study area**

Three of the four chapters in this thesis (Chapter 2, 4 and 5) were conducted in a semi-arid region in northeast Portugal (41º 21’ 0’’ N, 6º 58' 0’’ W), within the Baixo Sabor Long Term Ecological Research Site (LTER_EU_PT_002) and the Natura 2000 site of Sabor-Maçãs (PTCON0021). The study area included a 5-km buffer around the river Sabor and a 2-km buffer around its main tributaries totalizing 1 100 km2 (Figure 1.4A) as well as five *T. teniotis* roosts. These roosts consisted of three bridges located over the AHBS but safe from flooding and two bridges nearby (Figure 1.4A). The bridges offer several roosting possibilities for bats, mainly crevices of different types, but also box girders that mimic underground roosts (Amorim et al., 2013). All the bridges are of modern construction (the oldest was built in 1992) and are part of the Portuguese main road network (Figure 1.4B). The length and height of the bridges sampled ranged respectively from 60-600m and from 10-110m.

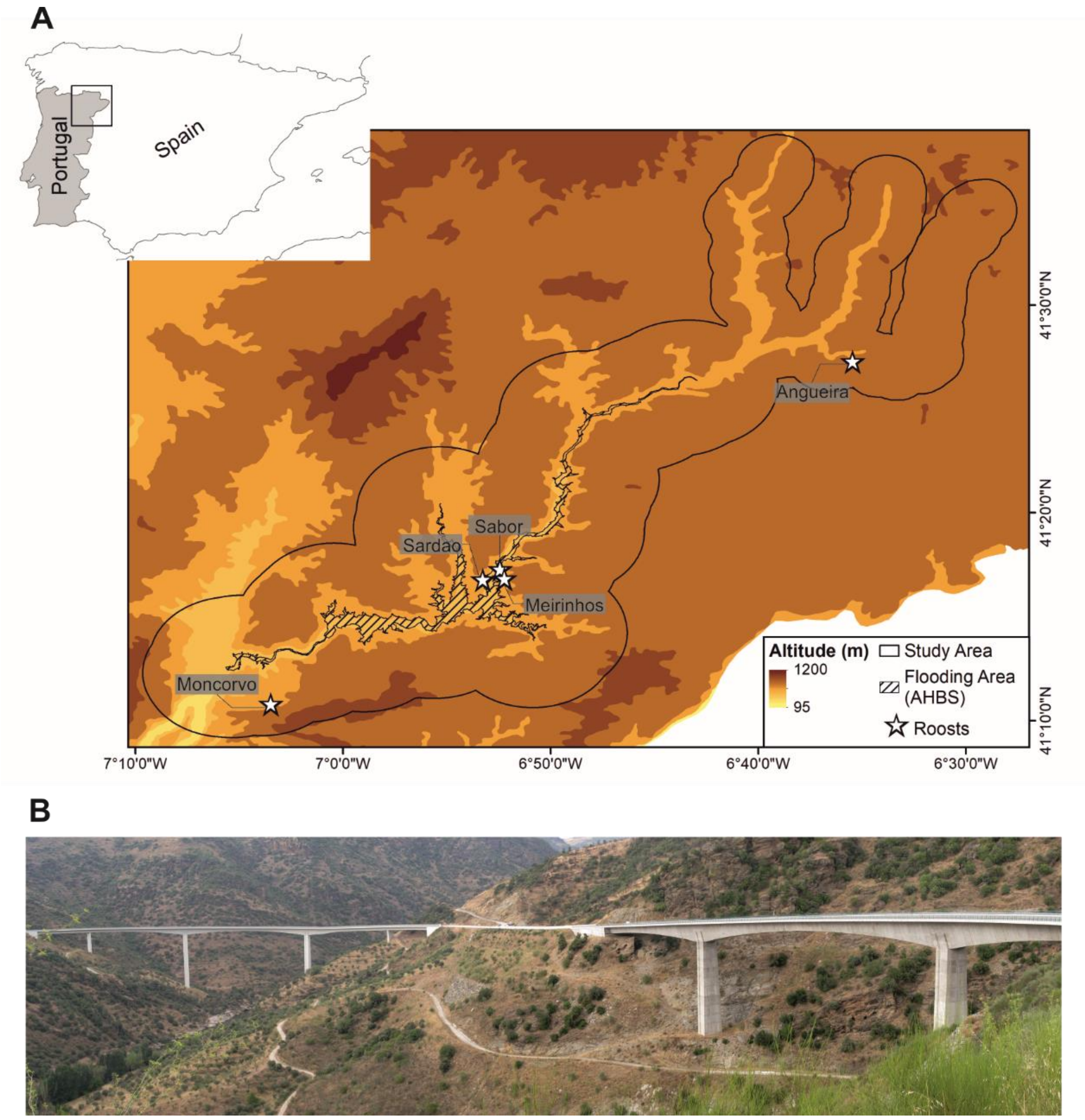

Figure 1.4 – (A) Study area (Chapter 2, 4 and 5) in northeastern Portugal, showing the limit of the study area (black line) the flooding area of the AHBS (line filled) and the *Tadarida teniotis* bridge roosts (stars). (B) Example of two safe bridge roosts over the AHBS.

The region is in the transition between meso- and supra-Mediterranean bioclimatic zones, with cold winters (average temperature of the coldest month <6ºC) and dry summers (total annual precipitation <600 mm, of which <5% in July–August), which are particularly hot in some valleys where monthly average temperatures exceed 21ºC (Monteiro-Henriques, 2010). The landscape is characterised by plateaus at about 700–800 m a.s.l., dissected by deep and narrow streams valleys. Land cover is dominated by a complex mosaic of natural vegetation patches, forest stands (mainly maritime pine *Pinus pinaster* plantations), permanent crops (mainly olive and almond groves), and pastures, which reflect a process of progressive agricultural land abandonment since the 1960s (Hoelzer, 2003). Natural vegetation is largely composed of shrublands of variable structure and species composition, remnants of native evergreen oak woodlands, and some well-developed riparian galleries (*Quercus suber*, *Q. rotundifolia*) (Hoelzer, 2003). Valleys are deep and narrow, and watercourses can have steep slopes and a highly variable hydrological regime, with many of them drying out seasonally while others persist year-round (Ferreira et al., 2016). From 2009 to 2013, the study area was impacted by the AHBS, and the subsequent flooding of the river valley, which started in the winter of 2014. The infrastructure includes a smaller and a larger dam, with the latter flooding a total length of river valley of 39 km and an area of about 3,000 ha (Figure 1.5). In 2014, before dam filling, all woody vegetation (shrublands, orchards, forests) located at the future reservoir area was removed to mitigate eventual eutrophication problems and enhance water quality in the reservoir.

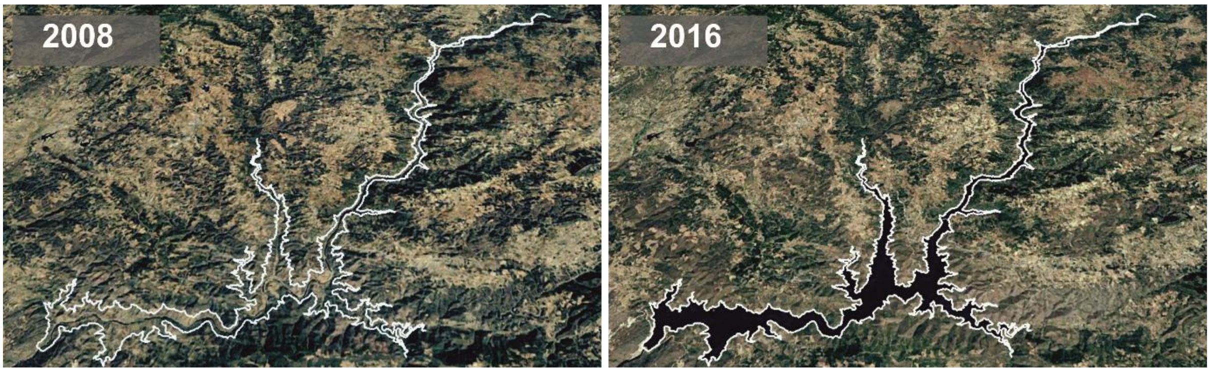

Figure 1.5 – River Sabor valley before (2008) and after (2016) the filling of the AHBS.

In Chapter 3 the study area was extend to the western range of the European free-tailed bat (Figure 1.6).

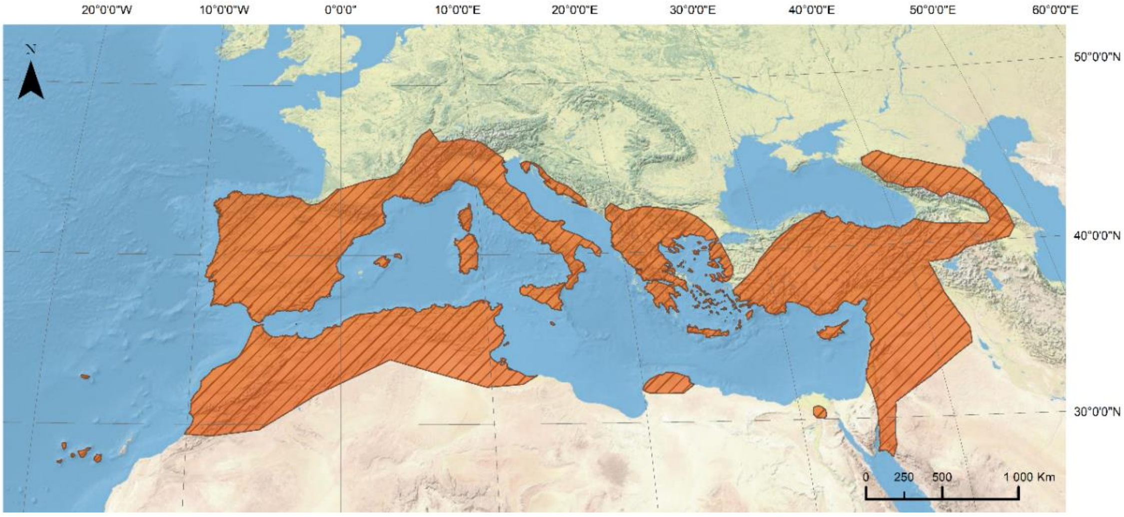

Figure 1.6 – Extended study area (Chapter 3) highlighting the western Palearctic range of the European free-tailed bat (*Tadarida teniotis*) according to the IUCN.

1.5 Objectives

The overall goal of this thesis is to understand how different stressors of riverine habitats affect bats at multiple levels, including species richness and abundance, population size, survival, reproduction and social structure. To address this general goal, the thesis will pursue the following specific objectives:

1 – Understand how bats living in semi-arid regions explore different landscape features throughout the year, particularly those where water is available in the dry summer season. This objective focuses on the whole bat community and examines whether bats become more dependent on riverine habitats over time in response to seasonal summer drought.

2 – understand the changes in the geographic range and population size of bats adapted to semi-arid regions in response to variations in ecological conditions mediated by large scale and long term climatic changes (LGM). This objective is justified by the need to have a better understanding on the model species (European free-tailed bat) used to address the remaining objectives, and how it responds to climate changes over long temporal frames.

3 – To determine the demographic and social consequences of river impoundment for crevice-dwelling species. This objective is addressed using a dataset collected over five years, before and after the construction of a hydroelectric dam, which is one of the main stressors currently affecting riverine environments.

4 – To determine how severe droughts may affect reproductive success of bat species adapted to semi-arid regions. This objective is important given the increased frequency of major droughts expected due to climate change, which may greatly affect bat populations.

5 – To develop a set of management guidelines that can promote the conservation of bats living in semi-arid regions. These guidelines specifically address the threats identified in the previous objectives and focus on their effects over the bat populations.

1.6 Thesis Outline

The first chapter is a general introduction to the problematic of biodiversity loss and the importance of riverine landscapes for biodiversity conservation in semi-arid regions. The second chapter in this thesis introduces the key point of the importance of freshwater habitats for the bat community, especially in the context of arid and semi-arid regions. More importantly, it is a step forward on our understanding about the seasonal use of these habitats, filling a knowledge gap clearly identified by Salvarina (2016). The third chapter is a study on the phylogeography of the European free-tailed bat (*T. teniotis*), the focal species in the subsequent chapters. The fourth and fifth chapters use *T. teniotis* as a model species and focus on measuring the demographic and social impacts of two major threats to riverine habitats in semi-arid regions: impoundments and extreme drought events. Finally, the last chapter provide a general discussion of the thesis. Bellow I present a short summary of the paper chapters of this thesis.

Chapter 2 (Amorim et al., 2018) investigates how the spatial distribution of ecological resources shapes species' diversity and abundance in human-modified landscapes, a central theme in conservation biology. We tested the hypothesis that bats in Mediterranean landscapes are strongly affected by the progressive reduction in water availability during the seasonal summer drought. The effects of landscape composition and structure on bat diversity and activity were analysed over three different phenological periods (pregnancy, lactation, and post-lactation). Results showed that the distribution of bat diversity and activity hotspots changed markedly over time. During pregnancy, there were no obvious bat diversity hotspots, while during lactation and particularly post-lactation, there was a concentration of hotspots along permanently flowing watercourses.

Chapter 3 (Amorim et al., *in press*) is an important contribution to fill the knowledge gap on whether or not species with tropical affinities living in temperate climates were able to survive in the Mediterranean region during the Quaternary climatic oscillations. Focusing on *Tadarida teniotis*, we aimed at identify the potential ancient populations and glacial refugia, determine the post-glacial colonization routes across the Mediterranean, and evaluate current population structure and demography. Mitochondrial and nuclear markers were used to understand *T. teniotis* evolutionary and demographic history. The results support a pre Last Glacial Maximum (LGM) colonization of Italy and the Anatolian/Middle East, while the remaining western Palearctic populations were colonized from Italy after the Younger Dryas and have subsequently expanded to its current circum-Mediterranean range.

Chapter 4 (*paper in preparation*) investigates how a large hydroelectric dam can affect the demography and social structure of terrestrial vertebrates. Focusing on *T. teniotis,* we were able to measure for the first time the extent of such impacts. We monitored roosts located in three road bridges crossing the main river valley and two bridges unaffected by the dam, both before, during and after habitat flooding. We captured and marked individuals, while also taking tissue samples for genetic analysis. Using Capture-Mark-Recapture models, we found an overall increase in the number of bats using bridge roosts after dam filling, but no changes in survival rates. By analysing bats' relatedness, we found a decrease at breeding roosts located over the flooded area, but not so on the nearby bridge roosts. The results suggest that a large number of individuals abandoned the river valley after flooding, possibly due to the loss of roosting areas. The concentration of an unusually large number of individuals at safe roosts over the flooded area appeared to have caused social disruption.

Chapter 5 (Amorim et al., 2015) addresses how a severe drought event has affected the reproductive success of species adapted to Mediterranean climates (*T. teniotis*). Unusually dry conditions occurred in 2012, when the annual precipitation was the lowest on record during three decades, and the normalized difference vegetation index (NDVI) was well below the long term median, particularly during the pre-breeding season. The number of pregnant or lactating females and the proportion of juveniles were significantly lower in 2012 than in 2013. The results suggest that the drought event largely impaired the reproduction of *T. teniotis*, though it only affected body condition early in the pre-breeding season. Possibly, dry conditions in 2012 resulted in reduced food resources during pre-breeding, limiting individuals' ability to restore their body condition after the winter and before breeding season. Consequently, most individuals did not produce offspring, probably using the food resources available during the dry breeding season to restore body condition before winter.

Chapter 6 presents the main conclusions from these studies and their implications for bat conservation in semi-arid regions. A set of management guidelines for semi-arid regions is provided alongside with some insights on specific questions that should be addressed in future research.

Chapter 2

Following the water? Landscape-scale temporal changes in bat spatial distribution in relation to Mediterranean summer drought

Francisco Amorim, Inês Jorge, Pedro Beja and Hugo Rebelo

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Keywords: acoustic monitoring, habitat use, landscape management, resource tracking, species diversity, water scarcity

Abstract

Understanding how the spatial distribution of ecological resources shape species' diversity and abundance in human-modified landscapes is a central theme in conservation biology. However, studies often disregard that such patterns may vary over time, thereby potentially missing critical environmental constraints to species persistence. This may be particularly important in highly mobile species such as bats, which are able to track temporal variations in spatial resource distribution. Here we test the hypothesis that bats in Mediterranean landscapes are strongly affected by the progressive reduction in water availability during the seasonal summer drought. We analysed the effects of landscape composition and structure on bat diversity and activity, during Pregnancy, Lactation and Post-lactation periods, and identified the most influential variables within and across periods. Water bodies showed the strongest positive effect on bats, followed by riparian habitats and areas with steeper (>30%) slopes. However, while during Pregnancy there were only small landscape effects, these increased during Lactation and Post-lactation, highlighting a progressively stronger association with water habitats during the summer drought. The spatial projection of habitat models showed that the landscape distribution of bat diversity and activity hotspots changed markedly over time. During Pregnancy the spatial pattern of hotspot distribution was weakly defined, while during Lactation and particularly Post-lactation there was a concentration of hotspots along permanently flowing watercourses. Our study highlights that permanently flowing watercourses are critical for bat conservation in Mediterranean landscapes, calling for measures to counteract their ongoing degradation due in particular to climate change, water abstraction and damming. More generally, our study underlines the importance of considering the temporal dimension in habitat selection studies, without which there is the risk of overlooking the importance of habitats that are key for species persistence only at certain times of the year.

2.1 Introduction

The long-term persistence of a species in a given landscape is conditional on the availability of resources at the appropriate temporal and spatial scales (Lynch & Ennis, 1983). As the resources and their spatial distribution change over time, it is highly likely that species' distributions change accordingly to track such resources (Benton *et al.*, 2003). For instance, the food resources required by a species often vary along the life cycle and among life stages (Rey, 1995; Loureiro et al., 2009), often associated to spatial changes in food availability. Such changes may be overcome either by species following the resources through different habitats, or by different habitats becoming available at the optimal time for a given species (Benton *et al.*, 2003). Both strategies will lead to temporal variation in species diversity and abundance across the landscape, which should be particularly evident for highly vagile organisms such as bats and birds. Understanding such spatiotemporal patterns is paramount for conservation, due to the need of protecting all habitats providing the resources to fulfil species' requirements across the whole year (Law & Dickman, 1998). However, such information is seldom available since most studies only provide snapshots from a single season or pool yearly data together into a single analysis, generally disregarding seasonal variations (Bissonette & Storch, 2007; Marra *et al.*, 2015, but see, e.g. Beja *et al.*, 2010; Russell & Ruffino, 2012).

Mediterranean landscapes provide an excellent setting to test hypotheses associated to species resource tracking. This is because the Mediterranean climate is naturally characterized by dry and hot summer periods (Blondel et al., 2010), and so seasonal water scarcity may strongly determine temporal variations in resource availability. During spring, water availability is usually high, either through precipitation (Mariotti et al., 2002; Magalhães et al., 2007) or soil moisture (Miller & Hajek, 1981), which in turn contributes to high levels of photosynthetic activity (Peñuelas et al., 1998) and primary productivity (Melillo *et al.*, 1993). During this season, water also flows in both temporary and permanent water bodies, though this is followed by a declining flow in late spring and subsequent summer drying of watercourses that ends with the first rains of the fall (Gasith & Resh, 1999). Consequently, from late spring to late summer soil moisture is at its lowest (Miller & Hajek, 1981) and surface water is restricted to the main tributaries, weirs and dams. This in turn leads to seasonal limitation of plant growth and yield (Galmés et al., 2007; Flexas et al., 2014), and may have consequences for the distributions of invertebrates and vertebrates in general, particularly flying insectivorous vertebrates that may track seasonal variations in resource availability (Bailey *et al.*, 2004; Baxter *et al.*, 2005). Therefore, understanding the responses of insectivorous vertebrates to the seasonal cycle of water availability is critical for conservation in Mediterranean landscapes since it allows identifying the key habitats that need to be maintained to assure sufficient resources throughout the year. This is particularly important given the current and predicted changes to the distribution of water in the Mediterranean due to water abstraction from rivers, large scale construction of dams, and climate changes that are expected to increase the frequency and intensity of summer droughts (Milly et al., 2005; Rebelo & Rainho, 2009; Dai, 2011; Hoerling et al., 2012).

Bats may be particularly adequate to understand resource tracking in the Mediterranean region because they are flying predators with high mobility, and potentially they respond fast to temporal changes in the spatial distribution of insect prey availability (Power et al., 2004). As a consequence, they may be responsive to the seasonal cycle of water availability, as they are known to be strongly influenced by the availability of aquatic habitats (Salvarina, 2016), particularly in arid and semi-arid environments (Razgour et al., 2010; Hagen & Sabo, 2012). This is supported by studies showing that ponds in Mediterranean forests have higher bat activity and diversity than the adjacent areas of the forest matrix (Lisón & Calvo 2014), and that permanent water bodies and riparian habitats are important for both bat species diversity and activity (Russo & Jones, 2003; Rainho, 2007; Razgour et al., 2011a). There is also evidence that small artificial water bodies such as farm dams may be beneficial to bats (Tuttle et al., 2006; Sirami et al., 2013), often attracting species that are widespread and abundant across the landscape (Lisón & Calvo, 2011; Hintze et al., 2016). Furthermore, water availability seem to have strong effects on the condition and reproductive output of individuals (Adams & Hayes, 2008; Amorim *et al.*, 2015), further stressing the importance to adjust habitat use to the availability of water resources. Despite these observations, there is still limited understanding on how bat distributions vary over time in Mediterranean landscapes, and it remains uncertain how these changes may be driven by temporal variations in the distribution of water resources (but see, e.g., Salvarina *et al.*, 2018; Dalhoumi *et al.*, 2017).

Here we tested the hypothesis that bats in Mediterranean landscapes are strongly affected by the progressive reduction in water availability during the seasonal summer drought. For that purpose, we evaluated changes in habitat use and the spatial distribution of both species richness and activity during bats' active phase (from spring to autumn). Our specific aims were: i) to determine which habitat variables are associated to species richness and bat activity considering three key phenological periods (Pregnancy, Lactation and Post-lactation); ii) to estimate whether the importance of habitat variables varied across the phenological periods; and iii) to estimate temporal variations in the spatial distribution of bat species richness and activity hotspots. We predict that the spatial distribution of bat diversity and activity should be largely independent of water availability in spring, during Pregnancy, but as summer progresses bats should be progressively more constrained by the spatial distribution of the remnant surface waters (Adams & Hayes, 2008; Adams, 2010).

2.2 Materials and methods

2.2.1 **Study area**

The study was carried out in north-east Portugal (41º 21’ 0’’ N, 6º 58' 0’’ W), within the Baixo Sabor Long Term Ecological Research Site (LTER_EU_PT_002). Specifically, we mainly focused on a 1,100 km2 area defined by a 5-km buffer around the main river and a 2-km buffer around its main tributaries (Figure 2.1), because we were interested in documenting bat activity relatively close to the main waterlines, and that could thus be more influenced by the seasonal changes in surface water availability. The region is in the transition between meso- and supra-Mediterranean bioclimatic zones, with cold winters (average temperature of the coldest month <6ºC) and dry summers (total annual precipitation <600 mm, of which <5% in July–August), which are particularly hot in some valleys where monthly average temperatures exceed 21ºC (Monteiro-Henriques, 2010) . The landscape is characterised by plateaus at about 700–800 m a.s.l., which are dissected by deep and narrow streams valleys. Land cover is dominated by a complex mosaic of natural vegetation patches, forest stands (mainly maritime pine *Pinus pinaster* plantations), permanent crops (mainly olive and almond groves), and pastures, which reflect a process of progressive agricultural land abandonment since the 1960s (Hoelzer, 2003). Natural vegetation is largely composed of shrublands of variable structure and species composition, remnants of native evergreen oak woodlands, and some well-developed riparian galleries (*Quercus suber*, *Q. rotundifolia*) (Hoelzer, 2003). Primary productivity peaks in winter and early spring, while the lowest values are observed in summer (Amorim *et al*., 2015).

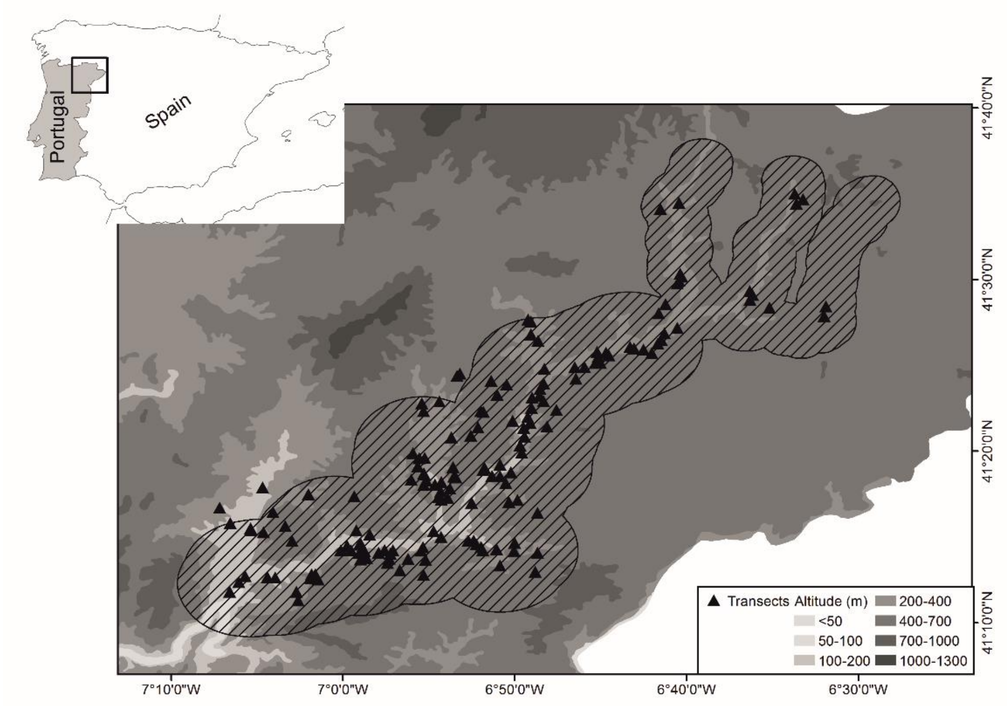

Figure 2.1 – Study area (line filled) in north-eastern Portugal and location of the acoustic transects (n=155) sampled for bats July to October 2011, and from May to September 2012.

2.2.2 **Study design**

The study was based on acoustic surveys carried out along 200 transects from July to October 2011, and from May to September 2012. Transects were distributed in the study area using a stratified randomization, in order to have a comparable sampling effort across the dominant land cover types. Each transect was surveyed only once during the study, in either the Pregnancy (May-June), Lactation (July-August) or Post-lactation (September-October) periods. These time windows cover the corresponding phenological period for most European bat species (Racey & Swift, 1985; Goiti et al., 2006; Pretzlaff et al., 2010; Amorim et al., 2015). The option to sample different transects in different periods was taken to maximise the coverage of environmental variability in the study area, under the logistic constraints limiting the maximum number of transects that could be sampled per period. However, temporal comparability of results was assured by sampling the same geographic areas, and by maintaining a similar representation of each land cover type across sampling periods. We used data from each phenological period to build seasonal habitat models, and pooled data across periods to build an annual habitat model. These models were then used to predict the distribution of species richness and bat activity across the landscape, for each time period.

2.2.3 **Bat acoustic surveys**

Bats were sampled using acoustic surveys, which started 45 minutes after sunset and lasted for three hours, corresponding to the period of highest bat activity (Vaughan et al., 1997; Duffy et al., 2000; Russo & Jones, 2003; Wickramasinghe et al., 2003; Rainho, 2007). Sampling was always made by the same observer (FM), accompanied by a second person. We only sampled during nights with favourable weather conditions for bat activity, specifically with no rain, low humidity, mild temperature and null or weak wind (Russo & Jones, 2003; Amorim et al., 2012). However, sometimes weather changed during a given night or at specific locations, and so the corresponding transects were discarded. Each transect was walked at low speed (ca. 2 km/h) for 15 minutes and all bat activity was recorded using a handheld ultrasound detector (D1000X, Pettersson Elektronik AB, Uppsala, Sweden) with a sampling frequency of 384 kHz. Species were identified using sound-analysis software (BatSound Pro 4.2, Pettersson Elektronik AB, Uppsala, Sweden) with a 1024 pt FFT and Hamming window for spectrogram analysis (Russo & Jones, 2003; Amorim et al., 2014). Acoustic identification of bat calls was based on Russo & Jones, 2002; Pfalzer & Kusch, 2003; Walters *et al.*, 2012; Rainho *et al.*, 2013. Bat calls that could not be assigned to a species or species group were considered as non-identified calls, and were only considered to estimate overall bat activity. Bat activity was measured at 10 seconds intervals.

2.2.4 **Landscape predictors**

We estimated variables describing landscape composition (land cover type) and structure (topography and configuration metrics), within a 500-m buffer around each sampling transect (Supporting Information, Table S2.1). This radius was chosen considering previous studies showing that bat presence at a site is highly influenced by habitat features within 100-500 m (Bellamy et al., 2013). All variables were extracted from digital thematic layers using QGIS 2.18.4 (QGIS Development Team, 2017) and the following R packages: rgdal (Bivand et al., 2016), maptools (Bivand & Lewin-Koh, 2016), raster (Hijmans, 2016) and sp (Pebesma & Bivand, 2005; Bivand et al., 2013). Topographic variables were estimated using a 25-m resolution digital elevation model (http://www.eea.europa.eu/dataand-maps/data/eu-dem). For each buffer, we computed the maximums, minimums, means, medians, ranges and standard deviations of elevation, slope and aspect. In addition, we estimated the proportion of the buffer occupied by high slopes, using 20º, 30º and 40º as alternative thresholds. Slopes were considered because they are expected to affect bats, as they provide roosting opportunities (Santos *et al.*, 2014), are used as landmark during commuting and foraging

(Russo et al., 2005), and may even assist bats to perform ascending flights while foraging (Roeleke et al., 2018). Land cover variables were extracted from the Portugal's digital Land Cover Map of 2007 (http://www.igeo.pt/), and were quantified as the proportion within the buffers of land cover classes aggregated into nine main categories judged *a priori* to reflect contrasting bat habitats (Rainho, 2007; Rebelo & Rainho, 2009): Mediterranean forest, riparian habitat, shrublands, water bodies, orchards, arable lands, conifers, eucalyptus plantations, urban areas (Supporting Information, Table S2.1). We only considered permanent water bodies, most of which are natural in the study area. Landscape structure was quantified by first reclassifying the land cover classes into "open" and "closed" habitats, and then computing patch richness, median patch area and edge density metrics computed with Fragstats 4.2 (McGarigal et al., 2012). Urban areas and closed and mixed forests were classified as "closed" habitats, while open forests, shrublands, water bodies, arable land and orchards were classified as "open" habitats (Supporting Information, Table S2.1). This reclassification was judged to provide a better description of landscape heterogeneity for bats than the original land cover classes, because echolocation limits the range of habitat structures a bat can explore and forage, leading to open or cluttered vegetation adaptation (Neuweiler, 1989).

2.2.5 **Data analysis**

Prior to habitat modelling, we assessed collinearity among variables by computing Variance Inflation Factors (VIF), and we dropped from subsequent analysis all variables with VIF > 7. In addition, we computed pairwise Pearson correlations (*r*) between variables, and dropped one variable from each pair showing *r* > 0.7. Finally, we inspected the histograms of variables for excess of zeros and outliers, and dropped the urban and eucalypt land cover classes due to their very low representation. These procedures reduced the environmental variables used in analysis from 50 to 22 (Table 2.1). Regarding the response variables, we confirmed that they were not spatially autocorrelated using spline correlogram plots with 95% pointwise confidence intervals calculated with 1000 bootstrap resamples (BjØrnstad & Falck, 2001) (Supporting Information, Figure S2.1), thereby indicating that autocorrelation did not contribute to biases in estimates of model coefficients and significance levels (Diniz-Filho et al., 2008; Rhodes et al., 2009).

Table 2.1 – Description and summary statistics (mean values and standard deviation) of landscape composition and structure variables used to model bat species richness and total activity in north-eastern Portugal.

Landscape composition	**Description**	**Mean ± stdev**
Mediterranean forest	Proportion of Mediterranean forest in 500-m buffer	0.17 ± 0.16
Riparian habitat	Proportion riparian habitat in 500-m buffer	0.01 ± 0.01
Shrublands	Proportion of shrublands in 500-m buffer	0.36 ± 0.22
Water bodies	Proportion of water bodies in 500-m buffer	0.02 ± 0.03
Orchards	Proportion of orchards in 500-m buffer	0.28 ± 0.21
Arable lands	Proportion of arable land in 500-m buffer	0.06 ± 0.13
Landscape structure		
Altitude		
Standard deviation	Altitude standard deviation	49.78 ± 20.38
Slope		
Median	Median slope	54.08 ± 2.89
Slope area		
>30º	Proportion of buffer area with slope higher than 30º	0.05 ± 0.08
Northness (aspect cosine)		
Median	Median northness	0.02 ± 0.03
Eastness (aspect sine)		
Median	Median eastness	0.03 ± 0.03
Number of closed patches	Number of land cover patches classified as closed weighted by total buffer area	0.03 ± 0.03
Area of open patches	Mean area of land cover patches classified as open weighted by total buffer area	0.06 ± 0.04
Edge density of closed patches	Edge density of land cover patches classified as closed weighted by total buffer area	0.01 ± 0.03
Closed patches richness	Number of land cover categories classified as closed weighted by total buffer area	0.01 ± 0.01
Number of open patches	Number of land cover patches classified as open weighted by total buffer area	0.16 ± 0.07
Edge density of open patches	Edge density of land cover patches classified as open weighted by total buffer area	0.79 ± 0.32
Area of closed patches	Mean area of land cover patches classified as closed weighted by total buffer area	0.04 ± 0.06
Open patches richness	Number of land cover categories classified as open weighted by total buffer area	0.03 ± 0.01

Seasonal and annual habitat relations were estimated using generalized linear models, with Poisson distribution and log link function for species richness, and negative binomial distribution and log link function for bat activity. No correction for overdispersion was needed for species richness models, while the negative binomial models adequately accounted for high overdispersion in bat activity data (Ver Hoef & Boveng, 2007). Models were built separately for the landscape composition and structure sets of variables, because combining the two might obscure the effects of landscape structure given the strong affinities of bats for particular habitat types (Russo & Jones, 2003). Model building was based on the model selection and averaging procedure of Burnham & Anderson (2002), which compares the relative support of a suite of candidate models using Akaike's information criterion (AIC) and the corresponding Akaike weights (w_i). Candidate models were built using all possible combinations of variables, and model building involved a two-step procedure. For each landscape model, we computed an average model based on the 95% confidence set of candidate models, and estimated the sum of the Akaike weights (w_i+) as a measure of its relative importance in the model. Variables with a probability of selection above 0.65 were then carried out to the second model building step, where we repeated the model selection and averaging procedure. Inferences were made considering the selection probability of each explanatory variable along with the uncertainty in parameter estimates with 95% confidence intervals (95% CI), with variables with CI overlapping zero considered to have equivocal meaning (Burnham & Anderson, 2002).

Analyses were performed in R 3.3.2 (R Core Team, 2018), using the usdm package (Naimi, 2015) to compute VIFs, the MASS package (Venables & Ripley, 2002) for generalized linear modelling, and the MuMIn package (Barton, 2016) for model selection and averaging.

2.2.6 **Species richness and bat activity mapping**

The seasonal habitat models were projected into the study area to identify hotspots of bat species richness and activity. To do this, we first created a hexagonal grid covering the whole study area (Birch et al., 2007), with hexagon area similar to that of the median transect buffer (c.a.109.21 m^2). The environmental variables were extracted for each polygon using the procedure described above, and then we used the habitat models to predict the species richness (number of species per 15-minutes interval) and bat activity (bat passes.minute^{-1}) for each polygon. Hotspots of species richness corresponded to hexagons with >3.5 species per 15-minutes interval, whereas hotspots of bat activity correspond to hexagons with >2 bat passes.minute^{-1}. Seasonal maps were then overlapped, and the consistency in hotspot location across seasons was estimated and depicted with Venn diagrams built using Venneuler R package (Wilkinson, 2011).

2.3 Results

2.3.1 **Acoustic surveys**

From the initial 200 transects sampled during a total of 50 hours, only 155 provided data with sufficient quality for subsequent analysis, due to low recording quality resulting for instance from equipment malfunction or background noise, local adverse weather conditions, and other field constraints such as terrain ruggedness. From these, 51 were sampled during Pregnancy, 60 during Lactation, and 44 during Post-lactation. A total of 6929 bat passes were recorded, of which 66% (4551) could be identified to species level, yielding a total of 19 species or species groups. Pipistrelle species had the highest activity levels, with *Pipistrellus*

pipistrellus representing 47% of the identified bat passes, followed by *P. kuhlii* (17%), *Tadarida teniotis* (17%) and *Myotis daubentonii* (13%). A total of 327 (7%) bat passes were assigned to the small *Myotis* group (*M. daubentonii*, *M. emarginatus*, *M. mystacinus*, *M. bechsteinii* and *M. escalerai*), though in most cases these probably belong to *M. daubentonii* that could not be reliably identified due to poor recording quality.

2.3.2 **Species richness**

Landscape composition models provided moderate ($0.9 > w_i+ > 0.7$) to high ($w_i+ > 0.9$) support for positive effects of cover by riparian habitats and water bodies on species richness in all phenological periods except Pregnancy (Figure 2.2, Supporting Information Table S2.2). The negative effects of orchards was moderately supported, but only during Lactation. Regarding landscape structure, there was moderate to high support for the positive effect of steeper slope (>30%) areas, more patches of open habitats, and higher richness of open patches, and for the negative effect of the mean size of closed habitat patches, but the effects were inconsistent across periods (Figure 2.2, Supporting Information Table S2.2). Global models combining composition and structure variables suggested that species richness was mainly affected by landscape composition, with consistently positive effects of riparian cover and water bodies, except during Pregnancy when there was no effect was supported (Figure 2.2, Supporting Information Table S2.2). During Lactation there was moderately supported positive effects of steeper slope (>30º) areas and the number of open patches, and negative effects of orchards and arable land cover. The effect of steeper slopes was also moderately supported in the annual model.

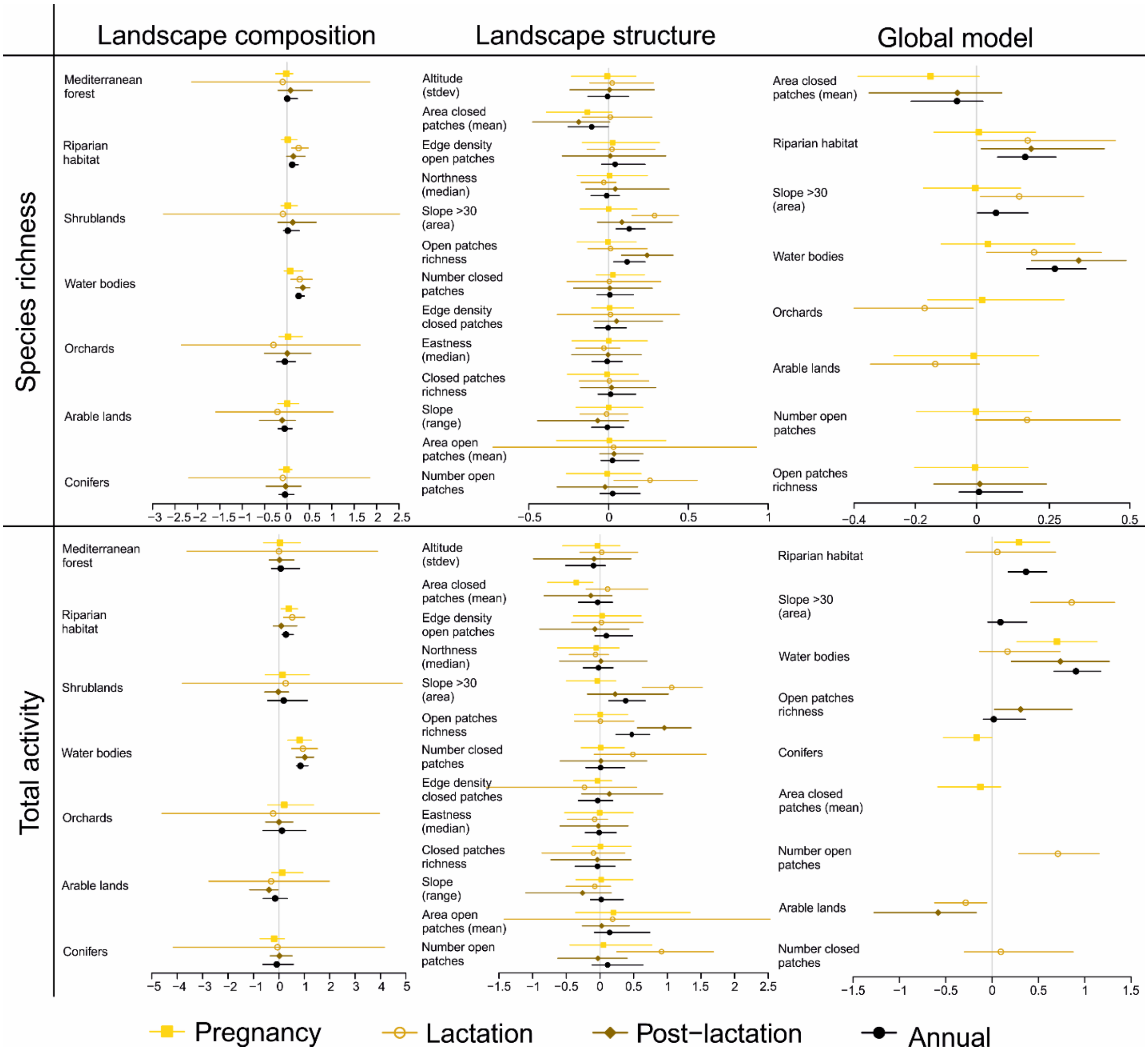

Figure 2.2 – Forest plots summarising average models relating bat species richness and total activity to either landscape composition, landscape structure, or a combination of landscape composition and structure (global) variables in north-east Portugal. Different models were built for each phenological period (Pregnancy – filled square, Lactation – empty square, Post-lactation – filled diamond) and for data combined over the annual cycle (Annual – filled circle). For each average model we plot the regression coefficient estimates and the corresponding 95% confidence interval for each variable included in the model. Details of each model are provided in Table S2.2 and Table S2.3 in Supporting Information.

2.3.3 **Bat activity**

Landscape composition models provided high support for the positive effects of cover by riparian habitats (except in Post-lactation) and water bodies in all phenological periods and in the annual model, while the negative effect of conifer plantations was only moderately supported during Pregnancy (Figure 2.2, Supporting Information Table S2.3). Landscape structure models provided moderate to high support for higher bat activity in areas of steeper slopes (>30%), with more patches of open habitats, and higher richness of open patches, and negative effects of the mean patch size of closed habitats (Figure 2.2, Supporting Information Table S2.3), but effects were inconsistent across periods. Global models provided moderate to high supported for positive effects of water bodies in all periods except Lactation and over the annual cycle, riparian habitats during Pregnancy and over the annual cycle, and for steeper slope areas and number of open patches during Lactation (Figure 2.2, Supporting Information Table S2.3). There was also a moderately supported negative effect of arable land during Lactation.

The modelling procedure for total bat activity was repeated after excluding data for *P. pipistrellus*, because this species represented 65% of the identified bat passes and could thus have a strong influence in the habitat associations uncovered. Likewise, we removed non-identified calls, because 80% of these were assigned to species groups including *P. pipistrellus*. The new landscape composition models provided strong support for the positive effects of water bodies, and moderate support for the negative effects of arable land, in all phenological periods except Pregnancy and over the annual cycle (Supporting Information Table S2.4). The landscape structure models provided high support for the effects of steeper slope (>30º) areas and the number of closed patches during Lactation, for open patch richness during Post-Lactation, and for steeper slope areas and open patch richness over the annual cycle (Supporting Information Table S2.4). Other variables showed only moderate support and their effects were inconsistent across periods.

2.3.4 **Hotspots of bat species richness and activity**

During Pregnancy, there was no obvious pattern in the spatial distribution of species richness hotspots, while during Lactation and Post-lactation there was a clear concentration of hotspots along the main river and its two largest tributaries (Figure 2.3). The hotspots of bat activity were similar to those of species richness and were always concentrated along the main river and its two largest tributaries, though this pattern was much weaker during Pregnancy than during Lactation and Post-lactation (Figure 2.3). There were also important differences between the two latter periods, with activity hotspots during lactation occurring all along the main river valley and its tributaries, while during Post-lactation it was concentrated almost exclusively in a narrow strip along the main river (Figure 2.3). Predictions considering the entire breeding season also identified the same areas as hotspots of bat activity, though the spatial patterns were less well defined than during either the Lactation or Post-lactation periods (Figure 2.3).

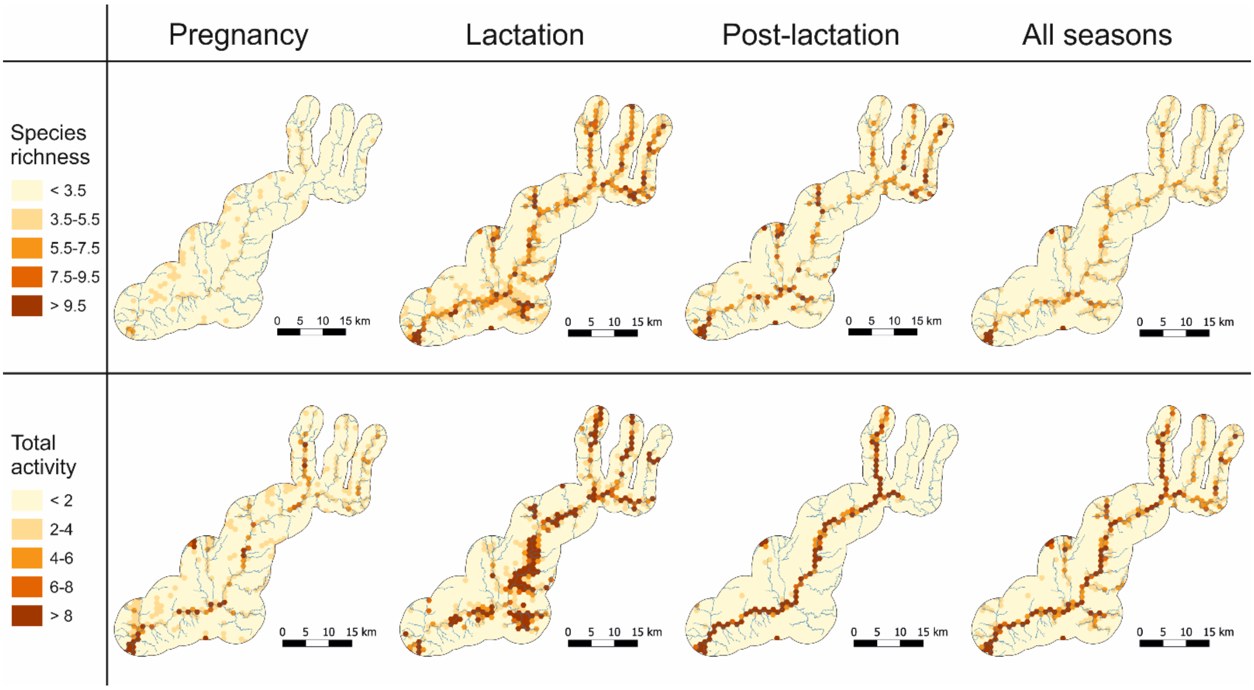

Figure 2.3 – Spatial distribution of hotspots (hexagons) of bat species richness (>3.5 species per 15-minutes interval) and total activity (>2 bat passes.minute^{-1}) in north-eastern Portugal, estimated from the spatial projection of the global landscape models provided in Table S2.2 and Table S2.3 in Supporting Information. Separate maps are provided for each phenological period (Pregnancy, Lactation and Post-lactation) and for data combined over the annual cycle.

Overall, there was a large temporal mismatch between the spatial distribution of hotspots, with only 16.3% and 24.6% being common across the three breeding periods for richness and activity, respectively (Figure 2.4). Most of these consistent hotspots are located along the main river (Figure 2.4). Spatial projection of the standard error can be found in Figure S2.2 in Supporting Information.

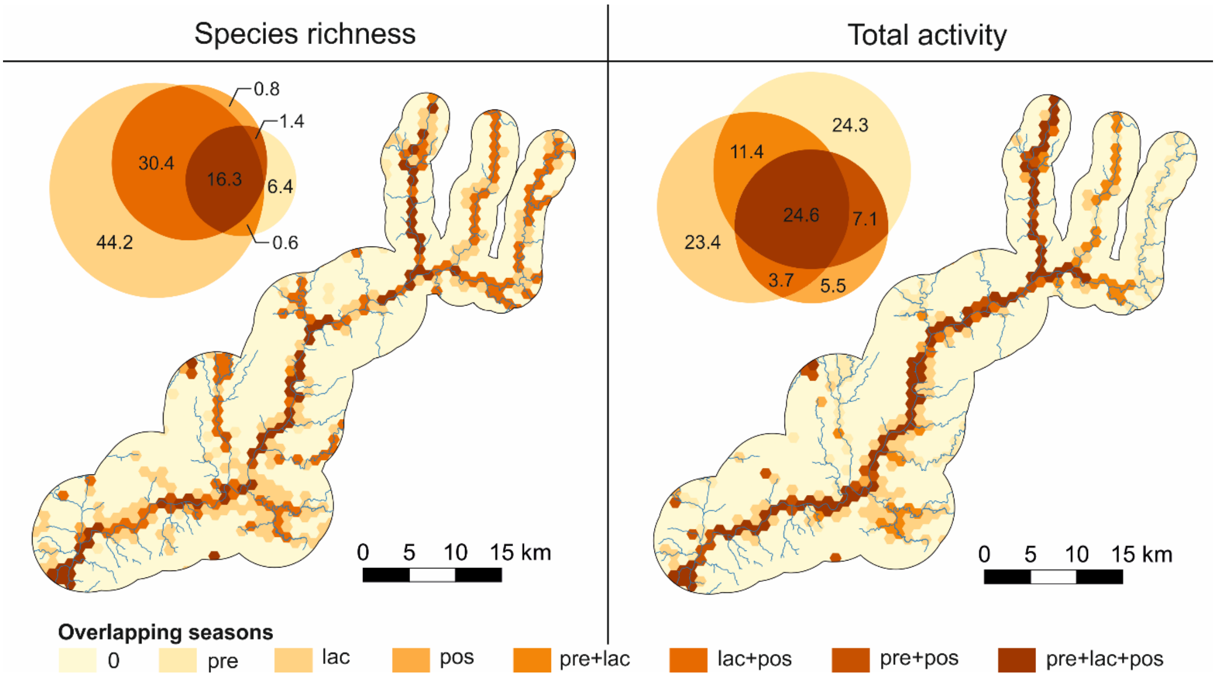

Figure 2.4 – Spatial overlap in the distribution of hotspots (hexagons) of bat species richness (>3.5 species per 15-minutes interval) and total activity (>2 bat passes.minute^{-1}) in north-eastern Portugal, across the three phenological periods considered in the study (Pre – Pregnancy, Lac – Lactation, Pos – Post-lactation). Zero represents areas without bat hotspots in any phenological period, while the remaining colours represent overlaps between different combinations of phenological periods. Venn diagrams shows the percentage overlap of hotspots among the three phenological periods.

2.4 Discussion

As predicted, our results have identified a seasonal pattern in habitat preferences of bats in a Mediterranean landscape of north-eastern Portugal, suggesting that bats may track the spatiotemporal dynamics of water availability. Overall, species richness and bat activity were mainly shaped by the habitats where water was available (water bodies, riparian galleries), with the strength of such associations peaking at the end of summer, when surface waters were mainly available in large streams and rivers (Ferreira *et al.*, 2016). In contrast, in spring, during Pregnancy, bats did not show strong associations to specific habitat features, probably due to higher water availability across the landscapes. Overall, our results point out the need to understand how vagile species such as bats modify their habitat associations and spatial distribution over the annual cycle, which is essential to determine the habitats that are needed year round to assure species persistence (Bissonette & Storch, 2007).

2.4.1 **Limitations and potential shortcomings**

Our study had some limitations and potential shortcomings, but we believe that they did not affect our key results in any significant way. First, although our sample size was relatively small, the number of transects surveyed was comparable to that of similar studies (e.g., Vaughan *et al.*, 1997; Davy *et al.*, 2007; Rainho, 2007; Mendes *et al.*, 2017; Salvarina *et al.*, 2018), and it was sufficient to detect 19 out of the 25 bat species occurring in continental Portugal. Therefore, it is unlikely that small sample sizes were responsible for the strong associations observed with water bodies and riparian habitats, or the marked variations in the spatial distribution of species richness and bat activity. Second, it should be considered that each transect was sampled only once, and so the sampling network varied across years and phenological periods. The sampling design was chosen to incorporate as much environmental variability as possible, while overcoming logistic limitations that prevented us from sampling every site during each period. This strategy is not without potential problems, however, as it might be argued that the patterns observed could be due to the sampling of different areas at different times of the year. We believe this is unlikely to have biased the results, because in each season we randomly distributed the transects across the study area, and stratified sampling so that at least three transects representative of each land cover type were visited in each season. In this way, we avoided time x space and time x habitat interactions that could have affected our results. Finally, our results on bat activity may be dominated by the spatial patterns of a single species, *P. pipistrellus*, which was by far the most frequently recorded. It should be noted, however, that our results for bat activity were largely consistent with those of obtained with species richness, though the latter variable should not be influenced by the abundance of a single species. Also, models developed after excluding *P. pipistrellus* still provided high support for the importance of water bodies, particularly during the Lactation and Post-lactation periods, though the effects of environmental variables in general were much less supported.

2.4.2 **Water is a key landscape feature for bats**

We found a strong positive association between bats and habitats where water is available (water bodies and riparian galleries), which was evident in analysis based on either species richness or total activity, and that was largely supported in most phenological periods and over the annual cycle. The association with these habitats had consequences for the landscape scale distribution of bats, with species richness and activity often peaking close to large rivers and streams. Reasons for these patterns are uncertain, but they may reflect the abundance of prey close to water bodies (Fukui et al., 2006; Goiti et al., 2008; Hagen & Sabo, 2012; Lisón et al., 2015; Salvarina et al., 2018), the need to drink water (Russo & Jones, 2003; Tuttle et al., 2006; Adams & Hayes, 2008; Greif & Siemers, 2010), or a combination of these and other ecological factors. Whatever the causes, the importance of aquatic habitats for bats has been reported in a large number of studies (review in Salvarina, 2016), including studies carried out in the Mediterraneam region. For instance, Russo & Jones (2003) showed that water sites corresponded to the habitat most used by bats, whilst a large number of endangered or vulnerable species occurred in riparian habitats, broadleaved woodlands and olive groves. Also, Rainho (2007) found that water sites during the summer period supported high species richness, while riparian habitats surrounded by autochthonous broad-leaved forests provided optimal foraging areas. Finally, Lisón & Calvo (2013) showed using ecological niche modelling that pipistrelle species have a strong preference for aquatic habitats, while a telemetry study by Salsamendi *et al.* (2012) concluded that *Rhinolophus mehelyi* foraged close to water bodies, where it was judged to have access to drinking water and higher insect abundances. Comparable patterns were found in other regions, with particularly strong associations between bats and water reported in arid and semi-arid environments, including for instance the Middle East, North Africa and parts of North America (Rebelo & Brito, 2007; Razgour et al., 2010; Hagen & Sabo, 2014; Korine et al., 2016).

The other landscape variables considered in our study had much weaker effects, and these were often inconsistent across phenological periods. One of the variables showing the most supported positive effects was the area with steeper slopes (>30%), but this may also reflect the presence of deep river valleys and thus the proximity to water and riparian galleries. However, this variable may also reflect the presence of bat roosts in cliffs and other steep areas (Santos *et al.*, 2014). This is supported by the strongest effect of steep slopes on both species richness and activity during lactation, a period when lactating females have smaller home ranges, fly shorter distances and return to roosts more often during the night, leading to increased activity near roosts (Henry et al., 2002; Lučan & Radil, 2010). Still weaker and more inconsistent effects were found for variables that describe landscape structure such as open patches richness, number of open patches and mean area of closed patches and the presence of edges, which are related to landscape heterogeneity, and may thus affect bat diversity and activity (Jantzen & Fenton, 2013; Stein et al., 2014). However, the effect of these variables may only be perceived at fine spatial scales, which may explain their modest contribution in our study.

2.4.3 **Water resource tracking by bats in Mediterranean landscapes**

Our results indicate that the effect of water bodies on bat species richness and activity increased consistently over the breeding season, and there was a progressive spatial concentration of diversity and activity hotspots close to permanently flowing waters. In fact, while in springtime, during Pregnancy, there were neither strong habitat effects or marked spatial patterns of hotspot distribution, in late summer, during Post-lactation, there were very strong effects of water bodies and the hotspots were distributed along the largest river in the region. These results suggest that bats may track spatial variations in water availability, which in the Mediterranean is at its highest in spring and at its lowest in late summer (Gasith & Resh, 1999; Magalhães *et al.*, 2007). This is also the case in our study area, where water availability progressively decreases during the summer, with smaller tributaries and upper reaches drying out, and surface waters remaining primarily in the main river and the largest tributaries (Ferreira *et al.*, 2016).

The reason for bats tracking the receding waters is unknown, but it may be a consequence of the changes in the availability of critical resources during the summer, coupled with changes in the requirements of individuals during the breeding season. One possibility is that insect prey is highest close to water bodies during the dry season, in a period of low primary productivity throughout most of the landscape (Amorim *et al.*, 2015). In fact, water availability is known to affect insect prey distribution and abundance (Hawkins & Porter, 2003; Bailey et al., 2004), and in summer the emergence of adult insects from streams may offer feeding opportunities for bats (Baxter et al., 2005; Fukui et al., 2006; Hagen & Sabo, 2012). Also, the availability of drinking water may be low throughout most of the landscape, which may constrain bats to areas close to the main rivers and streams where they can access this important resource (Russo & Jones, 2003; Tuttle et al., 2006; Adams & Hayes, 2008; Greif & Siemers, 2010). These factors may explain why there was no marked effects during the spring of water bodies, or actually of any habitat feature, as water availability and primary productivity tend to be high across the landscape. Furthermore, pregnant females may range more widely and forage for longer periods than during lactation and post-lactation (Henry et al., 2002; Encarnação et al., 2004; Daniel et al., 2010, but see, Vincent et al., 2011), thereby having the ability to move over large areas and thus presumably having weaker associations with specific habitat features. In contrast, during lactation females have the highest energetic demands (Anthony & Kunz, 1977; Kurta et al., 1990; Dietz & Kalko, 2006), which may constrain their foraging activity to areas with high prey availability close to roosts, thereby justifying the increased association with water bodies and riparian galleries. Clearly, these issues require further investigation to understand the extent to which the spatiotemporal tracking of water availability is a general pattern in dry landscapes, and to identify the factors driving such resource tracking. For this, it is important to develop more studies characterising habitat associations over the breeding season, because the usual practice of pooling all data into a single yearly data set (Russo & Jones, 2003; Salsamendi *et al.*, 2012) cannot detect eventual seasonal patterns in bat habitat use.

2.4.4 **Conservation implications**

Our results show that the habitat associations and distribution of bats in Mediterranean landscapes may change over the annual cycle, with species richness and activity progressively concentrating throughout the summer in the few habitats where water remains available. This has important consequences for conservation, as bat breeding season in the Mediterranean partly overlaps with the peak of dry conditions (Racey & Swift, 1985; Audet, 1990; Altringham, 1996; Ibáñez, 1997; Rodrigues et al., 2003; Amorim et al., 2015), thus reductions in water availability may reduce reproductive success compromising species persistence (Safi & Kerth, 2004; Adams & Hayes, 2008; Adams, 2010; Amorim et al., 2015). In fact, reduction in the availability of surface waters during the summer may decrease the opportunities for drinking (Rainho, 2007; Korine et al., 2016), reduce prey availability (Hagen & Sabo, 2012; Salvarina et al., 2018), and increase competition among individuals at remnant waters (Adams et al., 2003; Razgour et al., 2011b). Bats may thus be strongly affected by current trends of climate change, as the predicted increases in the frequency and severity of summer droughts in the Mediterranean region will likely reduce water flows (Milly *et al.*, 2005), thereby degrading habitat suitability for bats during critical periods. Increasing damming of rivers for hydroelectric power generation and to feed irrigated agriculture is also likely to reduce flowing waters and thus habitat suitability for bats (Rebelo & Rainho, 2009), although small scale artificial bodies of water may promote bat diversity and activity in arid environments (Tuttle et al., 2006; Razgour et al., 2010; Sirami et al., 2013). Finally, the degradation of water quality due to pollution may further reduce the availability of suitable water habitats for bats (Vaughan et al., 1996; Korine et al., 2015; Salvarina, 2016).

Overall, our results point out that rivers and larger streams that maintain water through the dry season should be considered a priority for bat conservation in the Mediterranean region, thereby further supporting the need to maintain their ecological integrity for a range of both aquatic and terrestrial species (Gasith & Resh, 1999; Baxter et al., 2005; Matos et al., 2009; Rebelo & Rainho, 2009; Carvalho et al., 2010). More generally, our results support the value of considering the temporal dimension of habitat studies, as ignoring spatiotemporal heterogeneities in resource use and availability may contribute for overlooking critical habitats for species persistence in dynamic landscapes (Bissonette & Storch, 2007).

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Supporting Information

Table S2.1 – Landscape composition and structure variables used to model bat habitats in NE Portugal. For each variable we provide a short description, summary statistics (Mean ± Sandard Deviation [SD]) and, where appropriate, its reclassification as "open" (O) or "closed" (C) according to habitat clutter to calculate configuration metrics.

Variables	Description	Mean ± SD	Clutter
Landscape composition			
Mediterranean forest	Proportion of Mediterranean forest in 500-m buffer	0.17 ± 0.16	C/O[a]
Riparian habitat	Proportion riparian habitat in 500-m buffer	0.01 ± 0.01	C
Shrublands	Proportion of shrublands in 500-m buffer	0.36 ± 0.22	O
Water bodies	Proportion of water bodies in 500-m buffer	0.02 ± 0.03	O
Orchards	Proportion of orchards in 500-m buffer	0.28 ± 0.21	O
Arable land	Proportion of arable land in 500-m buffer	0.06 ± 0.13	O
Conifers	Proportion of conifer plantations in 500-m buffer	0.09 ± 0.15	C/O[a]
Eucalyptus plantations	Proportion of Eucalyptus plantations in 500-m buffer	0.01 ± 0.04	C/O[a]
Urban areas	Proportion of urban areas in in 500-m buffer	0.00 ± 0.00	C
Landscape structure			
Altitude			
Standard deviation	Altitude standard deviation	49.78 ± 20.38	
Slope			
Median	Median slope	54.08 ± 2.89	
Slope area			
>20º	Proportion of buffer area with slope higher than 20º	0.28 ± 0.22	
>30º	Proportion of buffer area with slope higher than 30º	0.05 ± 0.08	
>40º	Proportion of bufferarea with slope higher than 40º	0.00 ± 0.01	
Northness (aspect cosine)			
Median	Median northness	0.02 ± 0.03	
Eastness (aspect sine)			
Median	Median eastness	0.03 ± 0.03	
Number of closed patches	Number of land cover patches classified as closed weighed by total buffer area	0.03 ± 0.03	
Area of open patches	Mean area of land cover patches classified as open weighed by total buffer area	0.06 ± 0.04	
Edge density of closed patches	Edge density of land cover patches classified as closed weighed by total buffer area	0.01 ± 0.03	
Closed patches richness	Number of land cover categories classified as closed weighed by total buffer area	0.01 ± 0.01	
Number of open patches	Number of land cover patches classified as open weighed by total buffer area	0.16 ± 0.07	
Edge density of open patches	Edge density of land cover patches classified as open weighed by total buffer area	0.79 ± 0.32	
Area of closed patches	Mean area of land cover patches classified as closed weighed by total buffer area	0.04 ± 0.06	
Open patches richness	Number of land cover categories classified as open weighted by total buffer area	0.03 ± 0.01	

[a] Forest patches (Mediterranean forest, Conifers and Eucalyptus plantations) were classified as either "closed" (closed and mixed forest) or "open" (open forest and agroforestry systems).

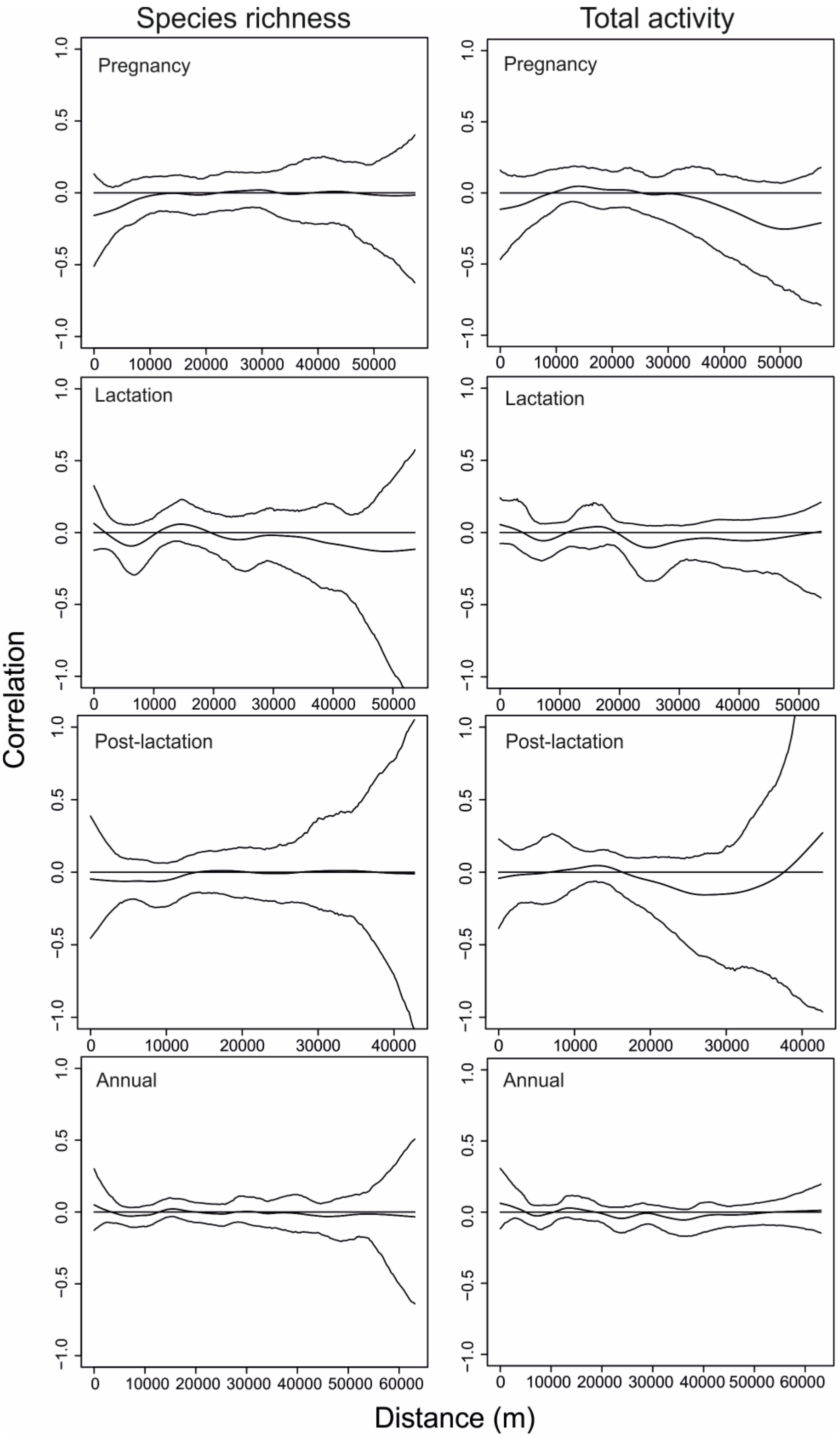

Figure S2.1 – Spline correlograms describing spatial autocorrelation in bat species richness and total activity. Separate correlograms are presented for each phenological period (Pregnancy, Lactation, Post-lactation) and over the annual cycle. Lines represent the estimate (in the middle) and the 95% confidence envelopes (external lines) using 1000 bootstrap resamples.

Table S2.2 – Summary results of average models relating bat species richness to landscape composition and structure variables. Models were built separately for each phenological period (Pregnancy, Lactation, Post-Lactation) and for data aggregated across periods (Annual). In each case, separate models were also built for landscape composition, landscape structure, and the combination of landscape composition and structure variables. For each variable we provide the estimate of the regression coefficient (B), its standard error (SE) and 95% confidence interval (95%CI), the sum of Akaike weights (w_i+), the number of models in which the variable was included (*N*), and the significance level. *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$. Variables with w_i+ >0.70 are highlighted in bold

Landscape composition models

Pregnancy

Variables	B	SE	95% CI	w_i+	*N*	*P*
(Intercept)	1.152	0.087	[9.77E-01, 1.33E+00]			<2.0E-16 ***
Mediterranean forest	-0.018	0.058	[-2.52E-01, 1.30E-01]	0.29	38	0.765
Riparian habitat	0.015	0.051	[-1.19E-01, 2.18E-01]	0.30	42	0.777
Shrublands	0.014	0.054	[-1.35E-01, 2.33E-01]	0.29	39	0.797
Water bodies	0.074	0.104	[-6.40E-02, 3.56E-01]	0.50	50	0.485
Orchards	0.025	0.080	[-1.77E-01, 3.43E-01]	0.30	39	0.762
Arable land	0.007	0.061	[-2.06E-01, 2.63E-01]	0.26	37	0.905
Conifers	-0.009	0.040	[-1.76E-01, 1.12E-01]	0.27	37	0.830

Lactation

Variables	B	SE	95% CI	w_i+	*N*	*P*
(Intercept)	1.261	0.089	[1.08E+00, 1.44E+00]			< 2.0E-16 ***
Mediterranean forest	-0.067	0.694	[-2.13E+00, 1.85E+00]	0.47	16	0.924
Riparian habitat	**0.290**	**0.093**	**[1.04E-01, 4.75E-01]**	**1**	**27**	**2.2E-03 ****
Shrublands	-0.061	0.945	[-2.76E+00, 2.51E+00]	0.5	16	0.949
Water bodies	**0.314**	**0.131**	**[8.88E-02, 5.62E-01]**	**0.96**	**26**	**1.8E-02 ***
Orchards	-0.273	0.884	[-2.36E+00, 1.63E+00]	0.75	17	0.761
Arable land	-0.184	0.551	[-1.59E+00, 1.03E+00]	0.66	13	0.741
Conifers	-0.064	0.629	[-2.19E+00, 1.85E+00]	0.38	14	0.920

Post-lactation

Variables	B	SE	95% CI	w_i+	*N*	*P*
(Intercept)	0.947	0.113	[7.19E-01, 1.18E+00]			< 2.0E-16 ***
Mediterranean forest	0.080	0.155	[-1.97E-01, 5.62E-01]	0.44	26	0.611
Riparian habitat	**0.143**	**0.124**	**[-7.01E-03, 4.03E-01]**	**0.72**	**32**	**0.254**
Shrublands	0.129	0.196	[-2.05E-01, 6.50E-01]	0.58	28	0.517
Water bodies	**0.355**	**0.078**	**[1.98E-01, 5.12E-01]**	**1**	**53**	**9.1E-06 *****
Orchards	0.006	0.153	[-5.00E-01, 5.35E-01]	0.34	23	0.970
Arable land	-0.105	0.175	[-6.14E-01, 1.85E-01]	0.49	29	0.555
Conifers	-0.030	0.126	[-4.63E-01, 3.10E-01]	0.39	26	0.816

Annual

Variables	B	SE	95% CI	w_i+	*N*	*P*
(Intercept)	1.109	0.047	[1.02E+00, 1.20E+00]			< 2.0E-16 ***
Mediterranean forest	0.045	0.069	[-5.85E-02, 2.34E-01]	0.52	14	0.513
Riparian habitat	**0.156**	**0.048**	**[6.04E-02, 2.51E-01]**	**1**	**29**	**1.4E-03 ****
Shrublands	0.057	0.083	[-7.72E-02, 2.75E-01]	0.57	15	0.499
Water bodies	**0.299**	**0.044**	**[2.13E-01, 3.86E-01]**	**1**	**29**	**< 2.0E-16 *****
Orchards	-0.008	0.066	[-2.29E-01, 1.88E-01]	0.38	14	0.906
Arable land	-0.014	0.049	[-1.96E-01, 1.14E-01]	0.33	13	0.782

Conifers	-0.004	0.047	[-1.78E-01, 1.55E-01]	0.30	13	0.940

Landscape structure models

Pregnancy

Variables	**B**	**SE**	**95% CI**	**w_i+**	***N***	***P***
(Intercept)	1.145	0.083	[9.77E-01, 1.31E+00]			<2.0E-16 ***
Altitude (stdev)	-0.008	0.054	[-2.32E-01, 1.71E-01]	0.27	1763	0.880
Area closed patches (mean)	**-0.133**	**0.120**	**[-3.90E-01, 2.15E-02]**	**0.72**	**2510**	**0.275**
Edge density open patches	0.025	0.077	[-1.65E-01, 3.18E-01]	0.32	1897	0.754
Northness (median)	0.006	0.056	[-1.96E-01, 2.43E-01]	0.26	1690	0.917
Slope >30 (area)	1.2E-5	0.045	[-1.78E-01, 1.78E-01]	0.26	1685	1
Open patches richness	-0.003	0.047	[-1.96E-01, 1.73E-01]	0.26	1679	0.95
Number closed patches	0.027	0.058	[-7.64E-02, 2.24E-01]	0.37	1904	0.64
Edge density closed patches	0.007	0.036	[-1.06E-01, 1.57E-01]	0.28	1763	0.85
Eastness (median)	0.002	0.060	[-2.29E-01, 2.43E-01]	0.26	1672	0.977
Closed patches richness	-0.010	0.061	[-2.58E-01, 1.86E-01]	0.28	1735	0.872
Slope (range)	0.001	0.054	[-2.04E-01, 2.14E-01]	0.27	1733	0.98
Area open patches (mean)	0.004	0.087	[-3.26E-01, 3.58E-01]	0.26	1684	0.962
Number open patches	-0.008	0.062	[-2.65E-01, 2.03E-01]	0.27	1701	0.897

Lactation

Variables	**B**	**SE**	**95% CI**	**w_i+**	***N***	***P***
(Intercept)	1.091	0.083	[9.24E-01, 1.26E+00]			< 2.0e-16 ***
Altitude (stdev)	0.027	0.069	[-1.17E-01, 2.81E-01]	0.33	930	0.698
Area closed patches (mean)	0.015	0.063	[-1.65E-01, 2.72E-01]	0.28	904	0.813
Edge density open patches	0.026	0.071	[-1.35E-01, 2.89E-01]	0.33	1087	0.721
Northness (median)	-0.026	0.047	[-1.72E-01, 4.67E-02]	0.41	1090	0.5854
Slope >30 (area)	**0.293**	**0.073**	**[1.47E-01, 4.38E-01]**	**1**	**2215**	**8.2e-05 *** **
Open patches richness	0.017	0.057	[-1.29E-01, 2.42E-01]	0.30	953	0.770
Number closed patches	0.009	0.077	[-2.61E-01, 3.27E-01]	0.26	884	0.910
Edge density closed patches	0.016	0.102	[-3.21E-01, 4.44E-01]	0.27	876	0.876
Eastness (median)	-0.025	0.053	[-2.06E-01, 7.10E-02]	0.37	974	0.643
Closed patches richness	0.009	0.059	[-1.84E-01, 2.52E-01]	0.28	921	0.876
Slope (range)	-0.008	0.041	[-1.79E-01, 1.19E-01]	0.28	901	0.844
Area open patches (mean)	0.036	0.252	[-7.24E-01, 9.27E-01]	0.35	1148	0.888
Number open patches	**0.265**	**0.152**	**[3.43E-02, 5.55E-01]**	**0.90**	**1642**	**0.086**

Post-lactation

Variables	**B**	**SE**	**95% CI**	**w_i+**	***N***	***P***
(Intercept)	1.100	0.097	[9.04E-01, 1.30E+00]			<2E-16 ***
Altitude (stdev)	0.006	0.070	[-2.42E-01, 2.87E-01]	0.28	1256	0.930
Area closed patches (mean)	**-0.188**	**0.142**	**[-4.76E-01, 6.42E-03]**	**0.80**	**1865**	**0.195**
Edge density open patches	0.011	0.092	[-2.90E-01, 3.58E-01]	0.31	1409	0.910
Northness (median)	0.042	0.095	[-1.42E-01, 3.78E-01]	0.36	1329	0.666
Slope >30 (area)	0.083	0.116	[-6.98E-02, 3.98E-01]	0.51	1544	0.481
Open patches richness	**0.241**	**0.081**	**[8.11E-02, 4.04E-01]**	**1**	**2964**	**3.82e-3 ** **
Number closed patches	0.008	0.065	[-2.19E-01, 2.73E-01]	0.28	1265	0.911
Edge density closed patches	0.050	0.091	[-9.22E-02, 3.37E-01]	0.41	1355	0.587
Eastness (median)	-0.004	0.056	[-2.31E-01, 2.03E-01]	0.27	1259	0.949
Closed patches richness	0.018	0.070	[-1.76E-01, 2.95E-01]	0.31	1272	0.799
Slope (range)	-0.068	0.121	[-4.46E-01, 1.24E-01]	0.42	1387	0.581

Area open patches (mean)	0.034	0.059	[-5.51E-02, 2.15E-01]	0.43	1464	0.569
Number open patches	-0.022	0.078	[-3.22E-01, 1.82E-01]	0.32	1291	0.780

Annual

Variables	**B**	**SE**	**95% CI**	w_i+	*N*	*P*
(Intercept)	1.126	0.047	[1.03E+00, 1.22E+00]			<2.0E-16 ***
Altitude (stdev)	-0.001	0.034	[-1.29E-01, 1.25E-01]	0.28	985	0.984
Area closed patches (mean)	**-0.100**	**0.077**	**[-2.53E-01, -2.64E-03]**	**0.78**	**1444**	**0.197**
Edge density open patches	0.047	0.067	[-4.37E-02, 2.28E-01]	0.51	1314	0.487
Northness (median)	-0.006	0.026	[-1.10E-01, 6.75E-02]	0.28	907	0.817
Slope >30 (area)	**0.135**	**0.049**	**[4.79E-02, 2.28E-01]**	**0.98**	**2081**	**0.006 ****
Open patches richness	**0.122**	**0.057**	**[3.19E-02, 2.28E-01]**	**0.94**	**1801**	**0.035 ***
Number closed patches	0.014	0.039	[-7.25E-02, 1.56E-01]	0.34	1029	0.719
Edge density closed patches	0.003	0.026	[-8.60E-02, 1.11E-01]	0.27	897	0.899
Eastness (median)	-0.003	0.025	[-1.05E-01, 8.42E-02]	0.26	877	0.915
Closed patches richness	0.019	0.044	[-6.57E-02, 1.69E-01]	0.36	1031	0.669
Slope (range)	-0.002	0.026	[-1.06E-01, 9.41E-02]	0.26	871	0.951
Area open patches (mean)	0.031	0.053	[-4.69E-02, 1.89E-01]	0.43	1047	0.563
Number open patches	0.032	0.055	[-5.32E-02, 1.97E-01]	0.44	1058	0.568

Global models

Pregnancy

Variables	**B**	**SE**	**95% CI**	w_i+	*N*	*P*
(Intercept)	1.155	0.086	[9.81E-01, 1.33E+00]			<2e-16 ***
Area closed patches (mean)	**-0.150**	**0.117**	**[-3.87E-01, 8.26E-03]**	**0.79**	**101**	**0.204**
Riparian habitat	0.007	0.045	[-1.39E-01, 1.93E-01]	0.27	69	0.874
Slope >30 (area)	-0.004	0.041	[-1.73E-01, 1.44E-01]	0.26	64	0.927
Water bodies	0.037	0.082	[-1.16E-01, 3.21E-01]	0.36	75	0.656
Orchards	0.018	0.066	[-1.59E-01, 2.85E-01]	0.29	70	0.786
Arable land	-0.009	0.063	[-2.70E-01, 2.03E-01]	0.27	62	0.89
Number open patches	-0.002	0.047	[-1.95E-01, 1.79E-01]	0.25	62	0.967
Open patches richness	-0.004	0.047	[-2.02E-01, 1.68E-01]	0.25	62	0.927

Lactation

Variables	**B**	**SE**	**95% CI**	w_i+	*N*	*P*
(Intercept)	1.184	0.088	[1.01E+00, 1.36E+00]			< 2.0E-16 ***
Riparian habitat	**0.171**	**0.139**	**[5.36E-03, 4.53E-01]**	**0.75**	**13**	**0.223**
Slope >30 (area)	**0.143**	**0.105**	**[1.34E-02, 3.49E-01]**	**0.79**	**16**	**0.176**
Water bodies	**0.192**	**0.115**	**[3.34E-02, 4.07E-01]**	**0.87**	**16**	**0.097**
Orchards	**-0.165**	**0.119**	**[-4.00E-01, -1.07E-02]**	**0.81**	**12**	**0.170**
Arable land	**-0.131**	**0.105**	**[-3.46E-01, 9.08E-03]**	**0.78**	**13**	**0.219**
Number open patches	**0.169**	**0.145**	**[-2.26E-03, 4.69E-01]**	**0.72**	**14**	**0.247**

Post-lactation

Variables	**B**	**SE**	**95% CI**	w_i+	*N*	*P*
(Intercept)	0.995	0.101	[7.91E-01, 1.20E+00]			< 2E-16 ***
Area closed patches (mean)	-0.063	0.099	[-3.51E-01, 8.27E-02]	0.47	3	0.536
Riparian habitat	**0.179**	**0.122**	**[1.56E-02, 4.17E-01]**	**0.82**	**4**	**0.151**
Water bodies	**0.334**	**0.077**	**[1.80E-01, 4.88E-01]**	**1**	**6**	**2.2E-05 *****
Open patches richness	0.011	0.049	[-1.40E-01, 2.28E-01]	0.24	2	0.831

Annual

Variables	**B**	**SE**	**95% CI**	w_i+	*N*	*P*

(Intercept)	1.106	0.047	[1.01E+00, 1.20E+00]			<2.00E-16 ***
Area closed patches (mean)	-0.058	0.066	[-2.13E-01, 2.09E-02]	0.61	3	0.378
Riparian habitat	**0.164**	**0.048**	**[6.88E-02, 2.59E-01]**	**1**	**6**	**7.3E-4 *****

Slope >30 (area)	0.069	0.050	[3.16E-03, 1.68E-01]	0.81	4	0.174
Water bodies	**0.261**	**0.049**	**[1.64E-01, 3.58E-01]**	**1**	**6**	**1.0E-7 *****
Open patches richness	0.013	0.035	[-5.68E-02, 1.50E-01]	0.29	2	0.706

Table S2.3 – Summary results of average models relating total bat activity to landscape composition and structure variables. Models were built separately for each phenological period (Pregnancy, Lactation, Post-Lactation) and for data aggregated across periods (Annual). In each case, separate models were also built for landscape composition, landscape structure, and the combination of landscape composition and structure variables. For each variable we provide the estimate of the regression coefficient (B), its standard error (SE) and 95% confidence interval (95%CI), the sum of Akaike weights (w_i+), the number of models in which the variable was included (*N*), and the significance level (*P*). *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$. Variables with w_i+ >0.70 are highlighted in bold

<u>Landscape composition models</u>

Pregnancy

Variables	B	SE	95% CI	w_i+	*N*	*P*
(Intercept)	3.458	0.172	[3.11E+00, 3.80E+00]			< 2.0E-16 ***
Mediterranean forest	0.042	0.223	[-6.03E-01, 8.46E-01]	0.35	16	0.852
Orchards	0.215	0.385	[-4.32E-01, 1.36E+00]	0.46	17	0.579
Riparian habitat	**0.389**	**0.183**	**[8.72E-02, 7.36E-01]**	**0.94**	**30**	**0.037 ***
Shrublands	0.146	0.336	[-5.41E-01, 1.20E+00]	0.44	20	0.665
Water bodies	**0.817**	**0.230**	**[3.57E-01, 1.28E+00]**	**1**	**39**	**5.0E-4 *****
Arable land	0.133	0.254	[-2.80E-01, 9.50E-01]	0.4	16	0.605
Conifers	**-0.188**	**0.236**	**[-7.41E-01, 2.18E-01]**	**0.72**	**21**	**0.431**

Lactation

Variables	B	SE	95% CI	w_i+	*N*	*P*
(Intercept)	3.631	0.208	[3.21E+00, 4.05E+00]			< 2.0E-16 ***
Mediterranean forest	0.057	1.247	[-3.62E+00, 3.88E+00]	0.43	19	0.964
Orchards	-0.171	1.585	[-4.60E+00, 3.97E+00]	0.53	18	0.915
Riparian habitat	**0.581**	**0.232**	**[1.86E-01, 1.02E+00]**	**0.96**	**30**	**0.014 ***
Shrublands	0.320	1.717	[-3.80E+00, 4.85E+00]	0.61	20	0.854
Water bodies	**0.998**	**0.278**	**[5.02E-01, 1.52E+00]**	**0.99**	**34**	**4.1E-4 *****
Arable land	-0.252	0.987	[-2.75E+00, 1.99E+00]	0.66	20	0.801
Conifers	0.000	1.135	[-4.16E+00, 4.16E+00]	0.29	15	1.000

Post-lactation

Variables	B	SE	95% CI	w_i+	*N*	*P*
(Intercept)	3.157	0.220	[2.71E+00, 3.60E+00]			<2.0E-16 ***
Mediterranean forest	0.028	0.132	[-3.83E-01, 5.95E-01]	0.26	19	0.839
Orchards	0.004	0.134	[-5.17E-01, 5.50E-01]	0.26	19	0.976
Riparian habitat	0.094	0.185	[-2.19E-01, 7.03E-01]	0.39	22	0.616
Shrublands	-0.022	0.124	[-5.54E-01, 3.82E-01]	0.26	19	0.862
Water bodies	**1.023**	**0.170**	**[6.79E-01, 1.37E+00]**	**1**	**48**	**<2.0E-16 *****
Arable land	-0.389	0.359	[-1.15E+00, -3.47E-02]	0.66	26	0.285
Conifers	0.024	0.116	[-3.36E-01, 5.20E-01]	0.26	19	0.840

Annual

Variables	B	SE	95% CI	w_i+	*N*	*P*
(Intercept)	3.429	0.101	[3.23E+00, 3.63E+00]			< 2E-16 ***
Mediterranean forest	0.144	0.247	[-2.92E-01, 8.19E-01]	0.55	15	0.560
Orchards	0.087	0.291	[-6.24E-01, 1.06E+00]	0.4	13	0.765
Riparian habitat	**0.349**	**0.111**	**[1.30E-01, 5.69E-01]**	**1**	**29**	**1.78E-3 ****
Shrublands	0.177	0.333	[-4.40E-01, 1.12E+00]	0.52	14	0.595
Water bodies	**0.920**	**0.113**	**[6.96E-01, 1.14E+00]**	**1**	**29**	**< 2E-16 *****
Arable land	-0.077	0.191	[-6.15E-01, 3.32E-01]	0.54	16	0.689
Conifers	-0.013	0.205	[-6.30E-01, 5.72E-01]	0.45	14	0.950

Landscape structure models

Pregnancy

Variables	B	SE	95% CI	w_i+	*N*	*P*
(Intercept)	3.355	0.170	[3.01E+00, 3.70E+00]			<2E-16 ***
Altitude (stdev)	-0.038	0.129	[-5.52E-01, 2.97E-01]	0.3	1857	0.774
Area closed patches (mean)	**-0.351**	**0.232**	**[-7.77E-01, -1.04E-01]**	**0.8**	**2813**	**0.134**
Edge density open patches	0.031	0.143	[-3.94E-01, 6.11E-01]	0.29	1854	0.832
Northness (median)	-0.055	0.152	[-6.30E-01, 2.83E-01]	0.32	1879	0.720
Slope >30 (area)	-0.040	0.117	[-4.97E-01, 2.34E-01]	0.3	1805	0.738
Open patches richness	0.005	0.103	[-3.77E-01, 4.11E-01]	0.27	1809	0.965
Number closed patches	0.011	0.085	[-2.79E-01, 3.61E-01]	0.27	1792	0.898
Edge density closed patches	-0.034	0.093	[-3.89E-01, 1.72E-01]	0.31	1787	0.721
Eastness (median)	-0.004	0.131	[-5.25E-01, 4.92E-01]	0.27	1756	0.974
Closed patches richness	0.007	0.113	[-4.09E-01, 4.63E-01]	0.27	1773	0.95
Slope (range)	0.018	0.114	[-3.58E-01, 4.90E-01]	0.27	1791	0.878
Area open patches (mean)	0.199	0.363	[-3.61E-01, 1.34E+00]	0.41	2124	0.587
Number open patches	0.052	0.188	[-4.45E-01, 7.70E-01]	0.32	1890	0.785

Lactation

Variables	B	SE	95% CI	w_i+	*N*	*P*
(Intercept)	3.026	0.184	[2.66E+00, 3.39E+00]			<2.0E-16 ***
Altitude (stdev)	0.038	0.130	[-2.98E-01, 5.60E-01]	0.29	811	0.774
Area closed patches (mean)	0.125	0.205	[-2.05E-01, 7.13E-01]	0.49	919	0.547
Edge density open patches	0.033	0.154	[-4.20E-01, 6.39E-01]	0.3	922	0.833
Northness (median)	-0.055	0.114	[-4.55E-01, 1.24E-01]	0.33	848	0.634
Slope >30 (area)	**1.076**	**0.223**	**[6.32E-01, 1.52E+00]**	**1**	**1942**	**2.0E-06 *****
Open patches richness	0.017	0.118	[-3.76E-01, 5.02E-01]	0.27	815	0.886
Number closed patches	0.500	0.490	[-8.48E-02, 1.58E+00]	0.67	1164	0.312
Edge density closed patches	-0.219	0.444	[-1.68E+00, 5.42E-01]	0.38	890	0.625
Eastness (median)	-0.068	0.126	[-4.81E-01, 1.11E-01]	0.37	854	0.595
Closed patches richness	-0.085	0.216	[-8.59E-01, 3.69E-01]	0.35	811	0.697
Slope (range)	-0.066	0.132	[-5.03E-01, 1.57E-01]	0.38	886	0.621
Area open patches (mean)	0.199	0.652	[-1.42E+00, 2.53E+00]	0.36	959	0.763
Number open patches	**0.926**	**0.405**	**[2.50E-01, 1.69E+00]**	**0.96**	**1648**	**0.024 ***

Post-lactation

Variables	B	SE	95% CI	w_i+	*N*	*P*
(Intercept)	3.664	0.218	[3.22E+00, 4.10E+00]			< 2.0E-16 ***
Altitude (stdev)	-0.088	0.242	[-9.88E-01, 4.58E-01]	0.33	1125	0.722
Area closed patches (mean)	-0.136	0.228	[-8.30E-01, 1.78E-01]	0.42	1273	0.556
Edge density open patches	-0.075	0.216	[-8.93E-01, 4.30E-01]	0.32	1121	0.734

Northness (median)	0.014	0.168	[-5.97E-01, 6.99E-01]	0.27	1057	0.937
Slope >30 (area)	0.226	0.301	[-1.85E-01, 1.01E+00]	0.54	1402	0.460
Open patches richness	**0.956**	**0.198**	**[5.56E-01, 1.36E+00]**	**1**	**2656**	**2.8E-06 *****
Number closed patches	0.014	0.167	[-5.89E-01, 6.94E-01]	0.27	1071	0.934
Edge density closed patches	0.140	0.254	[-2.70E-01, 9.31E-01]	0.42	1334	0.587
Eastness (median)	-0.023	0.135	[-5.94E-01, 4.19E-01]	0.26	1049	0.867
Closed patches richness	-0.040	0.172	[-7.28E-01, 4.60E-01]	0.3	1112	0.822
Slope (range)	-0.259	0.330	[-1.10E+00, 1.68E-01]	0.56	1345	0.439
Area open patches (mean)	0.025	0.101	[-2.62E-01, 4.35E-01]	0.29	1064	0.808
Number open patches	-0.030	0.143	[-6.25E-01, 4.05E-01]	0.28	1031	0.836

Annual

Variables	**B**	**SE**	**95% CI**	***w_i+***	***N***	***P***
(Intercept)	3.508	0.111	[3.29E+00, 3.73E+00]			< 2.0E-16 ***
Altitude (stdev)	-0.082	0.139	[-5.09E-01, 7.83E-02]	0.38	773	0.558
Area closed patches (mean)	-0.020	0.075	[-3.17E-01, 1.85E-01]	0.29	746	0.797
Edge density open patches	0.109	0.145	[-7.38E-02, 4.84E-01]	0.53	883	0.453
Northness (median)	-0.007	0.058	[-2.47E-01, 1.94E-01]	0.26	712	0.908
Slope >30 (area)	**0.397**	**0.143**	**[1.32E-01, 6.72E-01]**	**0.99**	**1631**	**5.9E-3 ****
Open patches richness	**0.487**	**0.127**	**[2.37E-01, 7.37E-01]**	**1**	**1727**	**1.4E-4 *****
Number closed patches	0.024	0.088	[-2.08E-01, 3.68E-01]	0.3	751	0.787
Edge density closed patches	-0.019	0.075	[-3.20E-01, 1.88E-01]	0.29	721	0.802
Eastness (median)	0.003	0.060	[-2.18E-01, 2.42E-01]	0.26	710	0.959
Closed patches richness	-0.022	0.089	[-3.68E-01, 2.22E-01]	0.3	743	0.805
Slope (range)	0.034	0.085	[-1.40E-01, 3.46E-01]	0.33	744	0.694
Area open patches (mean)	0.159	0.219	[-8.10E-02, 7.40E-01]	0.48	831	0.470
Number open patches	0.129	0.188	[-1.17E-01, 6.40E-01]	0.49	847	0.494

Global models

Pregnancy

Variables	**B**	**SE**	**95% CI**	***w_i+***	***N***	***P***
(Intercept)	3.197	0.159	[2.88E+00, 3.52E+00]			< 2.0E-16 ***
Riparian habitat	**0.289**	**0.173**	**[2.79E-02, 6.22E-01]**	**0.89**	**4**	**0.099 .**
Water bodies	**0.699**	**0.216**	**[2.67E-01, 1.13E+00]**	**1**	**6**	**1.5E-3 ****
Conifers	-0.169	0.164	[-5.26E-01, -4.25E-03]	0.64	3	0.310
Area closed patches (mean)	-0.127	0.174	[-5.87E-01, 9.19E-02]	0.51	3	0.468

Lactation

Variables	**B**	**SE**	**95% CI**	***w_i+***	***N***	***P***
(Intercept)	3.030	0.167	[2.70E+00, 3.36E+00]			< 2.0E-16 ***
Riparian habitat	0.068	0.169	[-2.79E-01, 6.84E-01]	0.33	6	0.693
Slope >30 (area)	**0.870**	**0.227**	**[4.17E-01, 1.32E+00]**	**1**	**14**	**1.7E-4 *****
Water bodies	0.178	0.223	[-1.35E-01, 7.33E-01]	0.59	8	0.430299
Number open patches	**0.721**	**0.216**	**[2.90E-01, 1.15E+00]**	**1**	**14**	**1.0E-3 ****
Arable land	**-0.274**	**0.184**	**[-6.21E-01, -5.78E-02]**	**0.81**	**8**	**0.141**
Number closed patches	0.106	0.226	[-2.99E-01, 8.75E-01]	0.37	7	0.642

Post-lactation

Variables	**B**	**SE**	**95% CI**	***w_i+***	***N***	***P***
(Intercept)	3.161	0.210	[2.74E+00, 3.58E+00]			< 2E-16 ***
Water bodies	**0.736**	**0.265**	**[2.07E-01, 1.26E+00]**	**1**	**4**	**6.39E-3 ****
Open patches richness	0.307	0.268	[2.51E-02, 8.63E-01]	0.69	2	0.257

Arable land	**-0.583**	**0.377**	**[-1.28E+00, -1.70E-01]**	**0.81**	**2**	**0.127**

Annual

Variables	B	SE	95% CI	*w_i*+	*N*	*P*
(Intercept)	3.313	0.101	[3.11E+00, 3.51E+00]			< 2.0E-16 ***
Riparian habitat	**0.381**	**0.105**	**[1.74E-01, 5.89E-01]**	**1**	**3**	**3.2E-4 *** **
Slope >30 (area)	0.105	0.116	[-4.57E-02, 3.74E-01]	0.64	2	0.367
Water bodies	**0.920**	**0.128**	**[6.69E-01, 1.17E+00]**	**1**	**3**	**< 2.0E-16 *** **
Open patches richness	0.033	0.081	[-9.53E-02, 3.59E-01]	0.25	1	0.685

Table S2.4 – Summary results of average models relating total bat activity, excluding *Pipistrellus pipistrellus* and Non Identified bat passes, to landscape composition and structure variables. Models were built separately for each phenological period (Pregnancy, Lactation, Post-Lactation) and for data aggregated across periods (Annual). In each case, separate models were also built for landscape composition, landscape structure, and the combination of landscape composition and structure variables. For each variable we provide the estimate of the regression coefficient (B), its standard error (SE) and 95% confidence interval (95%CI), the sum of Akaike weights (w_i+), the number of models in which the variable was included (*N*), and the significance level (*P*). *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$. Variables with w_i+ >0.70 are highlighted in bold

Landscape composition

Pregnancy

Variables	B	SE	95% CI	w_i+	*N*	*P*
(Intercept)	2.340	0.206	[1.93E+00, 2.75E+00]	0.29	41	<2.0E-16 ***
Arable land	-0.048	0.161	[-6.94E-01, 3.64E-01]	0.32	41	0.769
Conifers	-0.045	0.119	[-4.89E-01, 2.11E-01]	0.27	40	0.709
Riparian habitat	-0.016	0.105	[-4.49E-01, 3.30E-01]	0.5	47	0.880
Water bodies	0.177	0.243	[-1.18E-01, 8.25E-01]	0.31	42	0.471
Shrublands	-0.022	0.149	[-5.90E-01, 4.49E-01]	0.32	43	0.885
Mediterranean forest	0.054	0.143	[-2.55E-01, 5.90E-01]	0.47	46	0.710
Orchards	0.164	0.262	[-2.18E-01, 9.22E-01]	0.29	41	0.536

Lactation

Variables	B	SE	95% CI	w_i+	*N*	*P*
(Intercept)	2.426	0.179	[2.07E+00, 2.78E+00]			< 2.0E-16 ***
Arable land	**-0.252**	**0.442**	**[-1.33E+00, 6.02E-01]**	**0.70**	**31**	**0.569**
Conifers	0.064	0.491	[-1.35E+00, 1.67E+00]	0.40	25	0.896
Riparian habitat	0.210	0.228	[-3.98E-02, 7.40E-01]	0.60	29	0.363
Water bodies	**0.786**	**0.197**	**[4.31E-01, 1.16E+00]**	**0.99**	**52**	**8.6e-05 *** **
Shrublands	0.026	0.713	[-2.21E+00, 2.35E+00]	0.38	27	0.971
Mediterranean forest	-0.072	0.521	[-2.09E+00, 1.60E+00]	0.29	23	0.891
Orchards	-0.075	0.653	[-2.53E+00, 2.04E+00]	0.30	23	0.909

Post-lactation

Variables	B	SE	95% CI	w_i+	*N*	*P*
(Intercept)	2.303	0.225	[1.85E+00, 2.76E+00]			<2.0E-16 ***
Arable land	**-0.579**	**0.431**	**[-1.41E+00, -1.19E-01]**	**0.76**	**26**	**0.185**
Conifers	-0.119	0.228	[-8.52E-01, 2.53E-01]	0.40	18	0.605
Riparian habitat	0.003	0.112	[-4.48E-01, 4.74E-01]	0.24	17	0.979
Water bodies	**0.850**	**0.166**	**[5.15E-01, 1.19E+00]**	**1**	**46**	**7.0E-07 *** **
Shrublands	0.070	0.255	[-6.05E-01, 1.07E+00]	0.30	19	0.786
Mediterranean forest	0.050	0.204	[-5.24E-01, 8.77E-01]	0.28	20	0.808
Orchards	0.061	0.252	[-6.47E-01, 1.06E+00]	0.29	20	0.81

Annual

Variables	B	SE	95% CI	w_i+	*N*	*P*
(Intercept)	2.377	0.102	[2.17E+00, 2.58E+00]			<2.0E-16 ***
Arable land	**-0.183**	**0.156**	**[-5.04E-01, 1.83E-04]**	**0.73**	**29**	**0.243**
Conifers	-0.032	0.111	[-4.18E-01, 2.46E-01]	0.37	21	0.774
Riparian habitat	0.031	0.077	[-1.21E-01, 3.17E-01]	0.32	21	0.689
Water bodies	**0.733**	**0.108**	**[5.20E-01, 9.46E-01]**	**1**	**50**	**<2.0E-16 *****
Shrublands	0.085	0.175	[-2.32E-01, 6.40E-01]	0.41	25	0.629
Mediterranean forest	0.025	0.112	[-2.98E-01, 4.63E-01]	0.3	21	0.826
Orchards	0.047	0.149	[-3.11E-01, 5.90E-01]	0.34	24	0.751

Landscape structure models

Pregnancy

Variables	B	SE	95% CI	w_i+	*N*	*P*
(Intercept)	2.211	0.192	[1.83E+00, 2.60E+00]			<2.0E-16 ***
Altitude (stdev)	-0.105	0.239	[-9.34E-01, 3.52E-01]	0.36	2264	0.663
Area closed patches (mean)	-0.084	0.163	[-6.20E-01, 1.81E-01]	0.38	2419	0.611
Edge density open patches	0.187	0.288	[-2.13E-01, 1.00E+00]	0.47	2556	0.520
Northness (median)	0.060	0.183	[-3.81E-01, 7.76E-01]	0.3	2234	0.749
Slope >30 (area)	0.193	0.258	[-1.30E-01, 8.66E-01]	0.52	2812	0.459
Open patches richness	-0.013	0.115	[-4.83E-01, 3.85E-01]	0.27	2157	0.910
Number closed patches	**0.202**	**0.193**	**[-8.41E-02, 6.28E-01]**	**0.74**	**3328**	**0.305**
Edge density closed patches	-0.028	0.110	[-4.60E-01, 2.76E-01]	0.31	2180	0.802
Eastness (median)	-0.056	0.183	[-7.66E-01, 4.00E-01]	0.31	2248	0.761
Closed patches richness	0.003	0.214	[-7.14E-01, 7.31E-01]	0.35	2385	0.990
Slope (range)	0.075	0.178	[-2.82E-01, 7.03E-01]	0.36	2323	0.678
Area open patches (mean)	0.264	0.394	[-2.54E-01, 1.37E+00]	0.47	2483	0.508
Number open patches	-0.112	0.243	[-9.29E-01, 3.41E-01]	0.38	2337	0.648

Lactation

Variables	B	SE	95% CI	w_i+	*N*	*P*
(Intercept)	2.144	0.177	[1.79E+00, 2.50E+00]			< 2.0E-16 ***
Altitude (stdev)	0.049	0.143	[-2.86E-01, 5.87E-01]	0.32	920	0.737
Area closed patches (mean)	**0.286**	**0.243**	**[-5.85E-02, 7.96E-01]**	**0.78**	**1063**	**0.245**
Edge density open patches	0.294	0.292	[-5.13E-02, 9.39E-01]	0.66	1054	0.317
Northness (median)	-0.029	0.084	[-3.54E-01, 1.61E-01]	0.3	790	0.731
Slope >30 (area)	**0.588**	**0.218**	**[2.02E-01, 1.00E+00]**	**0.98**	**1654**	**7.9E-3 ****
Open patches richness	-0.013	0.113	[-4.67E-01, 3.75E-01]	0.28	815	0.911
Number closed patches	1.137	0.404	[3.60E-01, 1.93E+00]	0.99	1803	5.6E-3 **
Edge density closed patches	-0.235	0.412	[-1.51E+00, 3.61E-01]	0.41	860	0.572
Eastness (median)	-0.005	0.074	[-3.02E-01, 2.66E-01]	0.27	770	0.949
Closed patches richness	-0.211	0.275	[-9.21E-01, 1.31E-01]	0.53	955	0.448
Slope (range)	-0.089	0.143	[-5.03E-01, 1.20E-01]	0.46	864	0.538
Area open patches (mean)	-0.228	0.570	[-2.08E+00, 9.98E-01]	0.42	1023	0.692
Number open patches	0.338	0.372	[-7.02E-02, 1.20E+00]	0.6	1014	0.367

Post-lactation

Variables	B	SE	95% CI	w_i+	*N*	*P*
(Intercept)	2.792	0.216	[2.36E+00, 3.23E+00]			< 2.0E-16 ***
Altitude (stdev)	-0.029	0.185	[-7.63E-01, 5.65E-01]	0.29	1092	0.877
Area closed patches (mean)	**-0.373**	**0.298**	**[-9.67E-01, -5.25E-02]**	**0.73**	**1580**	**0.217**
Edge density open patches	-0.029	0.199	[-8.04E-01, 6.11E-01]	0.3	1104	0.886

Northness (median)	0.040	0.170	[-4.56E-01, 7.49E-01]	0.28	1042	0.816
Slope >30 (area)	0.099	0.217	[-3.15E-01, 8.36E-01]	0.38	1214	0.654
Open patches richness	**0.781**	**0.193**	**[3.91E-01, 1.17E+00]**	**1**	**2641**	**8.8E-05 *****
Number closed patches	-0.080	0.212	[-8.35E-01, 3.94E-01]	0.36	1136	0.712
Edge density closed patches	0.064	0.196	[-4.15E-01, 8.10E-01]	0.32	1100	0.75
Eastness (median)	-0.002	0.124	[-5.00E-01, 4.84E-01]	0.26	1037	0.987
Closed patches richness	-0.114	0.216	[-7.87E-01, 2.65E-01]	0.44	1295	0.603
Slope (range)	-0.264	0.321	[-1.07E+00, 1.46E-01]	0.57	1373	0.418
Area open patches (mean)	0.030	0.104	[-2.43E-01, 4.41E-01]	0.3	1120	0.777
Number open patches	-0.026	0.152	[-6.39E-01, 4.58E-01]	0.29	1051	0.867

Annual

Variables	B	SE	95% CI	w_i+	*N*	*P*
(Intercept)	2.484	0.109	[2.27E+00, 2.70E+00]			< 2.0E-16 ***
Altitude (stdev)	-0.033	0.097	[-4.07E-01, 1.91E-01]	0.3	634	0.736
Area closed patches (mean)	-0.001	0.068	[-2.62E-01, 2.53E-01]	0.27	604	0.987
Edge density open patches	0.168	0.181	[-4.59E-02, 5.82E-01]	0.63	847	0.355
Northness (median)	0.010	0.060	[-1.81E-01, 2.57E-01]	0.27	590	0.867
Slope >30 (area)	**0.367**	**0.135**	**[1.22E-01, 6.24E-01]**	**0.98**	**1372**	**6.7E-3 ****
Open patches richness	**0.413**	**0.126**	**[1.66E-01, 6.60E-01]**	**1**	**1474**	**1.1E-3 ****
Number closed patches	**0.227**	**0.192**	**[-8.76E-03, 6.20E-01]**	**0.74**	**839**	**0.237**
Edge density closed patches	-0.010	0.070	[-2.93E-01, 2.23E-01]	0.27	608	0.892
Eastness (median)	-0.031	0.079	[-3.26E-01, 1.24E-01]	0.31	618	0.694
Closed patches richness	**-0.239**	**0.202**	**[-6.53E-01, 1.32E-02]**	**0.75**	**937**	**0.237**
Slope (range)	0.017	0.070	[-1.79E-01, 2.98E-01]	0.29	601	0.808
Area open patches (mean)	0.147	0.174	[-3.31E-02, 5.66E-01]	0.55	764	0.400
Number open patches	-0.010	0.118	[-4.36E-01, 3.75E-01]	0.32	652	0.934

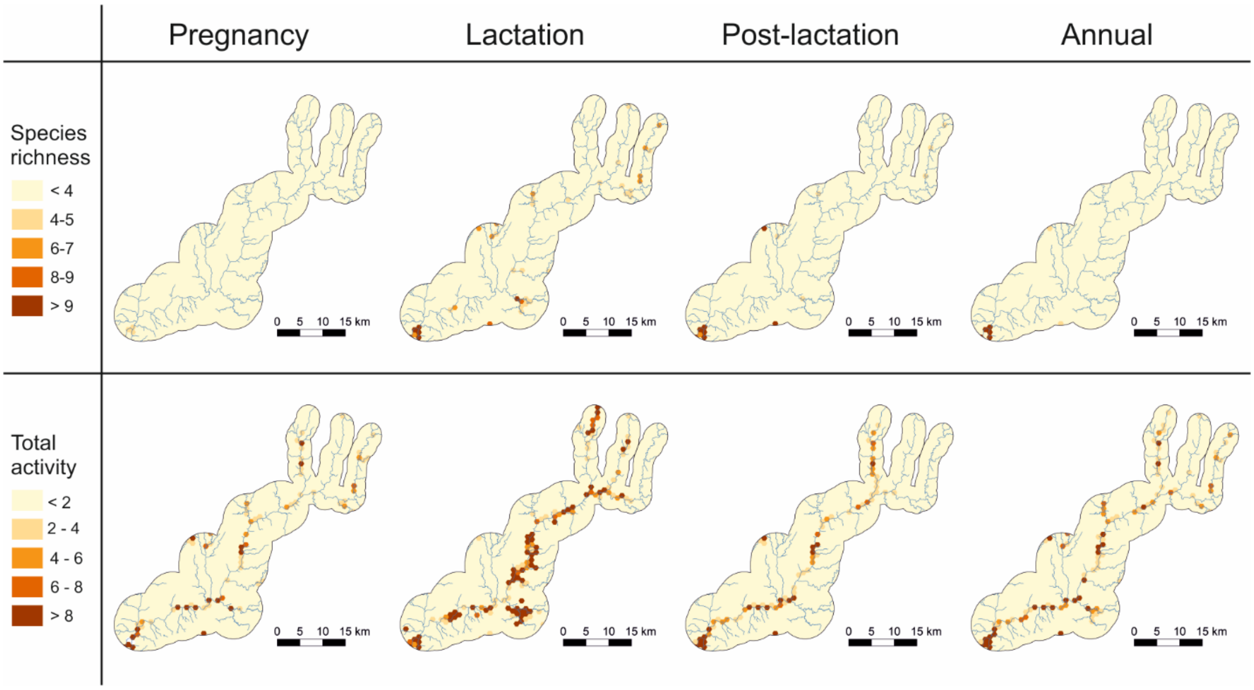

Figure S2.2 – Standard error of species richness and activity projections within the study area for each phenological stage (Pregnancy, Lactation and Post-lactation) and for the data set pooling the yearly data (Annual). Species richness values refer to 15 minutes sampling while total activity refers to bat passes.min^{-1}

Chapter 3

Evolutionary history of the European free-tailed-bat, a tropical affinity species spanning across the Mediterranean Basin

Francisco Amorim, Orly Razgour, Vanessa A. Mata, Susana Lopes, Raquel Godinho, Carlos Ibáñez, Javier Juste, Stephen J. Rossiter, Pedro Beja, Hugo Rebelo

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Abstract

The Mediterranean Basin is a global biodiversity hotspot, hosting a number of native species belonging to families that are found almost exclusively in tropical climates. Yet, whether or not these taxa were able to survive in the Mediterranean region during the Quaternary climatic oscillations remains unknown. Focusing on the European-free-tailed bat (*Tadarida teniotis*) we aimed to i) identify potential ancient populations and glacial refugia; ii) determine the post-glacial colonization routes across the Mediterranean; and iii) evaluate current population structure and demography. Mitochondrial and nuclear markers were used to understand *T. teniotis* evolutionary and demographic history. We show that *T. teniotis* is likely restricted to the Western Palearctic, with mitochondrial phylogeny suggesting a split between an Anatolian/Middle East clade and a European clade. Nuclear data pointed to three genetic populations, one of which is an isolated and highly differentiated group in the Canary Islands, another distributed across Iberia, Morocco and France, and a third stretching from Italy to the east, with admixture following a pattern of isolation by distance. Evolutionary and demographic reconstruction supports a pre Last Glacial Maximum (LGM) colonization of Italy and the Anatolian/Middle East, while the remaining populations were colonized from Italy after the Younger Dryas. We also found support for demographic expansion following the Iberian colonization. The results show that during the LGM *T. teniotis* persisted in Mediterranean refugia and has subsequently expanded to its current circum-Mediterranean range. Our findings raise questions regarding the physiological and ecological traits that enabled species with tropical affinities to survive in colder climates.

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3.1 Introduction

The Mediterranean Basin is a global biodiversity hotspot (Myers et al., 2000; Blondel et al., 2010). Despite being presently located in temperate latitudes, this region was mainly covered by tropical climates during the Tertiary (Blondel & Mourer-Chauviré, 1998). Nowadays, Europe still hosts a number of members belonging to several vertebrate groups that are almost exclusively associated with the tropics (defined here as tropical affinities), including reptiles such as geckos and chameleons, and birds such as rollers and bee-eaters (Blondel & Mourer-Chauviré, 1998; Townsend & Larson, 2002; Carranza & Arnold, 2006; Ammerman et al., 2012). However, the diversity of tropical species present in Europe is lower than that of other Holarctic areas like North America or eastern Asia (Blondel & Mourer-Chauviré, 1998). The reason for such pattern is that both North America and eastern Asia remained connected to the tropics over the whole Tertiary–Quaternary. In contrast, large geographical barriers (mountain ranges, seas and desert-belts) prevented the Palearctic tropical biota from expanding their range to tropical regions further south during glacial periods, and tropical species from colonizing northern regions during inter-glacial periods (Blondel & Mourer-Chauviré, 1998). Altogether, these led to a progressive decline of the tropical species during the Pleistocene (Blondel et al., 2010). Under such circumstances, it is remarkable that some of these species were able to persist in the western Palaearctic, although mostly restricted to the circum-Mediterranean area. The population history of such lineages during periods of glaciation is poorly understood and it is not known whether these taxa were able to survive in the Mediterranean region during the climatic oscillations of the Quaternary

Among non-flying mammals, only a small number of species in the western Palaearctic have tropical affinities (Dobson, 1998). Although in some cases this was the result of a longstanding human-mediated introductions across the Strait of Gibraltar, in others, such as the Egyptian mongoose (*Herpestes ichneumon*), this was the result of natural dispersal into the Iberian Peninsula during the Late Pleistocene (Gaubert et al., 2011). In bats, which are likely to be able to disperse over greater distances, there is a higher number of species shared between north-west Africa and Iberia (Dobson, 1998; García-Mudarra et al., 2009), but even for these mammals the number of species with tropical affinities occurring in temperate regions is relatively low. The European-free-tailed bat (*Tadarida teniotis* Rafinesque, 1814) is the only European representative of the Molossidae family that comprises more than 110 species (Ammerman et al., 2012). All the remaining molossids are restricted to tropical regions, apart from the Mexican free-tailed bat (*Tadarida brasiliensis*) and the Big free-tailed bat

(*Nyctinomops macrotis*), which reach similar Northern latitudes in the American continent. Molossidae is an ancient bat family that split into Old and New World molossids ca. 29 million years ago (Ammerman et al., 2012), and fossil records of the genus *Tadarida* in Europe date from the late Eocene ca. 25 million years ago (De Bonis et al., 1973).

Understanding phylogeographic patterns shaping the distributions and expansion of species is a powerful tool for predicting how future climatic changes will shape regional biodiversity (Hickerson et al., 2010). During the Quaternary ice ages, Europe experienced dramatic climatic fluctuations between glacial and interglacial cycles contributing to the contemporary distribution and genetic composition of biodiversity (Hewitt, 2000). The distributions of many animal species have been severely restricted to refugia to escape the harsh conditions of the glacial periods. The Last Glacial Maximum (LGM 18–20 ka BP), and the Younger Dryas (11.7-12.9 ka BP), correspond to the latest episodes where the ice sheets and cold temperatures reached their extremes. The Mediterranean region encompasses a high habitat diversity combined with topographic and geographic variability. Together with a dynamic palaeogeographic and climatic history these features contributed to marked environmental gradients (Blondel et al., 2010), strongly shaping current species and biodiversity spatial patterns, population structure and demography (Hewitt, 1999). Despite the increasing number of studies focusing on the phylogeography of species native to temperate environments, to the best of our knowledge, representatives from tropical families living in such environments have been seldom studied (but see Paulo, Pinto, Bruford, Jordan, & Nichols, 2002; Rato, Carranza, & Harris, 2011).

The European-free-tailed-bat is widespread throughout the Mediterranean and occurs in a variety of environments and habitats from the colder Alps to the border of the Sahara desert (Arlettaz et al., 2000; Bendjeddou et al., 2014; Amorim et al., 2018). However, during the Late Glacial Maximum (LGM), large parts of Europe had colder and drier habitats (Frenzel et al., 1992) with warmest month temperature being 10 ºC cooler than present, and coldest month temperature 20 ºC colder (Kageyama et al., 2006). These harsh conditions were likely unsuitable for most bat species (e.g., Bilgin et al., 2016; Kerth et al., 2008; Razgour et al., 2013; Rossiter, Benda, Dietz, Zhang, & Jones, 2007), thus raising the question of how species with tropical affinities were able to survive. Here we focus on the evolutionary history of *T. teniotis, which* belongs to a taxonomical family almost exclusively associated with the tropics and shows shorter duration of torpor bouts, and higher minimal body temperature in torpor than other temperate bats (Arlettaz et al., 2000). The high mobility and fast flight of these bats (McCracken et al., 2008; Mata et al., 2016) allows them to respond fast to environmental changes by shifting to more suitable areas. These features render *T. teniotis* a suitable model species to understand how species with topical affinity reacted to the climatic oscillations of the Quaternary in temperate and subtropical regions. Therefore, our main aims were to: i) identify the location of potential ancient populations and glacial refugia; ii) determine the post-glacial colonization routes across the Mediterranean; and iii) evaluate current population structure and demography in light of the post-glacial colonisation history.

3.2 Methods

3.2.1 Sample collection

A total of 154 genetic samples collected across the Western and Central Palearctic were obtained from researchers and museum collections. Samples spanned the entire range although coverage was uneven with few samples available from some regions, particularly from Asia, Eastern Mediterranean and North Africa. For a complete list of samples, origin and providers see Appendix 1 (GenBank accession numbers MK817165 to MK817272).

3.2.2 DNA extraction

Due to the different nature of the samples obtained (old museum specimens and recently collected wing tissue) we used different DNA extraction methods. For older museum specimens we followed the ancient DNA extraction protocol described in Rohland & Hofreiter, (2007) with modifications described in Dabney et al. (2013). For recent tissue samples, we used DNA Micro Kits (QIAGEN) following the manufacturer’s instructions.

3.2.3 Validation of species identity and mitochondrial genotyping

Given the poorly resolved taxonomic status of *Tadarida teniotis* (Mata et al., 2017), the identity of all samples were verified using mitochondrial markers prior to microsatellite genotyping. Due to taxonomic uncertainties (Mata et al., 2017), verification was considered to be especially important for putative *T. teniotis* samples obtained from the eastern part of the distribution (Kyrgyzstan and China). Additionally, samples from Laos previously identified as *T. latouchei* were also checked.

Four mitochondrial primer pairs were specifically designed using Geneious v9.1.7 (http://www.geneious.com, Kearse et al. 2012) based on an alignment of 37 mitogenomes covering the species range. The primers were designed to amplify the most variable regions of the mitogenomes (Supporting Information Table S3.1) and corresponded to three coding regions (*COI* - *cytochrome c oxidase subunit I*, *ATP6* - *ATP synthase subunit 6*, and *CytB* - *cytochrome b*) and one noncoding region (*D-loop*). While designing the primers took extra precautions and carefully examined the mitogenomic data to avoid the amplification of nuclear copies covering almost the entire mitogenome. We did this by comparing the sequences containing nuclear copies (identified by the high prevalence of stop codons) to those without nuclear copies and selecting the regions that did not amplify nuclear copies. This way the primers designed assure that only the mitochondrial haplotype were amplified, allowing the genotyping of samples through Sanger sequencing. For highly degraded museum samples that did not amplify using the regular primers, we further developed internal primers for the *COI* (*COI-mini*) and *D-loop* (*D-loop-mini*) regions targeting key SNPs that enable to differentiate *T. teniotis* and its different haplogroups from *T. latouchei* (Supporting Information Table S3.1).

The PCR reactions were carried in volumes of 10 µL, comprising of 5 µL of Multiplex PCR Master Mix (QIAGEN), with 0.4 µL of each 10 µM primer, and 1 µL of DNA extract. Cycling conditions for *COI*, *ATP6*, *CytB*, and *D-loop* used initial denaturing at 95 °C for 15 min, followed by 40 cycles of denaturing at 94 °C for 30 s, annealing at 59 °C for 45 s and extension at 72 °C for 45 s, with a final extension at 72 °C for 10 min. For *COI-mini* and *D-loop-mini* the cycling conditions were the same except the annealing temperature that was 52 ºC and the number of cycles was increased to 45. Successful amplifications were enzymatically purified, sequenced following the BigDye Terminator v3.1 Cycle sequencing protocol (Applied Biosystems), and sequencing products were separated using an automated Sequencer ABI3130xl Genetic Analyzer. Sequences were aligned and compared in the software SEQSCAPE 3.0 (Applied Biosystems).

3.2.4 **Microsatellite genotyping**

A custom microsatellite library was developed through 454 GS-FLX Titanium pyrosequencing of enriched DNA libraries based on 12 individuals along the distribution range of *T. teniotis* (Malausa et al., 2011). This process was developed by GenoScreen (http://www.pasteur-lille.fr/fr/recherche/plateformes/tordeux_plat.html) and included sequence data quality control, assembly and analyses, and primer design.

From the 159 candidate microsatellite loci, we selected 26 microsatellites with different numbers of repeat units, compatible allelic ranges and melting temperatures for multiplexing. We first tested the genotyping performance on four *T. teniotis* samples and discarded microsatellites that: i) showed no amplification, ii) had multiple bands and iii) had excessive slippage (many stutter bands). Those remaining were combined into two multiplex panels according to their allele size range and compatibility among primers, which was checked using Auto-Dimer (Vallone & Butler, 2004).

The optimisation of PCR conditions for multiplex loci and polymorphism detection was performed using 16 samples. From the 26 loci initially checked a total of 12 di and 2 tetra-nucleotides polymorphic markers (with more than 2 alleles) were selected and genotyped for 129 individuals in two multiplex panels with seven markers each. PCR fragments were fluorescent labelled following Schuelke (2000) but with FAM, VIC, NED, and PET dyes. A pig tail (GTTT) was added to the 5' end of the primer reverse in order to reduce stutter and drive the reaction to the ''plusA'' band (Brownstein et al., 1996). For additional details on microsatellite primers, see Supporting Information Table S3.2.

PCR amplifications were conducted as for mitochondrial fragments except that 1µL of primer mix was used per reaction. The PCR cycling profile was divided in four main steps: denaturation at 95 ºC for 15 min; 13 cycles with denaturation at 95 ºC for 30 s, annealing at 58 ºC for 90 s with a touchdown of 0.5 ºC per cycle and extension at 72 ºC for 45 s; 27 cycles with denaturation at 95 ºC for 30 s, annealing at 52 ºC for 60 s and extension at 72 ºC for 45 s; and a final extension at 60 ºC for 30 min. PCR products were later separated by capillary electrophoresis on the same automatic sequencer ABI3130xl Genetic Analyzer (AB Applied Biosystems). Fragments were scored using GENEMAPPER V4.0 (Applied Biosystems) and checked independently by two people.

3.2.5 **Genetic data analysis**

Mitochondrial data

Sequences from the four mitochondrial markers were concatenated and standard molecular diversity statistics calculated in ARLEQUIN 3.5 (Excoffier & Lischer, 2010). To test for geographical genetic structure, analyses of molecular variance (AMOVA) were carried out with 10,000 permutations and diversity measures were reported for geographic groups and assessed according to the degree of differentiation between regions (ΦCT), between populations within regions (ΦSC) and between all populations (ΦST). A median-joining (MJ) haplotype network was build using POPART (Leigh & Bryant, 2015) for each marker and for the concatenated sequences. Mitochondrial diversity was assessed considering seven geographic populations based on the common population structure of European bats (e.g. Bilgin et al., 2016; Razgour et al., 2013): 1) Canary Islands; 2) Iberian Peninsula (Portugal and Spain, excluding Canary Islands); 3) Morocco; 4) France; 5) Italy; 6) Greece; 7) Anatolia and 8) Middle East (Lebanon, Israel and Palestine).

Phylogenetic reconstruction was performed on the CIPRES Science Gateway V. 3.3 (Miller et al., 2010) using Bayesian inference implemented in BEAST V1.8.4 (Drummond et al., 2012) considering unique haplotypes only (n = 65) from the concatenated sequences and with inclusion of *T. latouchei* as outgroup (Mata et al., 2017, GenBank Accession numbers: NC_036331 and KY581662). The best substitution model scheme was determined using PARTITIONFINDER v2.1.1 (Lanfear et al., 2016). We used a coalescent tree prior under constant population. Three independent runs of 10^8 generations sampled every 1000 were combined in TRACER V1.7 (Rambaut et al., 2018) to confirm convergence on the same posterior distribution in the MCMC runs. The first 10^7 runs (10%) were discarded as burn-in.

Microsatellite data

To test for departures from Hardy–Weinberg and linkage equilibrium, both across the whole samples and within populations, we used the 'pegas' R package (Paradis, 2010). Loci that violated Hardy–Weinberg equilibrium in more than two populations were excluded from further analysis (Supporting Information Table S3.2). Allele frequencies and number of private alleles were estimated in GENETIX V4.05 (Belkhir et al., 2004) and_mean allele frequency across all loci was calculated for each population. Estimates of expected heterozygosity (H_e), observed heterozygosity (H_{obs}) and allelic richness within populations, and differentiation (F_{st}) among populations, were all calculated using the 'PopGenReport' R package (Adamack & Gruber, 2014). Relatedness among individuals was measured using the triadic maximum likelihood estimator (TrioML; Wang, 2007) implemented in 'related' R package (Pew et al., 2015). This estimator was chosen because it allows for inbreeding and accounts for genotyping errors in the data.

Population genetic structure was first examined using the principal component analysis in 'PopGenReport' R package (Adamack & Gruber, 2014) followed by the Bayesian clustering analysis implemented in STRUCTURE 2.3.4 (Pritchard et al., 2000) with all genotyped samples. We performed 10 replicate runs of structure for each value of K, from K = 1 to 10, and we applied the admixture model with a burn-in of 5×10^5 and a run length of 10^6 with and without the prior population information (LOCPRIOR). The later can often provide accurate inference of population structure and individual ancestry in datasets where the signal of structure is too weak to be found using the standard models (Hubisz et al., 2009). We used STRUCTURE HARVASTER V0.6.94 to visualize likelihood and detect the number of genetic groups that best fit the data (Earl & VonHoldt, 2012). The Greedy algorithm of CLUMPP (Jakobsson & Rosenberg, 2007) was used to derive symmetric similarity coefficients (SSC) among replicate runs within each value of K. Groups of runs with an SSC ≥ 0.8 were then combined and their outputs for each value of K were graphically displayed.

Spatial structuring was further analysed using multivariate analyses of spatial genetic patterns in 'adegenet' (Jombart, 2008). Spatial Analysis of Principal Components (sPCA) allows to find the individual scores that maximize the product of variance and spatial autocorrelation (Jombart et al., 2008). Isolation by Distance (IBD) across all individuals within the species range was tested in R using the package ‘ade4’ (Bougeard & Dray, 2018) and using a Mantel test.

3.2.6 **ABC inference of evolutionary and demographic history**

General overview

The evolutionary and demographic history of *T. teniotis* was reconstructed using Approximate Bayesian Computation (ABC) approach implemented in DIYABC V2.1 (Cornuet et al., 2014). We carried out two sets of analyses, aimed to: 1) infer the source population and patterns of range colonisation from putative refugia in the Western Palearctic; and 2) infer demographic history in the western range (Iberia, Morocco and France). In the first step, we modelled the probability of different scenarios considering 122 individuals from six populations (Iberia, Morocco, France, Italy, Greece and Anatolia/Middle East) and combining information from 12 microsatellites loci and two mitochondrial sequences (*COI* and *D-loop*). Multiple scenarios were compared representing a comprehensive range of alternative phylogeographic hypothesis and permuting the six geographic groups at the tips (Supporting Information Figure S3.1 and Table S3.3).

Using the scenario topology identified in the first step, we carried out a demographic history analysis of the western range to determine changes in population size during colonisation. We compared a null model of no change in population size (Scenario 1) to a model of colonization and expansion in all populations (Scenario 2), and two models of recent change with increase or decrease in Iberian population size (Scenario 3 and Scenario 4 respectively). For a schematic representation of the different scenarios, see Supporting Information Figure S3.2.

Each scenario was tested using the combined microsatellite and mtDNA datasets and running 10^6 simulations. The posterior probability of scenarios was then estimated using a weighted polychotomous logistic regression. Due to the criticism of ABC model choice outlined in Robert, Cornuet, Marin, & Pillai (2011) we empirically evaluated the power of the model to discriminate among scenarios by simulating pseudo-observed datasets and calculating false allocation rates (type1 and 2 errors, Cornuet, Ravigné, & Estoup, 2010). Further details on the methods, model specifications and run parameters are presented in the following sections and in Supporting Information Table S3.3.

Specific model parameters

Microsatellite loci were assumed to follow a Generalized Stepwise Mutation model (GSM) and mean mutation rate was bounded between 10^{-3} and 10^{-4} (Balloux & Lugon-Moulin, 2002; Storz & Beaumont, 2002). For mtDNA we only considered *COI* and *D-loop* due to computational requirements and sequence completeness. We used the best substitution model scheme determined using PARTITIONFINDER V2.1.1 (Lanfear et al., 2016) as follow: HKY for the coding region (*COI*) and K80 for non-coding region (*D-loop*). Generation time was set at three years, a value in between the age of first breeding for different bat families that can go from one to five years (Crichton & Krutzsch, 2000) which meets our expectations for *T. teniotis*. We considered a mean mutation rate (per site per generation) between $5.25E^{-8}$ and $7.2E^{-8}$ for *COI* (Ruedi & Mayer, 2001; Juste et al., 2004) and between $9.45E^{-8}$ and $3.75E^{-7}$ for *D-loop* (Petit et al., 1999).

In the colonization analysis, uniform priors were assumed for all demographic parameters. Effective population size (Ne) was kept as equal for all populations, ranging between $1E^{3}$ and $1E^{6}$. Population divergence time priors were bounded between $1E^{3}$ and $2E^{5}$ generations and varied depending on model analysis. Divergence times between source populations were set at either pre-LGM ($1E^{4}$ - $2E^{5}$) or flexible pre-post LGM ($1E^{3}$ - $2E^{4}$). Priors for admixture rates were bounded between 0.01 and 0.99. In the demographic history analysis, we used variable Effective population size ranging from 10 to $1E^{6}$. Population divergence time priors were bounded to post-LGM (10 and $1E^{4}$) and varied depending on model analysis.

In each ABC analysis we used 269 summary statistics. For the microsatellite loci we used three single sample statistics (mean number of alleles, mean Nei's genetic diversity index and mean allele size variance), and five between-sample statistics (F_{ST}, mean number of alleles, mean genic diversity, mean allele size variance and shared allele distance). For the mtDNA sequence we used seven single sample statistics (number of distinct haplotypes, number of segregating sites, mean pairwise differences, variance of pairwise distance, Tajima's D statistics, private segregating sites, mean of numbers of the rarest nucleotide at segregation site) and four between-sample statistics (Fst, number of haplotypes, number of segregating sites, mean within sample pairwise differences and number of segregating sites). The demographic history analysis included only 47 summary statistics due to the small number of groups compared.

The complete list of parameters used in the ABC analysis, respective priors and estimated results for the most supported colonization scenario (SC2) and the most supported demographic history scenario (SC2) can be found in Supporting Information Table S3.4.

Colonization analysis

This analysis included the potential range colonization from an ancient unsampled population with unknown origin. For a schematic representation of the different scenarios, see Supporting Information Figure S3.1.

Scenario 1 considered an Iberian colonization from an ancient unsampled population before the LGM, and a long-range colonization of the Eastern Mediterranean through an admixture event from Iberia and the ancient unsampled population. The Iberian population later colonized Morocco and the later colonized Italy. Admixture events between Iberia and Italy and between the Eastern Mediterranean and Italy resulted in the French and Greek populations, respectively.

Scenario 2 considered an Italian colonization from an ancient unsampled population before the LGM, and a colonization of the Eastern Mediterranean through an admixture event from Italy and the ancient unsampled population. The Italian population then colonized Morocco and France, while Iberia and Greece were colonized through admixture events between France and Morocco and Italy and Eastern Mediterranean, respectively.

Scenario 3 considered a colonization of the Eastern Mediterranean from an ancient unsampled population before the LGM, and a colonization of the Greek population through an admixture event between the Eastern Mediterranean population and the ancient unsampled population. Italy was later colonized from Greece, while Morocco and France were both be colonized from Italy. Finally, Iberia was colonized through an admixture event between the Moroccan and French populations.

Scenario 4 considered an Italian colonization from an ancient unsampled population before the LGM, and a colonization of the Greek population from an admixture event between the Italian population and the ancient unsampled population. Eastern Mediterranean was then be colonized from Greece, while Italy colonized both Morocco and France. Finally, Iberia was colonized through an admixture event between the French and Moroccan populations.

Scenario 5 considered a colonization of the Eastern Mediterranean from an ancient unsampled population before the LGM, and an Italian colonization through an admixture event between the Eastern Mediterranean and the ancient unsampled populations. Greece was also colonized through an admixture event, this time between the Italian and the Eastern Mediterranean populations. The Italian population then colonized France, while the Eastern Mediterranean population colonized Morocco. Finally, Iberia was colonized through an admixture event between the Moroccan and French populations.

3.3 Results

3.3.1 Mitochondrial data

We were able to amplify DNA from 136 samples. Samples from Kyrgyzstan were sequenced using *COI*-mini marker and showed a high mitochondrial divergence from *T. teniotis* (ca. 13%) and aligned with sequences belonging to *T. latouchei* from Laos (99% similarity, Mata et al., 2017). Additionally, from the four samples from China identified as *T. teniotis* in museum collections (Appendix 1), we were able to sequence two, both aligning with *Chaerephon plicatus* (vouchers: MVZ:Mamm:192571 and MVZ:Mamm:193379). According to the available information, the four samples were collected in the same event at a bat cave in Southern China, and thus assumed to belong to the same species. According to the International Union for Conservation of Nature (IUCN) the species has a highly fragmented distribution in central and eastern Asia (Benda & Piraccini, 2016) and our results suggest that *T. teniotis* could be absent or rare in this region. Therefore samples from Kyrgyzstan eastwards were excluded from further analysis.

A total of 120 samples belonging to *T. teniotis* were successfully sequenced for *COI* (566 bp final alignment) and *D-loop* (307 bp final alignment), 114 for *CytB* (509 bp final alignment) and 109 for *ATP6* (639 bp final alignment). The number of unique haplotypes ranged from 17 for *APT6* to 33 for *D-loop*. After concatenation the length of the resulting sequences was between 873 and 2020 bp (average = 1937 bp, Alignment in Supporting Information) and included 56 unique haplotypes (N = 109, 2020 bp). The Bayesian phylogenetic tree showed maximum posterior probability support (> 0.9) for the split of two main lineages, Anatolian/Middle East clade (AMh) and a European clade (EUh) further splitting into two subgroups but in this case with low support (EUh-A and EUh-B) (Figure 3.1).

The haplotype network divided the haplotypes into three separate groups, of which one was exclusive to Iberia and Morocco (EUh-A) and one was distributed elsewhere in central and western Mediterranean (EUh-B) (Figure 3.1 and Supporting Information Figure S3.3). The third group comprised all the haplotypes from Anatolia and Middle East and one additional haplotype from eastern Crete, broadly supporting the phylogenetic tree. The most common haplotypes from EUh-A and EUh-B were separated by only one mutational step (percent differences <0.05 %), while AMh shows a divergence of 0.70% from EUh-A and 0.59 % from EUh-B.

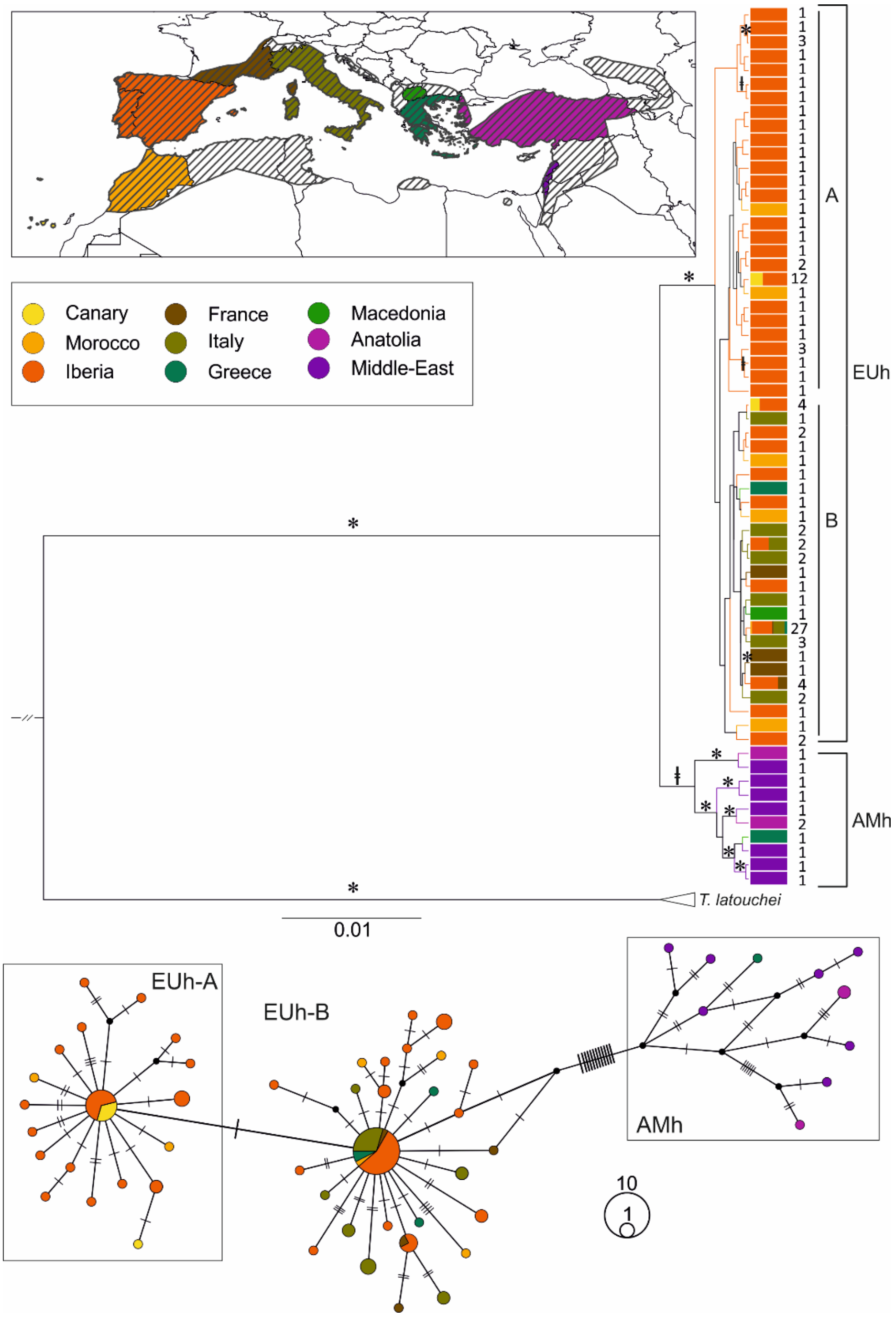

Figure 3.1 – Map showing the study area, with colour representing geographic origins of samples. Grey lined filled area represents IUCN species range within the study area. Bayesian phylogenetic tree and Median-joining haplotype network for *T. teniotis* based on 2020 bp of mtDNA (concatenated genes *COI*, *ATP6*, *CytB*, and *D-Loop*). Bayesian posterior probabilities (BPP) equal to 1 (∗) and greater than 0.9 (ǂ) are marked above branches. Proportional geographic origin of shared haplotypes is indicated in colour at the branch tips along with total number of samples. Major supported clades (EUh and AMh) and subgroups (EUh-A and EUh-B) are indicated (EUh and AMh). Median-joining haplotype networks for each supported clade as well as the European subgroups (EUh-A and EUh-A) are shown below where branch lengths are not proportional to base-pair changes. Sampling locations and haplotype frequency scale are shown in inset. The Bayesian phylogeny used unique haplotypes only (n = 56) and is shown with out-group (*T. latouchei*). For the median-joining network all concatenated mtDNA sequences (n = 109) were used.

Despite the split between the eastern and western clades, the phylogenetic tree and haplotype network based on mtDNA showed low levels of geographic structuring within each haplogroup. Mitochondrial haplotype diversity was highest and equal to one in the Middle East (N = 7), France (N = 4) and Morocco (N = 6), while nucleotide diversity was highest in Anatolia (Pi = 0.0040, N = 3) and the Middle East (Pi = 0.0036, N = 7) (Table 3.1). The lowest values for both haplotype and nucleotide diversity were found in the Canary Islands.

Genetic differentiation at mitochondrial DNA was seen between all populations ($\chi^2 = 532.49$, $P < 0.001$, overall $\theta_{ST} = 0.57$), with Anatolia and Middle East being genetically differentiated from all populations except for each other (Supporting Information Table S3.4). The general pattern showed a higher mitochondrial diversity in Anatolia/Middle East and equally low diversity in all the three peninsula.

3.3.2 Microsatellite data

A total of 129 individuals were successfully genotyped. Of the 14 microsatellite loci, two markers (TAD5 and TAD9) were removed due to violation of Hardy-Weinberg equilibrium (Supporting Information Table S3.2). After removing these markers all populations and markers were overall in Hardy-Weinberg. Our final dataset contained a total of 146 alleles, with an average number of 12.17 ± 2.44 alleles per locus (range 7-15) and 24 private alleles.

Table 3.1 – Genetic diversity of *T. teniotis* populations based on microsatellite (first five columns) and mtDNA (last two columns) datasets. Sample sizes in brackets. Mean allelic richness and mean allele frequency across all loci (± SD). H_e – Expected Heterozygosity; H_{obs} – Observed Heterozygosity.

	Mean Allele frequency	**Mean Allelic richness**	**Number of private alleles**	**H_e**	**H_{obs}**	**Haplotypic diversity**	**Nucleotide diversity (Pi)**
Canary (5)	0.34±0.10	2.62±0.45	0	0.58	0.63	0.40	0.0004
Morocco (6)	0.19±0.04	3.74±0.45	1	0.76	0.81	1.00	0.0022
Iberia (60)	0.1±0.020	3.91±0.37	14	0.80	0.78	0.92	0.0013
France (7)	0.19±0.03	3.72±0.31	1	0.76	0.78	1.00	0.0011
Italy (16)	0.12±0.03	4.00±0.40	3	0.80	0.77	0.83	0.0010
Greece (5)	0.22±0.06	3.55±0.47	3	0.73	0.73	0.90	0.0011
Turkey (3)	0.25±0.08	3.56±0.57	0	0.71	0.83	0.67	0.0040
Middle East (7)	0.15±0.03	4.00±0.44	2	0.79	0.78	1.00	0.0036

Genetic diversity in terms of allelic richness was highest in Anatolia and the Middle East, followed by Italy and the Iberian Peninsula (Table 3.1 and Supporting Information Figure S3.4). Expected heterozygosity was high in all populations with the exception of the Canary population, where the relatedness was particularly high (mean TrioML = 0.40). Overall population differentiation was low, suggesting a meaningful gene flow. Canaries showed the highest F_{ST} values with some degree of differentiation with Greek and Anatolian populations (Supporting Information Table S3.5).

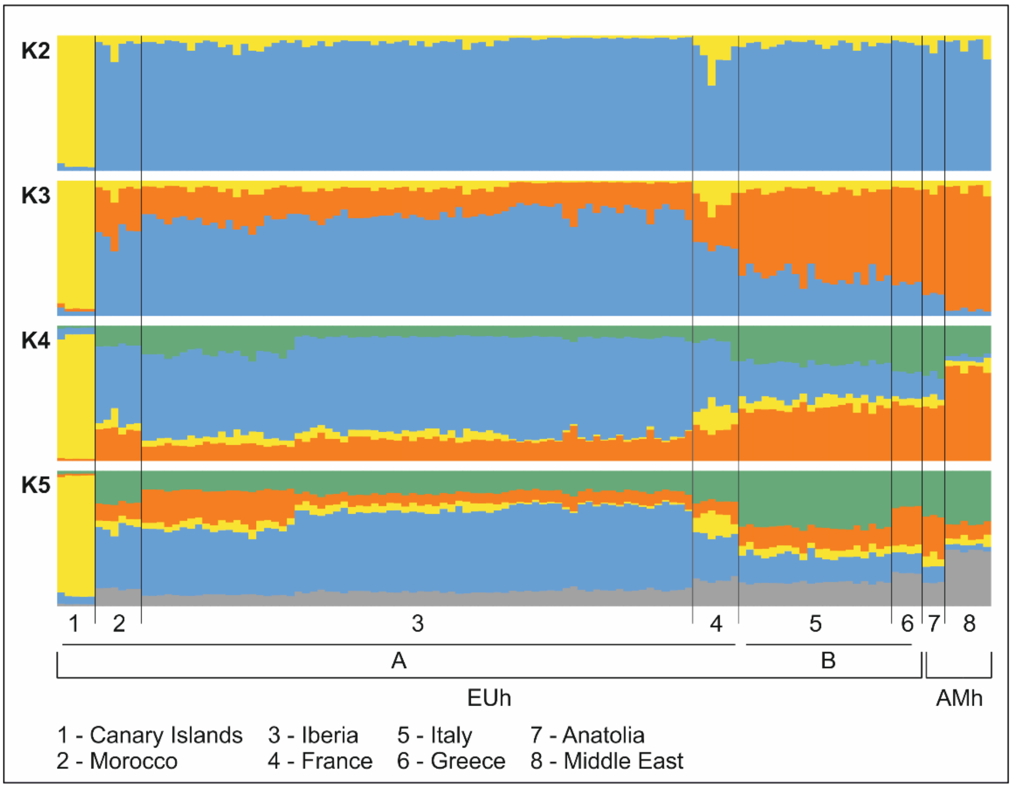

Figure 3.2 – *Tadarida teniotis* population structure based on the microsatellite dataset. Cluster membership plots from STRUCTURE analysis using prior population information (LOCPRIOR) including all samples. Results from 3 to 5 cluster are presented (K = 3 gets the highest rank according to the Evanno method, Supporting Information Figure S3.6 and Table S3.6).

Model-based clustering method implemented in STRUCTURE without prior population information did not identify any population structure (Supporting Information Figure S3.5). However, when using this prior, models revealed three main genetic populations (Supporting Information Figure S3.6 and Table S3.6). Individuals from the Canary Islands formed a separate population, while all individuals from the Iberian Peninsula, Morocco and France showed a higher estimated membership fraction to a second inferred cluster, and individuals from Italy eastwards consistently showed higher estimated membership fraction to a third inferred cluster (Figure 3.2). The three clusters topology was further supported by the Spatial Analysis of Principal Components (sPCA), although the pattern was not significant (Monte-Carlo test, p=0.082) (Figure 3.3). Both analyses showed that, except for the Canary population, most individuals had high levels of admixture, and only a west to east geographic gradient was evident. An overall observed pattern of isolation by distance was significant (Monte-Carlo test, p = 0.001) (Supporting Information Figure S3.7).

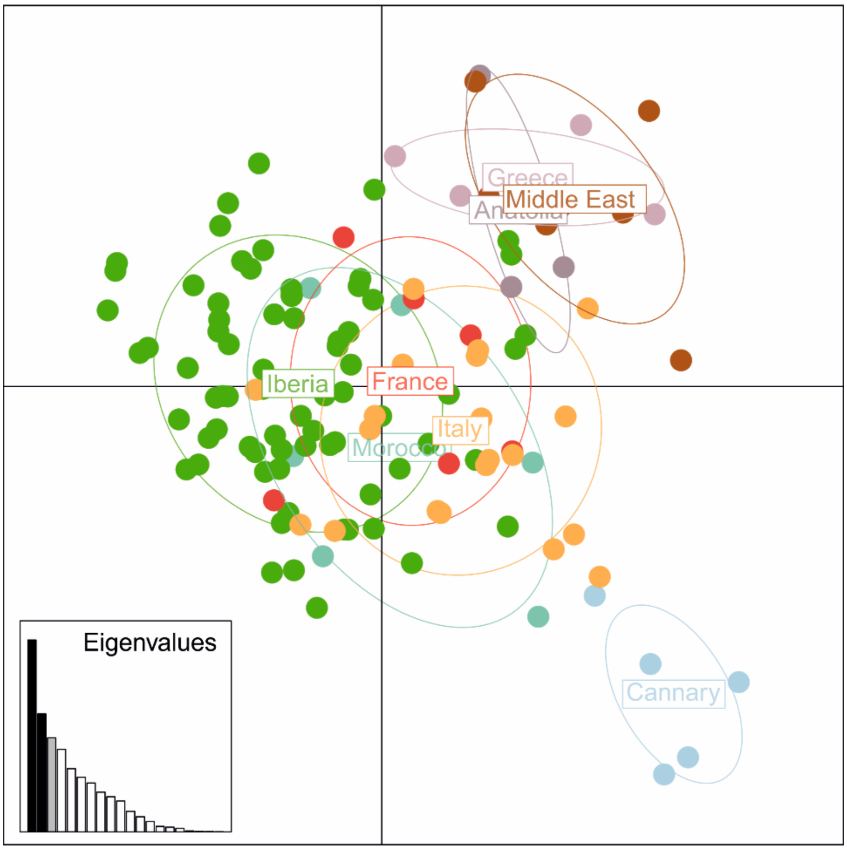

Figure 3.3 - Spatial Analysis of Principal Components (sPCA) showing the spatial genetic pattern of *Tadarida teniotis* population based on the microsatellite dataset. The Canaries form a separate cluster in the left down part, and with less support Greece, Anatolia and the Middle East also cluster together (top left). The two PCs explain 55.4% of the spatial genetic pattern. See also the sPCA Eigenvalues histogram in the inset. Dots indicate individual genotypes.

3.3.3 **ABC inference of evolutionary and demographic history**

Model-based inference showed high support (86 %) for a pre-LGM colonization of Italy from an unsampled population (Supporting Information Figure S3.1), while the Anatolian/Middle East population was also colonized pre-LGM from an admixture event between Italy and the unsampled population, with a similar contribution from both (proportion of admixture from unsampled population 0.46). The remaining European populations were colonized from Italy after the Younger Dryas, either directly or via a stepping stone manner with admixture (Figure 3.4). However, the Greek population showed some level of admixture between Italy and Anatolia/Middle East (Figure 3.4 and Supporting Information Table S3.3). Overall, our models identified two glacial refugia, in Italy and the Anatolia/Middle East with high confidence and low error rates (type I = 0.04; type II = 0.05).

Within the western edge of the range, ABC inference indicated a colonization and population expansion in Iberia with a generation time similar to that of the colonization analysis (Supporting Information Table S3.4). This scenario received high support (99 %) (Supporting

Information Figure S3.2) and error rates were estimated at 0.19 and 0.17 for type I and II errors respectively.

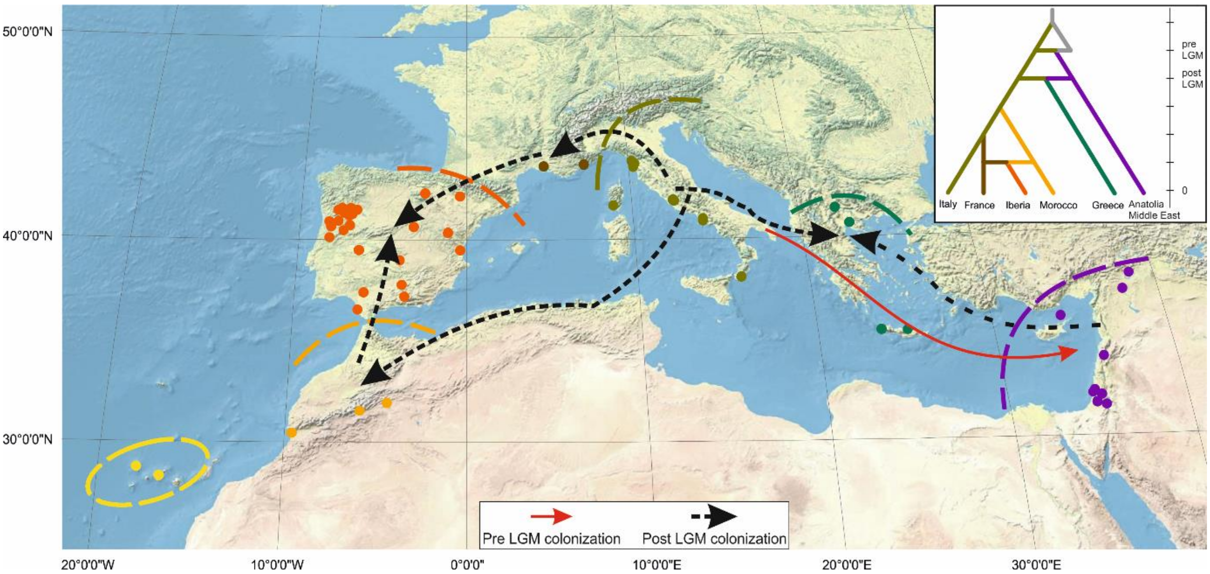

Figure 3.4 – Colonization patterns across the range of *T. teniotis* according to the best supported scenario (86 %) based on Approximate Bayesian Computation model inference (presented in the inset). The geographical location of T. teniotis genetic samples included in the study are plotted over an elevation map, with the location of the six populations marked and colour coded following the inset. Arrows indicate patterns of pre and post-Last Glacial Maximum range colonisation. Map coordinate system: Aitoff (sphere-based).

3.4 Discussion

We reconstructed the evolutionary history of a European bat species with tropical affinities. We show that *T. teniotis* populations were able to survive in Italy and Anatolia/Middle East during the LGM, and have subsequently colonized the current species range. The species has experienced a strong population expansion during the post-glacial colonization of its western range. Our results also point to the occurrence of another population in the Anatolian/Middle East area. Yet, the high haplotype diversity and network pattern found suggests that our samples did not cover the eastern refugium, which is likely located further east (Rossiter et al., 2007) or perhaps towards the Caucasus as suggested for the bat *Myotis bechsteinii* (Kerth et al., 2008).

3.4.1 **Postglacial colonization and demographic expansion**

Our inferences of demographic history indicate two main refugia during the LGM, one in the Italian Peninsula and another further east in the Anatolian/Middle East region. During this period, the species may have been extinct throughout the rest of southern Europe, with subsequent recolonization from the Italian Peninsula. Although the origin of the ancestral population is unclear, ABC indicates some degree of gene flow between Europe and Anatolia/Middle East before the LGM. Central and western Mediterranean areas were subsequently colonized in a stepping-stone manner, and through gene flow between populations originating from North Africa and France leading to an admixed population in the Iberian Peninsula. Although samples obtained provided a good coverage of the species range in the western Palaearctic, only a limited number of samples were available from North Africa. This is a common caveat of phylogeographic studies (Husemann et al., 2014) and we stress that our models do not negate the possibility of north African or Asian glacial refugium. While such a refugium could be the origin of the unknown ancestral population inferred in this study, our evolutionary history models show that a species with tropical affinities was able to survive in Italy during the LGM, from where it expanded across its current European range.

The inferred scenario of an Italian refugium and post-glacial European recolonization concurs with the widely accepted phylogeographic paradigms for the western Palearctic (Hewitt, 1999). Among bats, Italy has been identified as a glacial refugium for *Myotis myotis* (Ruedi et al., 2008) and a possible refugium for *Rhinolophus ferrumequinum* (Rossiter et al., 2007). In a recent paper, Bogdanowicz et al. (2015) suggested that this pattern might be widespread among bat species. Focusing on *Miniopterus schreibersii*, Bilgin et al. (2016) suggested a new paradigm of European colonization from Anatolian populations, and although we identified an ancient population in Anatolia/Middle East, our results do not support the hypothesis of a European recolonization from this region, a similar pattern to *R. ferrumequinum* (Rossiter et al., 2007). In fact, samples from Anatolia and the Middle East formed a distinct clade at the mitochondrial level (AMh), with no haplotypes shared with Europe. Interestingly, the high haplotype diversity (nine haplotypes in 10 samples) and the absence of a star-like pattern in the haplotype network for this region, suggests that the eastern refugium could be located further east.

High levels of relatedness and reduced genetic diversity in the Canary Islands likely reflect inbreeding in an isolated population. Increased inbreeding relative to mainland populations has been described for different taxa in insular populations (Frankham, 2008), including bats. Our results suggest that Canary Islands were colonized following a model of long-distance dispersal and establishment with limited subsequent gene flow from the parent population (Crisp et al., 2011). A general pattern of continental dispersion to the Canary Islands driven by stochastic events such as storms was described by Juan, Emerson, Oromí, & Hewitt (2000).

The star-like topology in the European mitochondrial groups (EUh-A and EUh-B) indicates population expansion (Slatkin & Hudson, 1991). This hypothesis was further supported by the ABC inference, which shows a demographic expansion following the Iberian colonization. Such expansion could be the result of a natural process (e.g., Bilgin et al., 2016; Razgour et al., 2013) or might be mediated by human activity, such as through increased roost availability from tall buildings and other structures including bridges, many of which were built during the 20th century (Amorim et al., 2013; Russo & Ancillotto, 2014).

Post-glacial population growth appear to be common in taxa with that underwent the same climatic changes since the LGM (Branco et al., 2002; Korsten et al., 2009), and was also suggested for another fast flying bat species, *Nyctalus noctula* (Petit et al., 1999). Microsatellites have a fast mutation rate when compared to other molecular markers, but it has been questioned whether this rate is fast enough to detect recent population changes (Barrett & Schluter, 2008). Therefore, it is difficult to ascertain if these populations, especially the ones located in the western edge of the species' range are still expanding.

3.4.2 **Barriers to gene flow**

Our results show high differentiation at mitochondrial markers between the populations from the Anatolia and Middle East region and those from central and western Mediterranean. We also found evidence of genetic differentiation within the European clade, whereby populations from Canary Islands, Morocco and Iberia seemed to form a distinct group from Central Mediterranean populations (Italy, France and Greece). Genetic structuring at the mitochondrial level suggests that, once established, females will not disperse freely, supporting some degree of philopatry, a common trait among several bat species (reviewed in Burland & Worthington Wilmer, 2001). In fact, the Iberian Peninsula seems to have been colonized following a first-come, first-served pattern, as indicated by the presence of haplotypes from both the central Mediterranean and North African haplogroups. Even though *T. teniotis* females are physically capable of crossing geographical barriers (e.g. mountain ranges and large bodies of water), phylopatric behaviour may have a strong effect on female dispersal, thus explaining the absence of Iberian/north African haplotypes in central Mediterranean. Contrary to mtDNA, at the nuclear level we confirmed some degree of gene flow between Europe and the Anatolia/Middle East. We also found high levels of gene flow within the European range and North Africa, whereas the Gibraltar strait does not act as a barrier to current or even past gene flow (García-Mudarra et al., 2009). Yet, the Canaries show high levels of isolation from mainland Africa. Combined, these results reflect a typical pattern of male-mediated gene flow (Castella et al., 2001).

Gene flow inferred from nuclear markers seemed to be solely restricted by geographic distance, showing a clear pattern of isolation by distance and the absence of strong geographic barriers to dispersal. *T. teniotis* performs fast and direct flights while foraging with median speeds of 50 km.h^{-1} and covering linear distances of up to 70 km (Marques et al., 2004). Although flight altitudes have not been reported for *T. teniotis*, the species is known to prey on large moths that migrate at high altitudes (Mata et al., 2016). Indeed the smaller congeneric species, *T. brasiliensis* (approx. 12 g compared to 30 g of *T. teniotis*), can fly up to 1 km above ground level (McCracken et al., 2008). Thus, the absence of geographic barriers to gene flow in our focal taxa is not surprising.

3.4.3 Implications for the phylogeography of Western Palearctic species with tropical affinity

The importance of refugia for conservation planning has been widely recognized because they can facilitate the persistence of biodiversity under changing climates (Keppel et al., 2012), and their relevance is even greater in the face of anthropogenic climate change. Common refugia in the Western Palearctic have been widely acknowledge for a number of species G. M. Hewitt, 1999; Husemann et al., 2014), however of the 914 studies focusing on taxa that occur in the western Palearctic (Keppel et al., 2012) only very few focus on species with tropical affinities (but see Rato et al., 2011). The location of refugia are often similar between species sharing climatic and environmental requirements, though it has been shown that species may respond differently to changes in habitat availability resulting from climatic changes at the end of the LGM (Taberlet et al., 1998). In a recent paper, Carstens, Morales, Field, & Pelletier (2018) showed that species' traits in bats can influence the response to climatic oscillations. Most importantly, they found that heavier bat species and those with longer wings were more likely to suffer a bottleneck at the LGM, and although this was mostly driven by frugivorous species from the neotropics, it highlights the importance of phylogeographic studies on species showing different traits in similar environments.

In this study we show that a species with tropical affinities was able to survive in the harsh environments of glacial Europe when a large area of the Western Palearctic was covered in ice sheets and permafrost, and temperatures were 10-20 ºC cooler than today (Kageyama et al., 2006). Yet, these results raise new questions regarding how these species survived in colder climates where the environment carrying capacity was lower (Frenzel et al., 1992). Moreover, free-tailed bats, such as *T. teniotis*, are thought to be poor hibernators. Although Arlettaz et al. (2000) found that in the Swiss Alps *T. teniotis* can go through torpor bouts that can last up to 8 days, average body temperature during hibernation and mean arousal frequency was much higher than in other temperate bat species.

This study contributes to understanding the evolutionary history of species with tropical affinities living in temperate regions, and raises questions regarding the physiological, behavioural and ecological traits that enabled them to survive in colder climates. The lack of phylogeographic studies focusing on these species highlights the importance of such studies for informing their population management and conservation, in particular under future environmental changes.

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Appendix 1

List of samples used in this study showing sample code, provider and corresponding voucher id (when available), geographic coordinates (WGS84), region and country (locality when geographic coordinates are not available). When mitochondrial sequencing was successful we provide GenBank accession number (for each gene) or ‘mini’ (sequenced obtained from internal primer), otherwise ‘n/a’ is shown for unsuccessful sequencing. Genotyping success (Micros) coded as ‘yes’ (successful genotyping) or ‘n/a’ (unsuccessful genotyping). When mitochondrial sequencing was unsuccessful for all genes samples were considered unidentified and were excluded from any further analysis. NOTE: Due to their short length, ‘mini’ sequences were not uploaded to GenBank and were not use in further analysis.

Sample Code	**Provider [voucher]**	**Lat / Long**	***COI***	***D-Loop***	***ATP6***	***CytB***	**Micros**	**Country / Region**
FG814	HNHM [HNHM2000.46.1]	41.41 / 22.24	MK817186	MK817240	MK817225	MK817216	yes	Macedonia / Demir Kapija
FG815	Eran Levin	31.58/ 34.88	MK817179	MK817264	MK817230	MK817198	yes	Israel / Yoav
FG816	Eran Levin	31.58 / 34.88	MK817180	MK817261	MK817229	MK817199	yes	Israel / Yoav
FG817	Eran Levin	32.03 / 34.77	MK817183	MK817262	MK817228	MK817198	yes	Israel / Tel-Aviv
FG818	Eran Levin	31.45 / 35.37	MK817185	MK817265	MK817227	MK817194	yes	Israel / Be'er Tuvia Regional
FG819	Eran Levin	32.17 / 34.9	MK817182	MK817260	MK817229	MK817199	yes	Israel / Kfar Sava
FG820	Groupe chiroptères corse	41.67 / 8.9	MK817186	MK817271	MK817219	MK817216	yes	France / Propriano (Corsica)
FG821	Muséum d'histoire naturelle Bourges	41.92 / 8.74	n/a	n/a	n/a	n/a	n/a	France / Ajaccio (Corsica)
FG822	Muséum d'histoire naturelle Bourges	41.92 / 8.74	n/a	n/a	n/a	n/a	n/a	France / Ajaccio (Corsica)
FG823	Muséum d'histoire naturelle Bourges	43.7 / 7.27	n/a	n/a	n/a	n/a	n/a	France / Nice
FG824	Muséum d'histoire naturelle Bourges	43.7 / 7.27	MK817186	MK817267	n/a	MK817215	yes	France / Nice
FG825	Muséum d'histoire naturelle Bourges	43.7 / 7.27	n/a	n/a	n/a	n/a	yes	France / Nice
FG826	Christian Dietz	40.64 / 22.94	MK817186	MK817240	MK817219	MK817216	yes	Greece / Thessaloniki
FG827	Christian Dietz	40.64 / 22.94	MK817186	MK817240	MK817219	MK817216	yes	Greece / Thessaloniki
FG828	Christian Dietz	31.54 / -5.92	MK817186	MK817240	MK817219	MK817216	yes	Morocco / Gorges du Dadès
FG829‡	Musée des Confluences [40001656]	-	mini	n/a	n/a	n/a	n/a	France / Villebois

FG830	FMNH [FMNH79280]	30.07 / 31.09	n/a	n/a	n/a	n/a	n/a	Egypt / Memphis
FG831	FMNH [FMNH79283]	30.07 / 31.09	n/a	n/a	n/a	n/a	n/a	Egypt / Memphis
FG832	FMNH [FMNH79284]	30.07 / 31.09	n/a	n/a	n/a	n/a	n/a	Egypt / Memphis
FG833	FMNH [FMNH79286]	30.07 / 31.09	n/a	n/a	n/a	n/a	n/a	Egypt / Memphis
FG834	FMNH [FMNH79288]	30.07 / 31.09	n/a	n/a	n/a	n/a	n/a	Egypt / Memphis
FG835	FMNH [FMNH79757]	30.01 / 31.21	n/a	n/a	n/a	n/a	n/a	Egypt / Memphis
FG836	FMNH [FMNH96280]	40.88 / 14.2	n/a	n/a	n/a	n/a	yes	Italy / Naples
FG837	FMNH [FMNH99291]	34.00 / 36.21	n/a	n/a	n/a	n/a	n/a	Lebanon / Baalbek
FG838	FMNH [FMNH99292]	34 .00 / 36.21	n/a	n/a	n/a	n/a	n/a	Lebanon / Baalbek
FG839	FMNH [FMNH99569]	34.00 / 35.83	MK817181	MK817261	MK817229	MK817199	n/a	Lebanon / Farayya
FG840	FMNH [FMNH111598]	27.13 / 57.09	n/a	n/a	n/a	n/a	n/a	Iran / Minab
FG841	Catherine Baby	43.72 / 7.26	MK817186	MK817240	MK817219	MK817200	yes	France / Nice
FG842	Catherine Baby	43.72 / 7.26	MK817186	MK817267	MK817219	MK817215	yes	France / Nice
FG843	Musée des Confluences [40002154]	47.08 / 9.33	n/a	n/a	n/a	n/a	n/a	Swiss / Gruyère
FG844	MNHM [ZM-MO-1984-369]	-	n/a	n/a	n/a	n/a	n/a	Italy
FG845	MNHM [ZM-MO-1999-762]	-	n/a	n/a	n/a	n/a	n/a	-
FG846	MNHM [ZM-MO-1999-765]	-	n/a	n/a	n/a	n/a	n/a	Spain
FG847	MNHM [ZM-MO-1983-1423]	-	MK817179	MK817259	MK817231	MK817198	yes	Palestine
FG848‡	MNHM [ZM-MO-1996-447]	-	n/a	mini	n/a	n/a	n/a	France
FG849	MNHM [ZM-MO-1984-1207]	-	n/a	n/a	n/a	n/a	n/a	Palestine
FG850	Panagiotis Georgiakakis	35.37 / 23.63	MK817171	MK817240	MK817219	MK817216	yes	Greece / Kissamos (Crete)
FG851	Panagiotis Georgiakakis	35.34 / 25.13	MK817182	MK817272	MK817229	MK817193	yes	Greece / Heraklion (Crete)
FG852	Leonardo Ancillotto	40.97 / 14.21	MK817186	MK817240	MK817219	MK817216	yes	Italy / Aversa
FG853	Leonardo Ancillotto	41.93 / 12.52	MK817170	MK817271	MK817222	MK817216	yes	Italy / Rome
FG854	Leonardo Ancillotto	41.93 / 12.52	MK817186	MK817240	n/a	MK817216	yes	Italy / Rome
FG855	Leonardo Ancillotto	41.93 / 12.52	MK817186	MK817240	MK817219	MK817216	yes	Italy / Rome
FG856	Leonardo Ancillotto	41.93 / 12.52	MK817170	MK817271	MK817222	MK817216	yes	Italy / Rome
FG857	Leonardo Ancillotto	41.93 / 12.52	MK817186	MK817240	MK817221	MK817216	yes	Italy / Rome
FG858	Leonardo Ancillotto	43.56 / 10.32	MK817186	MK817240	MK817221	MK817216	yes	Italy / Livorno
FG859	Leonardo Ancillotto	43.72 / 10.4	MK817186	MK817240	MK817219	MK817216	yes	Italy / Pisa

FG860	Leonardo Ancillotto	43.87 / 10.25	MK817186	MK817240	MK817219	MK817216	yes	Italy / Viareggio
FG861	Leonardo Ancillotto	41.88 / 12.57	MK817186	MK817240	MK817219	MK817216	yes	Italy / Rome
FG359	CIBIO	41.45 / -6.59	MK817176	MK817240	MK817235	MK817203	yes	Portugal / Mogadouro
FG373[†]	CIBIO	41.29 / -6.87	MK817186	MK817240	MK817219	MK817216	yes	Portugal / Alfândega da Fé
FG389	CIBIO	41.33 / -7.36	MK817186	MK817240	MK817219	MK817216	yes	Portugal / Carrazeda de Ansiães
FG432	CIBIO	41.55 / -6.98	MK817166	MK817248	MK817219	MK817216	yes	Portugal / Macedo de Cavaleiros
FG451	CIBIO	41.18 / -7.06	MK817186	MK817240	MK817219	MK817216	yes	Portugal / Torre de Moncorvo
FG530	CIBIO	41.55 / -6.98	MK817186	MK817270	MK817226	MK817216	yes	Portugal / Macedo de Cavaleiros
FG739	CIBIO	41.33 / -7.36	MK817186	MK817240	MK817219	MK817216	yes	Portugal / Carrazeda de Ansiães
FG793	CIBIO	41.28 / -6.89	MK817186	MK817240	MK817235	MK817216	yes	Portugal / Alfândega da Fé
FG862	Javier Juste & Carlos Ibáñez	-	MK817186	MK817257	MK817219	MK817214	yes	Spain / Cadiz
FG863	Javier Juste & Carlos Ibáñez	-	MK817186	MK817257	MK817219	MK817214	yes	Spain / Cadiz
FG864	Javier Juste & Carlos Ibáñez	-	MK817186	MK817240	MK817219	MK817216	yes	Spain / Granada
FG865	Javier Juste & Carlos Ibáñez	-	MK817186	MK817240	MK817219	MK817216	yes	Spain / Granada
FG866	Javier Juste & Carlos Ibáñez	-	MK817186	MK817240	MK817219	MK817216	yes	Spain / Granada
FG867	Javier Juste & Carlos Ibáñez	-	MK817186	MK817251	MK817235	MK817213	yes	Spain / Granada
FG868	Javier Juste & Carlos Ibáñez	-	MK817186	MK817240	MK817235	MK817216	yes	Spain / Granada
FG869	Javier Juste & Carlos Ibáñez	-	MK817169	MK817269	MK817235	MK817212	yes	Spain / Jaen
FG870	Javier Juste & Carlos Ibáñez	-	MK817175	MK817250	MK817235	MK817211	yes	Spain / Huesca
FG871	Javier Juste & Carlos Ibáñez	-	MK817186	MK817271	MK817219	MK817216	yes	Spain / Huesca
FG872	Javier Juste & Carlos Ibáñez	-	MK817186	MK817270	MK817235	MK817216	yes	Spain / Teruel
FG873	Javier Juste & Carlos Ibáñez	-	MK817186	MK817240	MK817219	MK817216	yes	Spain / Teruel
FG874	Javier Juste & Carlos Ibáñez	-	MK817167	MK817240	MK817223	MK817216	yes	Spain / Teruel
FG875	Javier Juste & Carlos Ibáñez	-	MK817186	MK817240	MK817219	MK817216	yes	Spain / Teruel
FG876	Javier Juste & Carlos Ibáñez	-	MK817186	MK817271	MK817219	MK817216	yes	Spain / Teruel
FG877	Javier Juste & Carlos Ibáñez	-	MK817186	MK817271	MK817219	MK817216	yes	Spain / Teruel
FG878	Javier Juste & Carlos Ibáñez	-	MK817186	MK817270	MK817234	MK817216	yes	Spain / Tenerife (Canary)
FG879	Javier Juste & Carlos Ibáñez	-	MK817186	MK817240	MK817235	MK817216	yes	Spain / Tenerife (Canary)
FG880	Javier Juste & Carlos Ibáñez	-	MK817186	MK817240	n/a	n/a	yes	Spain / La Palma (Canary)
FG881	Javier Juste & Carlos Ibáñez	-	MK817186	MK817240	MK817235	MK817216	yes	Spain / La Palma (Canary)

FG882	Javier Juste & Carlos Ibáñez	-	MK817186	MK817240	MK817235	MK817216	yes	Spain / La Palma (Canary)
FG883	Javier Juste & Carlos Ibáñez	-	MK817186	MK817240	MK817235	MK817216	yes	Spain / La Palma (Canary)
FG884	Javier Juste & Carlos Ibáñez	-	MK817186	MK817240	MK817235	MK817216	yes	Spain / Ciudad Real
FG885	Javier Juste & Carlos Ibáñez	-	MK817173	MK817240	MK817235	MK817213	yes	Spain / Guadalajara
FG886	Javier Juste & Carlos Ibáñez	-	MK817186	MK817240	MK817235	MK817210	yes	Spain / Guadalajara
FG887	Javier Juste & Carlos Ibáñez	-	MK817186	MK817240	MK817219	MK817216	yes	Spain / Guadalajara
FG888	Javier Juste & Carlos Ibáñez	-	MK817186	MK817240	MK817232	MK817216	yes	Spain / Guadalajara
FG889	Javier Juste & Carlos Ibáñez	-	MK817186	MK817243	MK817235	MK817216	yes	Spain / Guadalajara
FG890	Javier Juste & Carlos Ibáñez	-	MK817186	MK817240	MK817235	MK817216	yes	Spain / Guadalajara
FG891	Javier Juste & Carlos Ibáñez	-	MK817186	MK817241	MK817235	MK817209	yes	Spain / Sevilla
FG892	Javier Juste & Carlos Ibáñez	-	MK817186	MK817251	MK817219	MK817216	yes	Spain / Caceres
FG893	Javier Juste & Carlos Ibáñez	-	MK817186	MK817270	MK817235	MK817216	yes	Spain / Caceres
FG894	Javier Juste & Carlos Ibáñez	-	MK817186	MK817253	MK817219	MK817216	yes	Spain / Caceres
FG895	Javier Juste & Carlos Ibáñez	-	MK817186	MK817240	MK817219	MK817216	yes	Spain / Caceres
FG896	Javier Juste & Carlos Ibáñez	-	MK817186	MK817251	MK817235	MK817216	yes	Spain / Caceres
FG897	Javier Juste & Carlos Ibáñez	-	MK817186	MK817251	MK817235	MK817216	yes	Spain / Caceres
FG898	Javier Juste & Carlos Ibáñez	-	MK817186	MK817248	MK817219	MK817216	yes	Spain / Caceres
FG899	Javier Juste & Carlos Ibáñez	-	MK817186	MK817240	MK817219	MK817216	yes	Spain / Caceres
FG900	Javier Juste & Carlos Ibáñez	-	MK817186	MK817249	MK817219	MK817216	yes	Spain / Caceres
FG901	Javier Juste & Carlos Ibáñez	-	MK817186	MK817251	MK817235	MK817216	yes	Spain / Caceres
FG902	Javier Juste & Carlos Ibáñez	-	MK817186	MK817240	MK817219	MK817216	yes	Italy / Samo
FG903	Javier Juste & Carlos Ibáñez	-	MK817186	MK817240	MK817219	MK817216	yes	Italy / Samo
FG904	Javier Juste & Carlos Ibáñez	-	MK817177	MK817245	MK817219	MK817216	yes	Italy / Samo
FG905	Javier Juste & Carlos Ibáñez	-	MK817178	MK817240	MK817219	MK817208	yes	Italy / Samo
FG906	Javier Juste & Carlos Ibáñez	-	MK817186	MK817240	MK817220	MK817216	yes	Italy / Samo
FG907	Javier Juste & Carlos Ibáñez	-	MK817186	MK817240	MK817219	MK817216	yes	Italy / Samo
FG908	Javier Juste & Carlos Ibáñez	-	MK817178	MK817240	MK817219	MK817208	yes	Italy / Samo
FG909	Javier Juste & Carlos Ibáñez	-	MK817186	MK817244	MK817219	MK817216	yes	Italy / Samo
FG910	Javier Juste & Carlos Ibáñez	-	MK817178	MK817240	MK817219	MK817208	yes	Italy / Samo
FG911	Javier Juste & Carlos Ibáñez	-	MK817177	MK817245	MK817219	MK817216	yes	Italy / Samo

FG912	Javier Juste & Carlos Ibáñez	-	MK817186	MK817240	MK817233	MK817207	yes	Spain / Valencia
FG913	Javier Juste & Carlos Ibáñez	-	MK817175	MK817249	MK817235	MK817216	yes	Spain / Valencia
FG914	Javier Juste & Carlos Ibáñez	-	MK817186	MK817240	n/a	n/a	yes	Spain / Valencia
FG915	Javier Juste & Carlos Ibáñez	-	MK817186	MK817240	n/a	n/a	yes	Spain / Valencia
FG916	Javier Juste & Carlos Ibáñez	-	MK817186	MK817240	n/a	MK817216	yes	Spain / Valencia
FG917	Javier Juste & Carlos Ibáñez	-	MK817186	MK817240	n/a	n/a	yes	Spain / Valencia
FG918	Javier Juste & Carlos Ibáñez	-	n/a	n/a	n/a	n/a	n/a	Spain / Valencia
FG919	Javier Juste & Carlos Ibáñez	-	MK817168	MK817256	n/a	n/a	yes	Spain / Valencia
FG920	Javier Juste & Carlos Ibáñez	-	MK817186	MK817240	MK817235	MK817206	yes	Spain / Valencia
FG921	Javier Juste & Carlos Ibáñez	-	MK817174	MK817240	MK817219	MK817216	yes	Spain / Valencia
FG922	Javier Juste & Carlos Ibáñez	-	MK817186	MK817240	MK817219	MK817216	yes	Spain / La Rioja
FG923	Javier Juste & Carlos Ibáñez	-	MK817186	MK817252	MK817219	MK817216	yes	Spain / La Rioja
FG924	Javier Juste & Carlos Ibáñez	-	MK817186	MK817240	n/a	MK817196	yes	Spain / La Rioja
FG925	Javier Juste & Carlos Ibáñez	-	MK817184	MK817263	MK817229	MK817197	yes	Turkey / Şanlıurfa
FG926	Javier Juste & Carlos Ibáñez	-	MK817185	MK817266	MK817227	MK817195	yes	Turkey / Adıyaman
FG927	Javier Juste & Carlos Ibáñez	-	MK817184	MK817263	MK817229	MK817197	yes	Turkey / Şanlıurfa
FG928	Javier Juste & Carlos Ibáñez	-	MK817186	MK817251	MK817219	MK817205	yes	Morocco / Agadir-Ida
FG929	Javier Juste & Carlos Ibáñez	-	MK817186	MK817255	MK817235	MK817216	yes	Morocco / Agadir-Ida
FG930	Javier Juste & Carlos Ibáñez	-	MK817172	MK817240	MK817219	MK817202	yes	Morocco/ Agadir-Ida
FG931	Javier Juste & Carlos Ibáñez	-	MK817186	MK817247	MK817224	MK817204	yes	Morocco/ Agadir-Ida
FG932	Javier Juste & Carlos Ibáñez	-	MK817186	MK817246	MK817235	MK817201	yes	Morocco / Errachidia
FG01	Plecotus	40.84 / -8.19	MK817186	MK817268	MK817219	MK817216	yes	Portugal / São Pedro do Sul
FG02	Plecotus	40.64 / -8.13	MK817186	MK817240	MK817219	MK817216	yes	Portugal / Tondela
FG11	CIBIO	41.39 / -7.45	MK817186	MK817240	MK817219	MK817216	yes	Portugal / Tinhela
FG64	CIBIO	41.45 / -6.59	MK817186	MK817240	MK817235	MK817216	yes	Portugal / Mogadouro
FG92	Plecotus	40.45 / -7.36	MK817186	MK817240	MK817235	MK817216	yes	Portugal / Trancoso
FG93	Bio3	40.93 / -7.69	MK817166	MK817248	MK817219	MK817216	yes	Portugal
FG94	Bio3	41.44 / -7.00	MK817186	MK817254	MK817219	MK817216	yes	Portugal
FG95	Plecotus	40.10 / -8.18	MK817186	MK817240	MK817235	MK817216	yes	Portugal / Lousã
FG96	Plecotus	40.88 / -8.25	MK817166	MK817248	n/a	MK817216	yes	Portugal / Arouca

FG164	Ecosativa	41.53 / -7.51	MK817186	MK817240	MK817235	MK817216	yes	Portugal / Vila Pouca de Aguiar
FG165	Ecosativa	41.52 / -7.51	MK817186	MK817251	MK817219	MK817216	yes	Portugal / Vila Pouca de Aguiar
FG166	Ecosativa	41.55 / -7.51	MK817186	MK817242	MK817235	MK817216	yes	Portugal / Vila Pouca de Aguiar
FG167	Ecosativa	41.45 / -7.75	MK817166	MK817248	MK817219	MK817216	yes	Portugal / Vila Pouca de Aguiar
FG933	MVZ [MVZ:Mamm:192570]	27.92 / 101.33	n/a	n/a	n/a	n/a	yes	China / Yunnan
FG934[§]	MVZ [MVZ:Mamm:192571]	27.92 / 101.33	MK817187	n/a	MK817192	MK817190	n/a	China / Yunnan
FG935	MVZ [MVZ:Mamm:192573]	27.92 / 101.33	n/a	n/a	n/a	n/a	yes	China / Yunnan
FG936[§]	MVZ [MVZ:Mamm:193379]	27.92 / 101.33	MK817188	n/a	MK817191	MK817189	n/a	China / Yunnan
FG1052	NHM-AUB	-	n/a	n/a	n/a	n/a	yes	Lebanon
FG1053	NHM-AUB	-	n/a	n/a	n/a	n/a	n/a	Lebanon
FG1054	NHM-AUB	-	n/a	n/a	n/a	n/a	n/a	Lebanon
FG1551[†¶]	Toni Guillén [ROM:MAM:118321]	-	MK817165	MK817238	MK817237	MK817218	yes	Laos
FG1552[¶]	Toni Guillén	-	MK817165	MK817239	MK817236	MK817217	yes	Laos
FG1735	Sébastien Puechmaille	43.62 / 4.77	MK817186	MK817240	MK817219	MK817216	yes	France / Saint-Martin-de-Crau
FG16	CIBIO	41.17 / -7.05	MK817186	MK817258	n/a	n/a	yes	Portugal / Torre de Moncorvo
FG3144	Senckenberg [38739]	-	n/a	n/a	n/a	n/a	n/a	Afghanistan / Kabul
FG3145	Senckenberg [33526]	-	n/a	n/a	n/a	n/a	n/a	France
FG3146	Senckenberg [77805]	-	n/a	n/a	n/a	n/a	n/a	Kyrgyzstan
FG3147[‡]	Senckenberg [77806]	-	mini	n/a	n/a	n/a	n/a	Kyrgyzstan
FG3148	Senckenberg [77807]	-	n/a	n/a	n/a	n/a	n/a	Kyrgyzstan
FG3149[‡¶]	Senckenberg [77808]	-	mini	n/a	n/a	n/a	n/a	Kyrgyzstan
FG3150[‡¶]	Senckenberg [91142]	-	mini	n/a	n/a	n/a	n/a	Kyrgyzstan
FG3151[‡¶]	Senckenberg [91143]	-	mini	n/a	n/a	n/a	n/a	Kyrgyzstan

[†] Complete mitochondrion genome available on genbank (Mata V.A *et al.*, (2017) First complete mitochondrial genomes of molossid bats (Chiroptera: Molossidae). *Mitochondrial DNA Part B*, **2**(1), 152–154)

[‡] This samples were amplified using internal primers that differentiate *T. teniotis* from *T. latouchei*. Due to differences in sequence length, the two samples from *T. teniotis* amplified using these primers were not included in the phylogenetic and evolutionary analysis.

[§] Samples identified as belonging to *Chaerephon plicatus*

[¶] Samples identified as belonging to *T. latouchei*

Supporting Information

Table S3.1 – Mitochondrial primers designed for *COI*, *ATP6*, *CytB* and *D-Loop* regions. Forward and reverse primers are presented, as well as the amplicon position in the mitogenome and its length (excluding primers). Internal primers designed for highly degraded museum samples are also presented (*COI-mini* and *D-Loop-mini*).

Fragment	Primer (5'-3')		Mitogenome position(1)	Length (bp)
COI	Tten-COI-F	CCCAYGCAGGAGCTTCAGTT	5761-6373	612
	Tten-COI-R	TGTTAGGCCYCCTACTGTGA		
ATP6	Tten-ATP6-F	CAAMCAACTCCTTGAGAYAYC	7931-8620	689
	Tten-ATP6-R	CTATATGRTARGCATGTGTTTGG		
Cytb	Tten-Cytb-F	CGCAGCCATAGYCATAGTC	14740-15303	563
	Tten-Cytb-R	CAAGACCGRRGTAATGTATATATAC		
Dloop	Tten-Dloop-F	CGTAAACGGCATCTGGTTCT	16011-16349	338
	Tten-Dloop-R	GCGTATCTGATTRTGTTATATGTCCTG		
COI-mini	Tten-COI-mini-F	RGTCCTAGCAGCMGGAATCAC	621-718	97
	Tten-COI-mini-R	GGATTAGAATATAAACTTCGGGGTG		
Dloop-mini	Tten-Dloop-mini-F	AATTAARGGTCCCAGGACATTCA	16278-16349	71
	Tten-Dloop-R	GCGTATCTGATTRTGTTATATGTCCTG		

(1) Mata et al. (2017) First complete mitochondrial genomes of molossid bats (Chiroptera: Molossidae). Mitochondrial DNA Part B, 2(1), 152–154. https://doi.org/10.1080/23802359.2017.1298419.

Table S3.2 – Microsatellite loci and primer sequences used in the study, including information on motif, concentration of forward (F) primer and tail, allele size range, observed (H_{obs}), expected (H_{exp}) heterozygosity, P values for deviation from Hardy-Weinberg equilibrium (P(HW)), and estimated frequency of null alleles. Concentration of reverse primer was 10x the forward primer.

	Primer name	**motif**	**Sequence (5'-3'), F: 10 µM, R: 100 µM**	**Tail**	**Concentration (pM)**		**Allele size range**	**Number of alleles**	**H_{obs}**	**H_{exp}**	**P (HW)**	**F (null allele)**
					Primer	**Tail**						
Mix1	**TAD1**	ac	F: GGCTACCTGAGCTTCTTAGTCTTC R: TGTTGCTTTCTAGGCTGTTGC	FAM	0.11	4.00	145-183	15	0.86	0.87	0.073	0.01
	TAD5	tatc	F: ATTCCTGGTCAGTGCATCGT R: TCACTGACACAGATGATAATAGAGACA		0.19		322-354	9	0.49	0.80	0.00^{*}	0.20
	TAD6	ac	F: TTCTTAACCACACTGGCCCT R: TGCAAGCCAAACATAAGTCG		0.11		221-247	13	0.67	0.88	$0.00^{†}$	0.13
	TAD23	ctat	F: GAGCACAGTTCTCCTGATTGG R: TTCAGTCCTAAACTAGGATCAAAGAC	VIC	0.08	0.80	132-156	7	0.76	0.76	0.73	0.00
	TAD2	ac	F: TCATCGGTATCCTCAAAGAAATG R: GCATTGATGGAGACAGAAATATAAA	NED	3.20	33.20	177-199	11	0.81	0.85	0.11	0.03
	TAD4	ag	F: ACCTTTGATTACGCAGCACC R: AATTTTGAGAAGTTTGAATGCCA		0.12		99-127	14	0.73	0.77	0.08	0.02
	TAD7	ac	F: AAAAGCAGATGGTCAGCAGG R: TGCCAGGCCTAAGATGCTAC	PET	0.12	1.20	96-126	13	0.85	0.84	0.52	0.00
Mix2	**TAD13**	atct	F: TTTCTCTTTTATGGGAAATCCA R: CCTCAGAAGTAGCCGCAAGT	FAM	0.08	1.60	183-219	10	0.78	0.81	0.46	0.02
	TAD19	ca	F: TGTTCAATTACACTGTTACATGCG R: CAACCTTGGTCAATTTTAAAGC		0.08		104-130	10	0.79	0.78	$0.00^{†}$	0.00
	TAD9	tg	F: CCCTTCTGCCAGTCCAAATA R: CATTGCCACCAAATTCCTCT	VIC	0.08	1.60	136-154	10	0.63	0.78	0.00^{*}	0.09
	TAD18	gaag	F: CCTCAGCTATCTTGGGATTGTC R: AAGCACCACCGATCTGAATC		0.08		186-242	13	0.88	0.86	0.76	0.00
	TAD26	tg	F: TTGAAACACCACATTCAGTCG R: AGTTGAATAAGTTAATTGAAATAAGCC	NED	0.2	2.00	138-156	9	0.79	0.81	0.53	0.01
	TAD12	ag	F: CGCTAATCTCTGCTAATCTCTGG R: GCCTTACCTATACTTCGCTTGG	PET	0.12	3.60	172-200	14	0.83	0.83	0.18	0.00
	TAD24	ag	F: GATTGTTCCATTGTTATTCCTATTTG R: TGACTGTGGGCAGGTTGTTA		0.24		108-138	14	0.69	0.78	$0.02^{†}$	0.05

[*] Deviation from Hardy-Weinberg equilibrium (P < 0.05 no correction for multiple tests applied) in more than two out of the eight populations. These loci were removed from further analysis

[†] Deviation from Hardy-Weinberg equilibrium (P < 0.05 no correction for multiple tests applied) in two or less populations. These loci were included in further analysis.

Table S3.3 – ABC Scenarios parameters for inferring 1) colonization analysis; and 2) demographic history analysis. Only the best scenarios for each analysis are presented. Parameters as follow: N – effective population size parameters, where *e* denotes equal population size for all populations, *Ib* and *Fr* correspond to Iberian and French populations respectively; t – time as number of generations where numbers represent the scenario and time step; r – admixture rate corresponding to time step *t*.

Colonization analysis (SC2)

Parameters	Priors		Results				
	Conditions	Distribution [min-max]	mean	median	mode	Q_5	Q_{95}
N_e		Uniform [1E^3 - 1E^6]	5.16E^4	4.99E^4	4.68E^4	3.18E^4	7.70^4
$t_{2.1}$		Uniform [1E^4 - 2E^5]	6.06E^4	4.51E^4	2.50E^4	1.65E^4	1.60E^5
$t_{2.2}$	$t_{2.2} < t_{2.1}$	Uniform [1E^3 - 2E^4]	1.27E^4	1.29E^4	1.32E^4	5.62E^3	1.92E^4
$t_{2.3}$	$t_{2.3} \leq t_{2.2}$	Uniform [1E^3 - 2E^4]	3.93E^3	3.32E^3	2.53E^3	1.45E^3	8.56E^3
$t_{2.4}$	$t_{2.4} \leq t_{2.1}$	Uniform [1E^3 - 2E^4]	5.34E^3	4.34E^3	3.04E^3	2.01E^3	1.26E^4
$t_{2.5}$	$t_{2.5} \leq t_{2.1}$	Uniform [1E^3 - 2E^4]	6.16E^3	5.15E^3	3.62E^3	2.33E^3	1.39E^4
$t_{2.6}$	$t_{2.6} < t_{2.4}$ $t_{2.6} \leq t_{2.5}$	Uniform [1E^3 - 2E^4]	1.96E^3	1.76E^3	1.53E^3	1.17E^3	3.37E^3
$r_{2.1}$		Uniform [0.001 - 0.999]	4.72E-1	4.60E-1	3.63E-1	7.85E-2	9.03E-1
$r_{2.3}$		Uniform [0.001 - 0.999]	5.48E-1	5.57E-1	6.25E-1	1.66E-1	8.89E-1
$r_{2.6}$		Uniform [0.001 - 0.999]	5.07E-1	5.12E-1	5.47E-1	9.49E-2	9.01E-1

Demographic history analysis (SC2)

Parameters	Priors		Results				
	Conditions	Distribution [min-max]	mean	median	mode	Q_5	Q_{95}
N_{Fr1}		Uniform [1E^2 - 5E^5]	1.14E^3	8.90E^2	5.80E^2	2.92E^2	2.73E^3
N_{Fr2}	$N_{Fr2} > N_{Fr1}$	Uniform [1E^2 - 1E^6]	1.76E^6	1.88E^6	2.00E^6	1.05E^6	1.99E^6
N_{Ib}	$N_{Ib} > N_{Fr1}$	Uniform [1E^2 - 1E^6]	3.22E^5	2.41E^5	2.74E^4	2.74E^4	8.62E^5
$t_{2.1}$		Uniform [10 - 5E^3]	1.45E^3	1.09E^3	7.93E^2	3.60E^2	3.84E^3

Table S3.4 – Genetic differentiation at the mitochondrial DNA level between *Tadarida teniotis* geographical groups based on θ_{ST} values.

	Canary	**Iberia**	**Morocco**	**France**	**Italy**	**Greece**	**Anatolia**
Morocco	0.094						
Iberia	0.065	0.023					
France	**0.442****	**0.049***	**0.068***				
Italy	**0.343****	**0.093***	**0.105****	0.013			
Greece	**0.179***	0.016	**0.181***	0.031	**0.13***		
Turkey	**0.831***	**0.72***	**0.843****	**0.819***	**0.533***	**0.841****	
Middle-East	**0.75****	**0.685****	**0.816****	**0.744****	**0.507***	**0.79****	0.142

$^*p<0.05$; $^{**}p<0.001$

Table S3.5 – Genetic differentiation at nuclear (microsatellite) level between *Tadarida teniotis* populations based on F_{ST} values. The bottom diagonal includes all individuals, while in the top diagonal values of F_{ST} were calculated after the removal of close relatives (TrioML > 0.5). Values above 0.1 (moderate to strong population differentiation) are marked in bold

	Canary	**Morocco**	**Iberia**	**France**	**Italy**	**Greece**	**Anatolia**	**Middle-east**
Canary		0.093	0.093	0.066	0.067	**0.144**	**0.130**	0.093
Morocco	0.067		-0.004	-0.002	-0.005	0.039	0.012	0.014
Iberia	0.067	-0.005		0.000	0.012	0.046	0.025	0.037
France	0.042	-0.002	0.000		0.005	0.038	0.005	0.004
Italy	0.047	-0.005	0.011	0.005		0.055	0.010	0.027
Greece	**0.106**	0.039	0.046	0.038	0.055		0.020	0.003
Turkey	0.093	0.012	0.025	0.005	0.010	0.020		0.023
Middle East	0.074	0.014	0.037	0.004	0.027	0.003	0.023	

Table S3.6 – Mean LnP(K) and Delta K (Evanno method) results for the Bayesian clustering analysis with prior population information, as implemented in STRUCTURE HARVESTER. Bold highlights the largest value in the Delta K for K = 3.

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
1	10	-5842.78	0.38	NA	NA	NA
2	10	-5799.81	11.54	42.97	30.37	2.63
3	**10**	**-5787.21**	**12.01**	**12.60**	**108.49**	**9.03**
4	10	-5883.10	34.42	-95.89	103.69	3.01
5	10	-5875.30	36.12	7.80	38.18	1.06
6	10	-5905.68	43.03	-30.38	24.09	0.56
7	10	-5911.97	66.55	-6.29	15.39	0.23
8	10	-5902.87	40.91	9.10	32.29	0.79
9	10	-5926.06	81.41	-23.19	18.08	0.22
10	10	-5967.33	86.42	-41.27	NA	NA

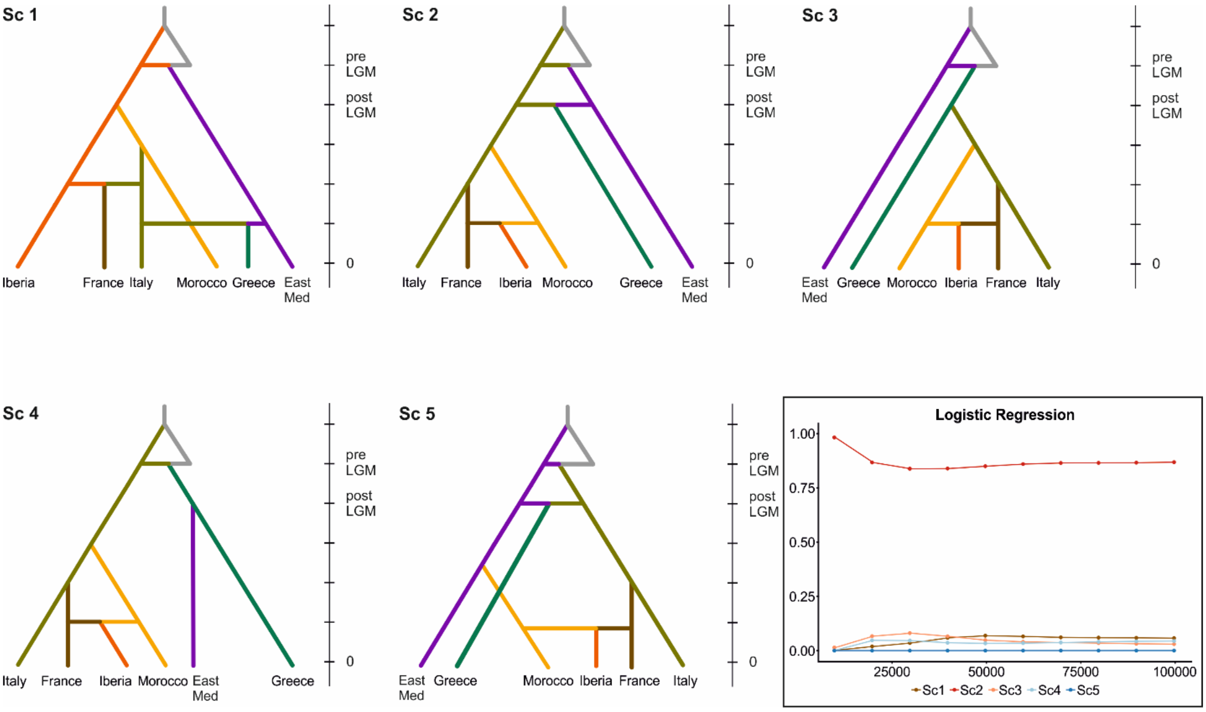

Figure S3.1 – Schematic representation of colonization scenarios compared using Approximate Bayesian Computation and the logistic regression of the posterior probabilities of the five scenarios as a function of number of simulated datasets. For further details, see Methods in main text and Table S3.3.

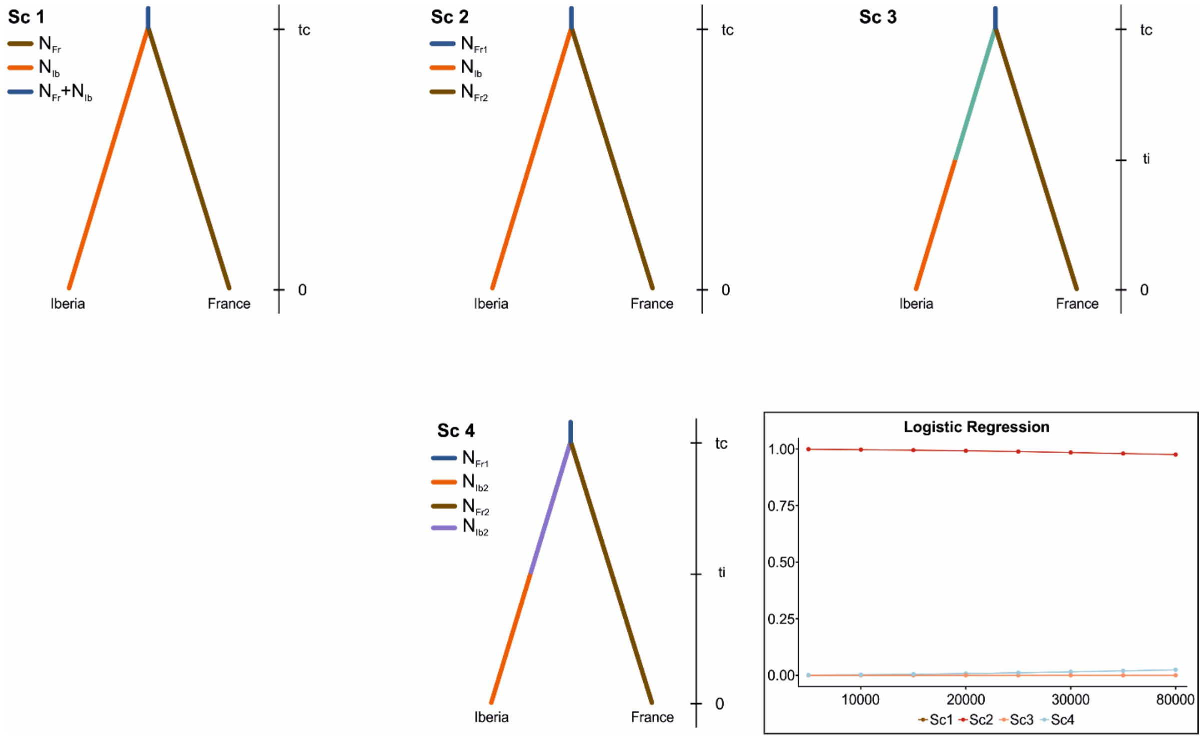

Figure S3.2 – Schematic representation of the demographic history scenarios of the western range compared using Approximate Bayesian Computation and the logistic regression of the posterior probabilities of the five scenarios as a function of number of simulated datasets. Subscripts *Ib* and *Fr* correspond to Iberian and French populations respectively. For further details, see Methods in main text and Table S3.3.

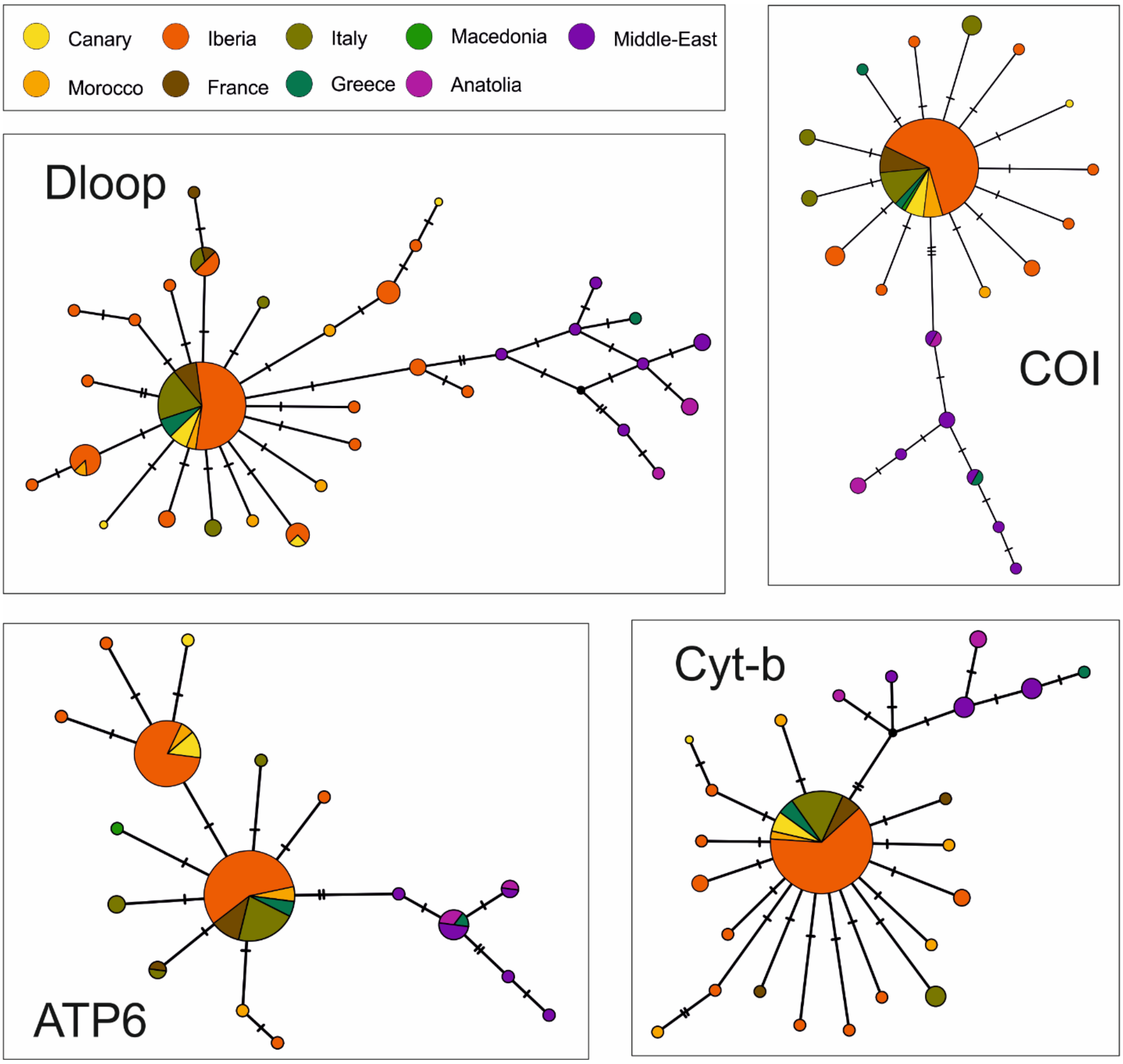

Figure S3.3 – Median-joining haplotype networks for *T. teniotis* mtDNA coding regions (*cytochrome c oxidase subunit I, ATP6*, and *cytochrome b*) and noncoding region (control region *Dloop*). All concatenated mtDNA sequences (n = 109) were used in the Median-joining networks in which branch lengths are not proportional to base-pair changes.

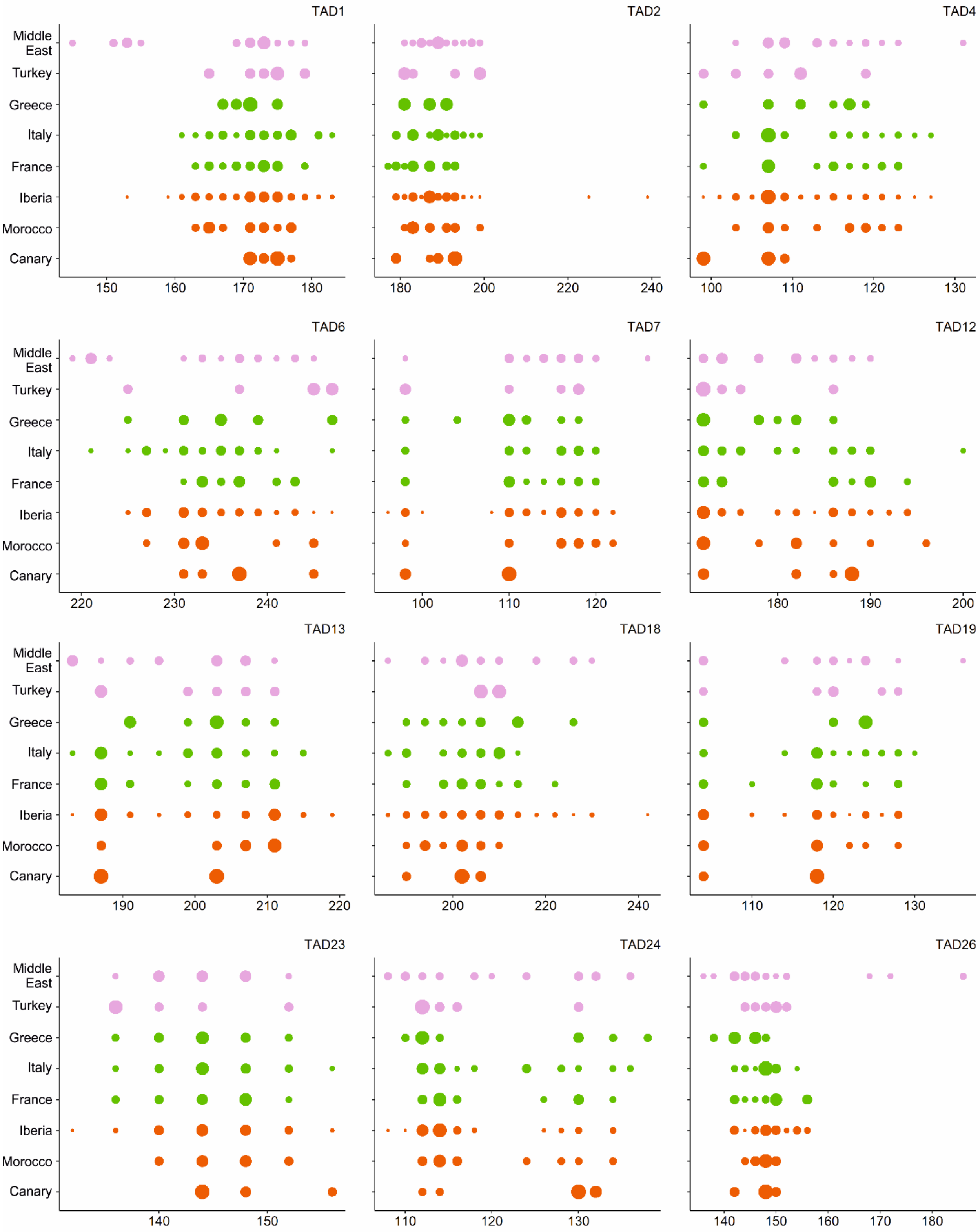

Figure S3.4 – Allele frequency per population and per locus corrected by sample size

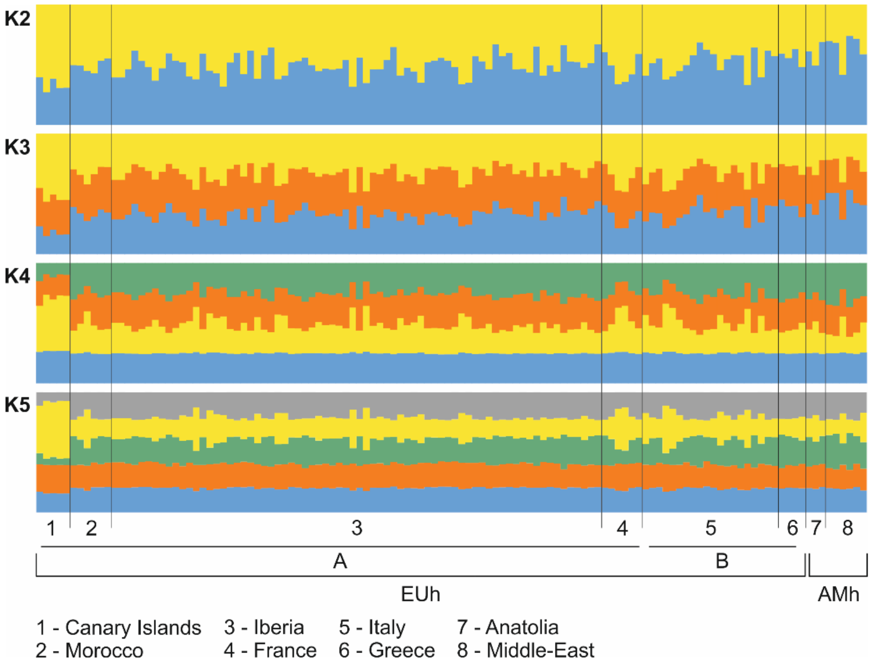

Figure S3.5 – *Tadarida teniotis* population structure based on the microsatellite data set. (A) Individual membership assignment plots resulting from STRUCTURE analysis without prior population information including all samples (K = 2 to K = 5).

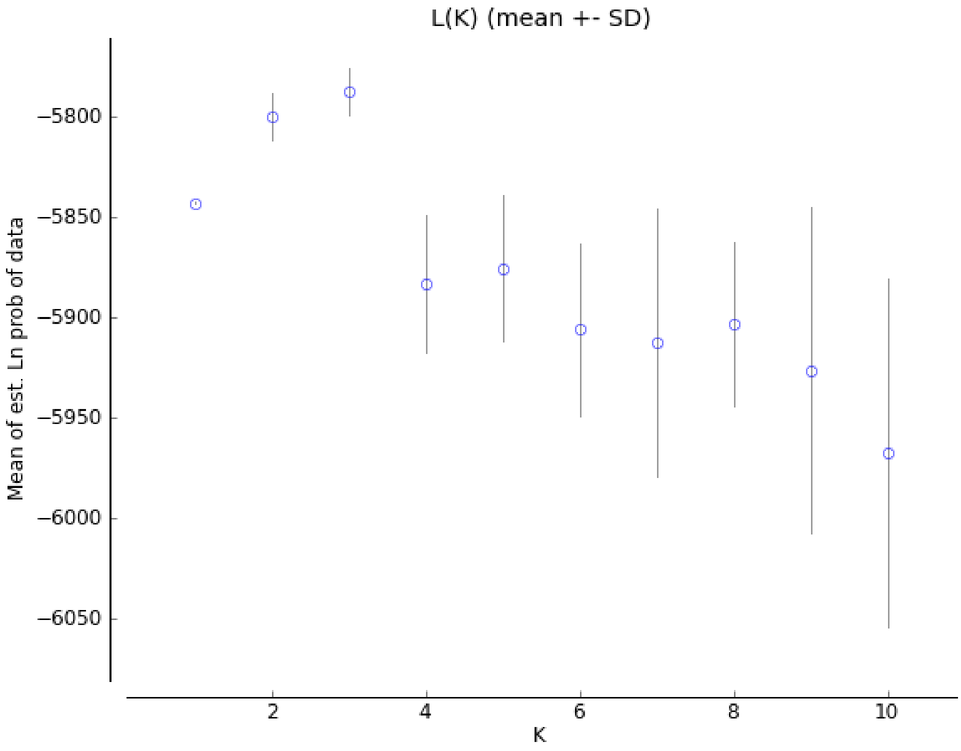

Figure S3.6 – Results of the STRUCTURE analysis for the *Tadarida teniotis* microsatellite dataset using a prior for population information. Log-likelihood probability for each K from 1–10 clusters, showing means and standard deviations of the ten replicate runs for each number of clusters.

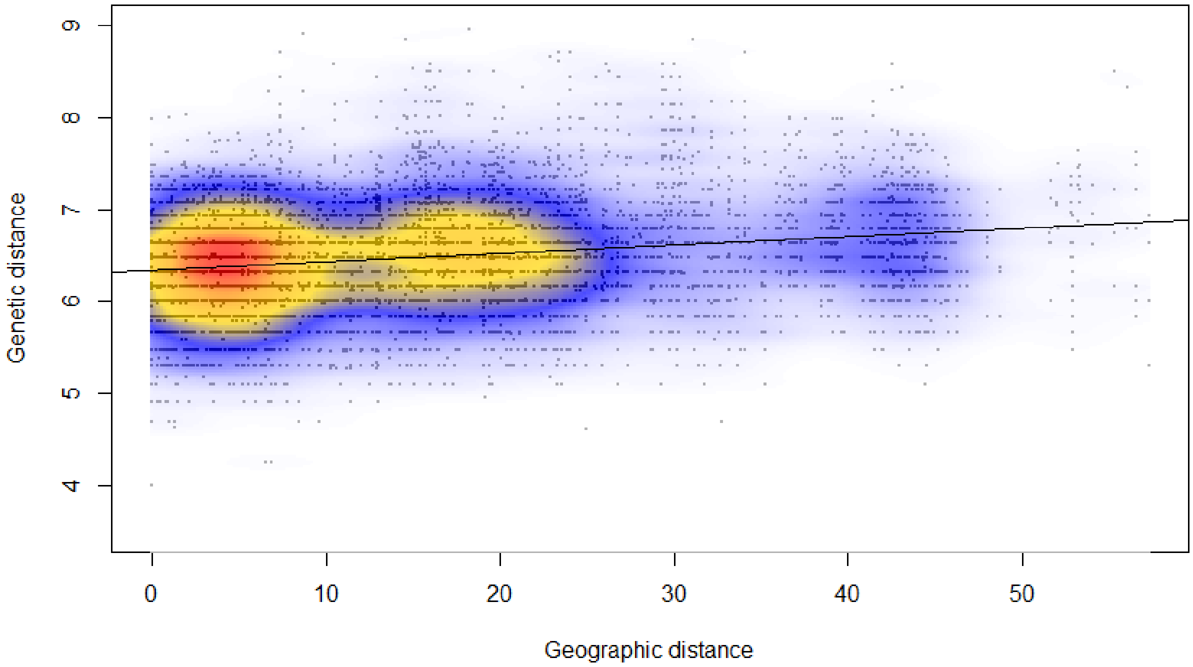

Figure S3.7 – Scatterplot of genetic and geographic distance. Colours represent points' density. The observed discontinuities in the cloud of points suggest a pattern of IBD, confirmed by the Monte-Carlo test with 1000 permutations (p = 0.001).

Chapter 4

Rapid changes in bat demography and social structure after river valley flooding by a large hydroelectric dam

Francisco Amorim, Ricardo Pita, Vanessa A. Mata, Pedro Beja and Hugo Rebelo

Amorim F., Pita R., Mata V.A., Beja P., & Rebelo H. Rapid changes in bat demography and social structure following large-scale habitat disturbance: the case of hydroelectric dam flooding. *Paper in preparation*

Keywords: breeding roosts, capture-mark-recapture, population size, roost loss, survival, *Tadarida teniotis*,

Abstract

Growing energy demands and the mitigation of global climate change is leading to a worldwide increase in investment towards ‘clean’ energy, particularly hydropower generation. The negative consequences of dams on aquatic species have been widely documented, but much less is known about impacts on terrestrial species. In particular, it is poorly known how habitat loss caused by flooding of river valleys affects population processes at the landscape scale. Here we investigate the impacts of a new hydroelectric dam on demographic parameters and social structure of the European free-tailed bat (*Tadarida teniotis*). We monitored free-tailed bat roosts at three road bridges crossing the main river valley and at two bridges farther from the dam, both before, during and after habitat flooding. We captured and marked (PIT tags) 3821 individuals, of which 1407 were genotyped for 14 microsatellite markers. Capture-Mark-Recapture models revealed an overall increase in the number of bats using bridge roosts after dam filling, but no changes in survival rates. Movement probability among bridges was low suggesting high roost fidelity. Genetic analysis revealed a decrease in relatedness at breeding roosts located over the flooded area, but not on the two bridge roosts far from the reservoir. Taken together, these results suggest that many individuals abandoned the river valley after flooding, possibly due to the loss of roosting and/or foraging habitats. The concentration of an unusually large number of individuals at safe nearby roosts appeared to have caused social disruption, recommending that long-term effects on breeding output and population size should be further investigated. By measuring the immediate impact of disturbance events on species demography and social structure, we provide a baseline for effective mitigation measures and management actions.

4.1 Introduction

In a world with increasing human population and consumption per capita, the need to meet growing energy demands while trying to tackle climate change is leading nations worldwide to promote renewable energy sources, in particular hydropower (REN21, 2017). Among renewable energy sources, hydropower has the most serious impacts on biodiversity and ecosystems, particularly where it affects biodiversity hotspots (Winemiller et al., 2016; Gasparatos et al., 2017; Gibson et al., 2017). Thus, understanding how hydropower facilities affect biodiversity is crucial to inform managers and practitioners on whether such facilities should be implemented at a particular site, on less damaging alternative locations, and on the best management actions and mitigation measures in future projects (Gibson et al., 2017).

While there is extensive bibliography on the effects of dams on freshwater biodiversity (i.e. Graf, 1999; Johnson et al., 2008; Liermann et al., 2012), few studies have assessed their impacts on terrestrial vertebrates (Gibson et al., 2017), and those who have done so are mostly focused on reservoir land-bridge islands (for a compreensive review see Jones et al., 2016). One of the most relevant impacts on terrestrial species caused by hydropower is the permanent habitat loss due to flooding of upstream terrestrial and riparian areas that are used by countless species (Naiman & Decamps, 1997). The sudden and extensive habitat loss can severely affect species composition and abundance at a local or even regional scale (Nilsson & Berggren, 2000). For instance, habitat loss due to a large hydroelectric dam located in southern Portugal caused a reduction in the regional distributions of the European wildcat (*Felis silvestris*) and European polecat (*Mustela putorius*), although the European otter (*Lutra lutra*) expanded its range (Santos et al., 2008). Another study found that bird communities surrounding a hydroelectric reservoir in Thailand were dominated by generalist and disturbance-resistant species, while species intolerant to disturbance were rare or missing (Irving et al., 2018). Despite the undeniable value of these studies, interpreting low abundance as a metric of sensitivity to a pressure may be misleading, especially for highly mobile animals like bats and birds (Henry et al., 2007). Ultimately, it is the specific demographic processes involved in their population dynamics and trajectories of change that may drive species long-term population viability (Lampila et al., 2005; Selwood et al., 2015).

Bats are flying predators with high mobility, and are acknowledged as excellent indicators of environmental change due to their fast response to disturbance and ecological changes (Jones et al., 2009; Bader et al., 2015). For instance, several studies have shown that climatic extreme events have immediate effects on bat demographic parameters, such as reproductive success (Rhodes, 2007; Amorim et al., 2015). Thus, bats can be particularly suitable to understand how sudden habitat alteration can influence species persistence in a given area (Bobrowiec & Tavares, 2017). Large-scale habitat changes stemmed from impoundment reservoirs are known to potentially affect the survival of bat populations through the permanent loss of roosts and foraging areas (Cosson et al., 1999). In a large hydropower reservoir in southern Portugal, Rebelo & Rainho (2009) found a clear decline in bat activity over the submerged area and an increase on the activity of disturbance-resistant species in the area surrounding the reservoir. Their findings suggest that bats were affected simultaneously by the extensive loss of habitat and roosts. Although roost loss has been previously identified as one of the major impacts of dams on bats, the direct effects on species demography and social structure have never been assessed.

Roost loss may result in increased energetic costs associated with finding new roosts (Arnold, 2007), and force the use of poorer quality roosts (Sedgeley, 2001; Sedgeley & O'Donnell, 2004), or roosts already occupied, potentially increasing resource competition and species movements within the matrix (Rebelo & Rainho, 2009). Consequently, roost loss can impact species demographic parameters, by decreasing survival, reproductive success, and population size (Brigham & Fenton, 1986a; Sedgeley, 2001; Sedgeley & O'Donnell, 2004) but also by disrupting social structure (Silvis et al., 2016). Species vulnerability to roost loss may vary according to dispersal abilities or even the type of roost they use (e.g. underground, trees, crevice). For cave-dwellers living in landscapes where underground roosts are rare, roost loss can lead to local or even regional extinction (Sagot & Chaverri, 2015). On the other hand, species using ephemeral roosts (e.g. furled leaves, trees, or snags) show higher resilience to roost loss, although this will depend on the extent of roost loss in a given area (Silvis et al., 2016). For hydroelectric dams located in mountain regions with deep valleys, upstream rising water levels can lead to a dramatic loss of crevice roosts that are usually clustered along rocky areas. Although there is very little information on roosting behaviour of crevice-dwellers species, we would expect them to maintain an array of suitable roosts and switch roost within a given area, similarly to tree-dwellers (Willis & Brigham, 2004; Russo et al., 2005; Reckardt & Kerth, 2007). However, the impact on roost switching may be influenced by roost availability, and the stability of the social groups (Silvis et al., 2016). Species living in colonies may benefit from improved foraging efficiency and reduced predation, but this will in part depend on their social stability (Altringham & Senior, 2006). Because both roost fidelity and sociality through cooperative behaviour are frequently linked to breeding (Lewis, 1995; Kerth et al., 2011), social stability is likely to be greater at breeding colonies and among adult females. In theory these colonies should show higher resistance to roost switching (Silvis et al., 2016), but events such as roost loss, forcing individuals to move to new roosts, are likely to disrupt the social structure on both the colonies that are subject to change and the ones they are moving to.

One of the most important variables that influences associative behaviour in some animal societies is genetic relatedness, with individuals preferentially associating with relatives (Silk, 2002). The relationship between genetic relatedness and social association is widespread in bats, although the strength of the association seems to depend on the degree of roost switching, with species in which individuals change roosts frequently exhibiting higher levels of association among relatives (Wilkinson et al., 2019)

In this study, we evaluated the short-term demographic and social responses of a bat species to the flooding of a river valley by a hydroelectric dam. We focused on the Baixo Sabor Hydroelectric Infrastructure (NE Portugal), which flooded about 39 km of a river valley, destroying a large extent of natural and semi-natural habitats such as riparian galleries, and Mediterranean forests and shrublands (Santos et al., 2017). Previous studies showed that this valley was important for the local bat community (Amorim et al. 2018), and that there was a particularly large population of European free-tailed bat (*Tadarida teniotis*) roosting in bridges crossing or near the main river valley (Amorim et al., 2013, 2015). This population was monitored before (2012 and 2013), during (2014 and 2015) and after (2016) the deforestation and flooding of the valley, providing an excellent opportunity to assess how large-scale habitat disturbance caused by hydroelectric infrastructures impacts on bat populations. The European free-tailed bat was considered particularly suitable for this study, because although it is generally considered a habitat generalist (Russo & Jones, 2003; Mata et al., 2016), it roosts in rock crevices such as those found along the Baixo Sabor river valley. Therefore, we hypothesised that flooding will cause many individuals to abandon the river valley, eventually converging to nearby safe roosts, thus affecting their population demography and social structure. Specifically, we predicted that: i) the number of individuals at bridge roosts would increase, reflecting the arrival of individuals from flooded rock crevice roosts; ii) individual survival would decrease, due to lower resource availability (roosts and foraging areas); iii) social structure among females (measured as genetic relatedness) would decrease because of a higher number of females arriving at the roosts. Finally, we expected the magnitude of these effects to be different for roosts within and outside the flooded area, as well as for breeding and non-breeding roosts.

4.2 Materials and Methods

4.2.1 **Study area**

The study was carried out in northeast Portugal (41º 21’ 0’’ N, 6º 58' 0’’ W), within the Baixo Sabor Long Term Ecological Research Site (LTER_EU_PT_002) and the Natura 2000 site of Sabor-Maçãs site (PTCON0021) (Figure 4.1). The region is in the transition between meso- and supra-Mediterranean bioclimatic zones, with cold winters (average temperature of the coldest month <6ºC) and dry summers (total annual precipitation <600 mm, of which <5% in July–August), which are particularly hot in some valleys where monthly average temperatures exceed 21ºC (Monteiro-Henriques, 2010). The landscape is characterised by plateaus at about 700–800 m a.s.l., dissected by deep and narrow streams valleys. Land cover is dominated by a complex mosaic of natural vegetation patches, forest stands (mainly maritime pine *Pinus pinaster* plantations), permanent crops (mainly olive and almond groves), and pastures, which reflect a process of progressive agricultural land abandonment since the 1960s (Hoelzer, 2003). Natural vegetation is largely composed of shrublands of variable structure and species composition, remnants of native evergreen oak woodlands, and some well-developed riparian galleries (*Quercus suber*, *Q. rotundifolia*) (Hoelzer, 2003). The study area was impacted by the construction of the Baixo Sabor hydroelectric infrastructure (2009-2013), and the subsequent flooding of the river valley, which started in the winter of 2014. The infrastructure includes a primary and a secondary reservoir, flooding a total length of the main river and its' tributaries of about 60 km and an area of approximately 3,000 ha (Santos et al., 2017). In 2014, before dam filling, all woody vegetation (shrublands, orchards, forests) was removed to mitigate eventual eutrophication problems and enhance water quality in the reservoir.

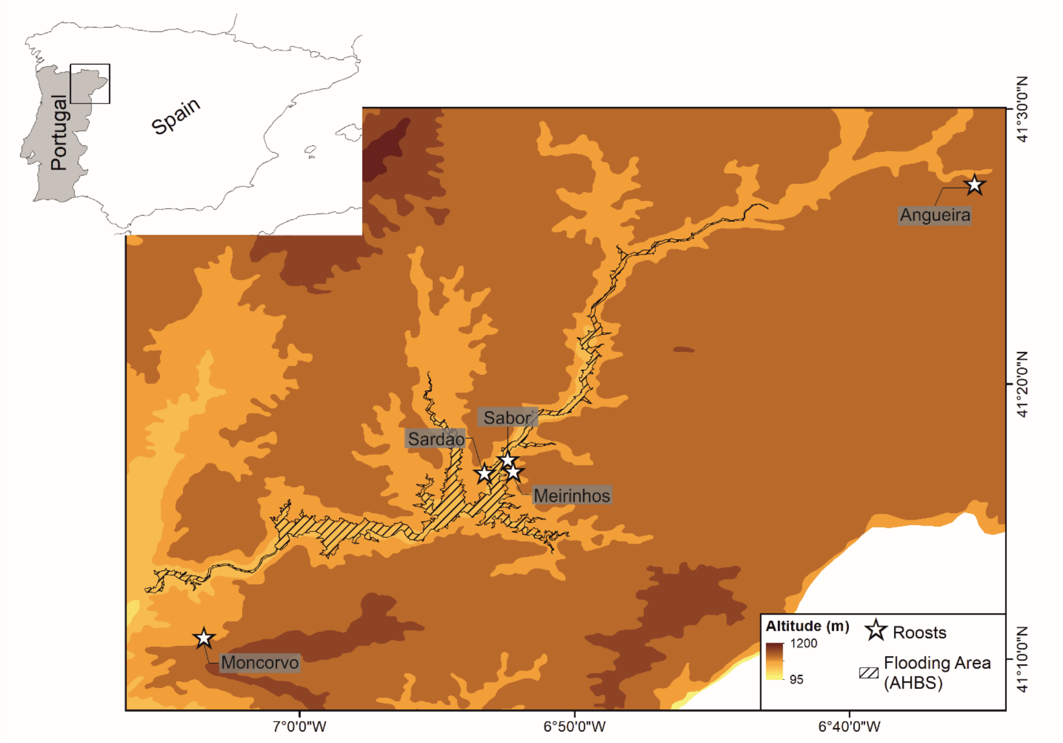

Figure 4.1 – Location of *Tadarida teniotis* bridge roosts in North-eastern Portugal sampled in 2012-2016. Flooding area of the AHBS is also shown (line filled). Safe roosts located at the Impact Area: Meirinhos, Sabor and Sardao; Roosts at the Control area: Angueira and Moncorvo.

4.2.2 Sampling

Sampling focused on *T. teniotis* roosts found in three bridges located over the hydroelectric reservoir but safe from flooding (Meirinhos, Sabor and Sardao, hereafter roosts in the impact area) and two bridges nearby (Angueira and Moncorvo, hereafter roosts in the control area) (Figure 4.1). We focused on bridges rather than on natural crevices in cliffs both because they provided a setting where it was feasible to capture a large number of individuals in a relatively short time, and because they were unaffected by the flooding of the river valley. The bridges offer several roosting possibilities for bats, mainly crevices of different types, but also box girders that mimic underground roosts (Amorim et al., 2013). All the bridges are of modern construction (the oldest was built in 1992) and are part of the Portuguese main road network. The length of the bridges sampled ranged from 60-600m, while height ranged from 10-110m.

The study was based on monthly live-trapping of *T. teniotis* at each roost covering bats active season, from May to October, during five years (2012 to 2016), totalling 138 trapping nights. Captures were carried out from dusk to dawn using a trap designed by ourselves that allows maximizing capturing efficiency of bats roosting in bridges. The trap is easily assembled by two people and consists of a steel frame for mist nets that can be secured to the bridge rails or walls (Amorim & Rebelo, 2011). Bats were captured in different places (sections) within each bridge and, to avoid excessive disturbance of the individuals, the minimum time interval between captures in the same section was two months. This interval was determined based on the experience gathered from 2011 to 2012. For a detailed sampling scheme in the five bridges, see Figure S4.1 in Supporting Information. Sampling effort, measured as the total mist-net area, varied between occasions (164.97 ± 49.96 m^2, 62.4 – 374.4 m^2). Total section length, thus mist-net length, could vary to avoid for example capturing colonies of other species, or to capture in areas of the bridges where *T. teniotis* were absent. Although effort by section was approximately constant, in some cases we had to change to mist-nets of different length due to poor mist-net condition (details on sampling effort in Supporting Information Table S4.1).

Captured bats were kept in individual cotton bags until they were processed. Individuals were marked using Passive Integrated Transponder tags (PIT tags), specifically the High Performance Tag, 9mm FDX-B 134.2 kHz (HTP9, BIOMARK). Each PIT tag has a unique ID, and marked individuals were identified using a BIOMARK 601 handled reader. For each specimen we collected a tissue sample from the wing membrane using a 3 mm biopsy punch (BP-30F, KAI MEDICAL). PIT tagging and tissue sampling followed protocols described by Kunz & Parsons (2009).

For each individual we determined the sex, age and reproductive status following Kunz & Parsons (2009). Pregnancy was assessed by palpation, distention and size of the abdomen, though early pregnancy was difficult to diagnose. Evidence for lactation included the presence of milk and the enlargement of nipples. Juveniles were identified through the presence of unfused epiphyses. All individuals captured before September were considered adults since young of the year were only observed from that month onwards, and individuals born in the previous year (sub-adults) were no longer distinguishable (Amorim et al., 2015). In October, some individuals could no longer be classified as juveniles solely by epiphyses observation, and so in young males we used as additional characteristics the presence of a small non-secreting gular gland and the smaller size of testes (Kunz & Parsons, 2009; Amorim et al., 2015). During this month juvenile females were easily distinguishable by their small nipples and smaller size (Amorim et al., 2015).

4.2.3 **Capture Mark Recapture Models**

Population estimates

We estimated the number of adult bats occupying the five bridge roosts each year using Huggins closed population models (Huggins, 1991) implemented in the ‘RMark’ R package (Laake, 2013). Closed population models assume that no birth, death, immigration or emigration are observed during the sample period. To control for the birth effect we excluded all juveniles of the year from the analysis. Regarding mortality, we assumed that the probability of death of individuals was negligible within each sampling year, given the high longevity of bats (Wilkinson & South, 2002), including *T. teniotis* (Ibañez & Pérez-Jordá, 1998),. Finally, capture data suggested that individuals have strong roost fidelity and even if they use satellite roosts the sampling effort within a period of interest (year) allowed us to avoid missing those individuals. Similar to other studies estimating bats population size using closed population estimates (Vonhof & Fenton, 2004; Puechmaille & Petit, 2007), we then considered that the implied demographic closure among adults within each year could be reasonably assumed. Nonetheless, the geographical closure assumption could not be assured, due to the expected income of individuals from inundated roosts. We therefore assumed our population estimates referred to a ‘super-population’ of individuals associated to the sampled bridge roots during each particular year (e.g. McClintock, 2018). The Huggins estimator was selected because it allows capture probability to be a function of both individual (e.g. sex, reproductive status) and time varying covariates (e.g. sampling effort), thus allowing more robust estimates than other closed population models also assuming heterogeneity of capture probability (Boulanger & McLellan, 2001).

We evaluated model fit using the Akaike Information Criterion adjusted for low sample sizes (AICc). The model with the lowest AICc was considered to be the most supported by the data (Burnham & Anderson, 2002). Changes in AICc (DAICc) were also used to assess the fit of different models, and all models with DAICc less than 2 were considered for population estimation. To account for model uncertainty we estimated population size and the associated variance using the model averaging procedure in 'RMark' and considering the previously identified models. For the average model we computed log-based corrected CIs using the unconditional SE from model averaged estimates following Williams et al. (2002).

Annual survival and inter-bridge movement

We used multistate open Cormack-Jolly-Seber (CJS) capture-recapture models to estimate annual recapture probability (p), survival (S), and movement probability (Psi) via maximum likelihood methods (Lebreton et al., 1992; Nichols & Kendall, 1995; Lebreton & Cefe, 2002), implemented in 'marked' R package (Laake, 2013). Since we were interested in inter-annual differences in demographic parameters, we collapsed individual capture histories within each year into a single capture-recapture occasion, as required for CJS-type studies (Schwarz & Arnason, 2018). We considered the bridges where bats were sampled as five different states, with transitions among states representing the movements among bridges. Those individuals that were captured in more than one year were considered 'Residents', while individuals captured in only one year were considered 'Transients'.

We first tested the goodness-of-fit (GOF) of a general multistate CJS model, here the Jolly MoVe (JMV) model, assuming all parameters being time and state dependent (Brownie et al., 1993; Pradel et al., 2003). The GOF tests were performed using the U-CARE v2.3.4 (Choquet et al., 2009), which incorporates tests for transience effects (test 3G.SR), memory effects (test WBWA), and trap-dependence effects (test M.ITEC) (Pradel et al., 2003). The overall GOF test indicated a significant lack of fit of the data to the JMV model (χ^2=142.1, df=56; p<0.01). When examining the GOF components separately, although test WBWA (memory) was non-significant (χ^2=5.5, df=4; p=0.243), the data failed both test 3G.SR (transience) and test M.ITEC (trap-dependence), with most of the overdispersion attributed to the presence of transients (3G.SR: χ^2=94.2, df=15; p<0.01; M.ITEC: χ^2=30.5, df=9; p<0.01). This indicates that a large proportion of marked bats within a year were never captured again in the following years, and that recapture probability of an individual depended on whether or not it has been caught in the previous year. After removing the first encounter and thus eliminating all potential transients, the GOF based on overall and subcomponent tests suggested adequate fit (overall test: χ^2=20.8, df=17; p=0.234), corresponding to an acceptable variance inflation factor c-hat=1.22. We therefore fitted models incorporating the presence of transients, and corrected for the remaining overdispersion by scaling models' deviances with the estimated variance inflation factor (Burnham & Anderson, 2002). Transience effects were included by considering two age classes for survival, with individuals that were captured only once being part of the first age class (Transients), and all the others being part of the second (Residents). Survival in the multistate CJS models with transience effects is therefore a weighted average between the survival of transient bats, equal to zero by definition, and the survival of resident bats (Pradel et al., 1997).

Starting from a multistate CJS model including only the transience effects on survival [p(.),S(trans),Psi(.)], our model building and selection procedure followed a 3-step hierarchical approach based on quasi-Akaike Information Criterion corrected for small sample sizes (QAICc, Burnham & Anderson, 2002). This consisted in first building several multisate CJS models including all combinations of relevant main effects on recapture probability (i.e. covariates that when included alone in the submodel of recapture probability resulted in a lower QAICc's relative to our starting model), while keeping only the transience effects on survival and no covariates on movement probability. Tested covariates of recapture probability included the sampling effort (given as the sum of mist net area per bridge and year), time, sex, overall population size (provided by population estimates using Huggins models), and bridge (Table 4.1). In the next step, we used the best model explaining recapture probabilities, and built another set of models including all possible combinations of relevant main effects on survival (i.e. covariates that when included alone in the submodel of survival adjusted for transience resulted in a lower QAICc relative to the best model found in the previous step), while keeping movement probability constant. Variables considered in survival analyses included time (interval between years), sex, overall population size, and bridge (Table 4.1). Finally, using the best model structure found for both recapture probability and survival, we build a further set of models including all possible combinations of relevant main effects on movement probability (i.e. those that when included alone in the submodel of movement probability resulted in a lower QAICc relative to the best model fond in the previous step). Predictors of inter-bridge movement probability considered included time, sex, overall population size, bridges, and distance among bridges (Table 4.1).

Table 4.1 – Covariates initially considered for each parameter of the multistate CJS models (recapture probability, *p*; survival, *S*; and inter-bridge movement probability, *Psi*) with transience effects on survival, and description of associated hypotheses

Covariate	**Parameters**			**Description/hypothesis**
	p	***S***	***Psi***	
Time	X	X	X	Parameters are time-dependent
Effort (/time/stratum)	X			*p* varies with the sampling effort employed
Sex	X	X	X	Parameters differ between sexes
Stratum (bridge)	X	X	X	Parameters differ according to the bridge
Population size/time	X	X	X	Population size increases *p* and *Psi*, and decreases *S*
Inter-bridge distance			X	*Psi* decreases with the distance among bridges

4.2.4 **Molecular data and relatedness analysis**

We used DNA Micro Kits (QIAGEN) following the manufacturer’s instructions for DNA extraction methods from tissue samples. In total 1407 individuals were genotyped at 14 polymorphic microsatellite loci specifically designed and optimized for *T.* teniotis by Amorim et al. (*in press*). Laboratory protocol followed Amorim et al. (*in press*), and is presented in Methods S1 in Supporting Information.

After genotyping, we performed relatedness analysis to assess differences in social structure between years and within each roosts. Relatedness analyses were done using COANCESTRY software (Wang, 2011) and ‘related’ R package (Pew et al., 2015). We first evaluated the performance of four different estimators (Lynch and Li, Lynch and Ritland, Queller and Goodnight and Wang) to select the one that best represented our dataset. This was done by simulating a specified number of individuals (N = 500) of known relatedness, based on observed allele frequencies, and estimating relatedness using the four estimators. Finally, we compared the values obtained for each known relatedness value and accessed the correlation coefficients between observed and expected relatedness. The Wang estimator (Wang, 2002) showed the best performance for our study system and data with the highest correlation values between observed and expected values and the best fit for simulated data sets (Figure S4.2 in Supporting Information). Using this estimator, we then analysed relatedness between groups, with groups defined as individuals captured within a given year and individuals captured in the remaining years. This was done in COANCESTRY by comparing the observed mean relatedness difference between both groups with that of 1000 bootstrapping and considering 5 % confidence limits to ascertain significant differences.

4.3 Results

4.3.1 **Overall patterns**

Adult bats represented 68.4 % of the total number of captures. Juveniles were found from late August to October, representing 13.3 % of the captures. Age could not be assigned to 18.3% of individuals, though most of them were likely sub adults (individuals born in the previous year). Females represented 71.6 % of the adult individuals, and 52.3 % of the juveniles. Considering adult females, 43.8 % were reproductively, but their proportion varied widely across bridges from 22.9% (Sardão) to 55.2% (Meirinhos) (Figure S4.3 in Supporting Information). The roosts with the highest proportion of reproductive females also had the highest recapture rates, which increased over time (Figure S4.4 in Supporting Information).

Recaptures (n = 1173) represented 29.7 % of the total number of individuals captured, of which 69.2% were females. Among recaptured adult females, most of them were reproductively active (62.7%). The proportion of recaptured individuals increased over time (R = 0.776, p = 0.002), up to about 50% at the end of the study (Figure S4.5 in Supporting Information). Transients included 24.4 % juveniles, 20.3% males, 17.8 % reproductive females and 41.9 % other adult females (either non-reproductive or with unknown status). Residents included 45.8 % reproductive females, 25.4 % males, only 2.4 % juveniles and 26.4 % of other adult females. Reproductive females represented 63.5 % of Resident adult females but only 32.2 % of Transients. Inter bridge movements were only detected in 3.6 % of the recaptures, while shifts among roost locations within a bridge were detected in 14.6 % of the recaptures. Among moving individuals, the proportion of males was slightly higher than that of the overall proportion of males, particularly for intra bridge movements.

4.3.2 **Capture Mark Recapture Models**

Population estimates

There were striking inter-annual differences in population size estimates, with one marked peak in 2015, after the start of dam filling, and a smaller one in 2012, while the remaining years had similar values (Figure 4.2). Confidence limits were narrow, suggesting robust estimates (details in Table S4.3 in Supporting Information). Females accounted for 73-76% of the population estimates, with an even higher percentage in 2016 (84%).

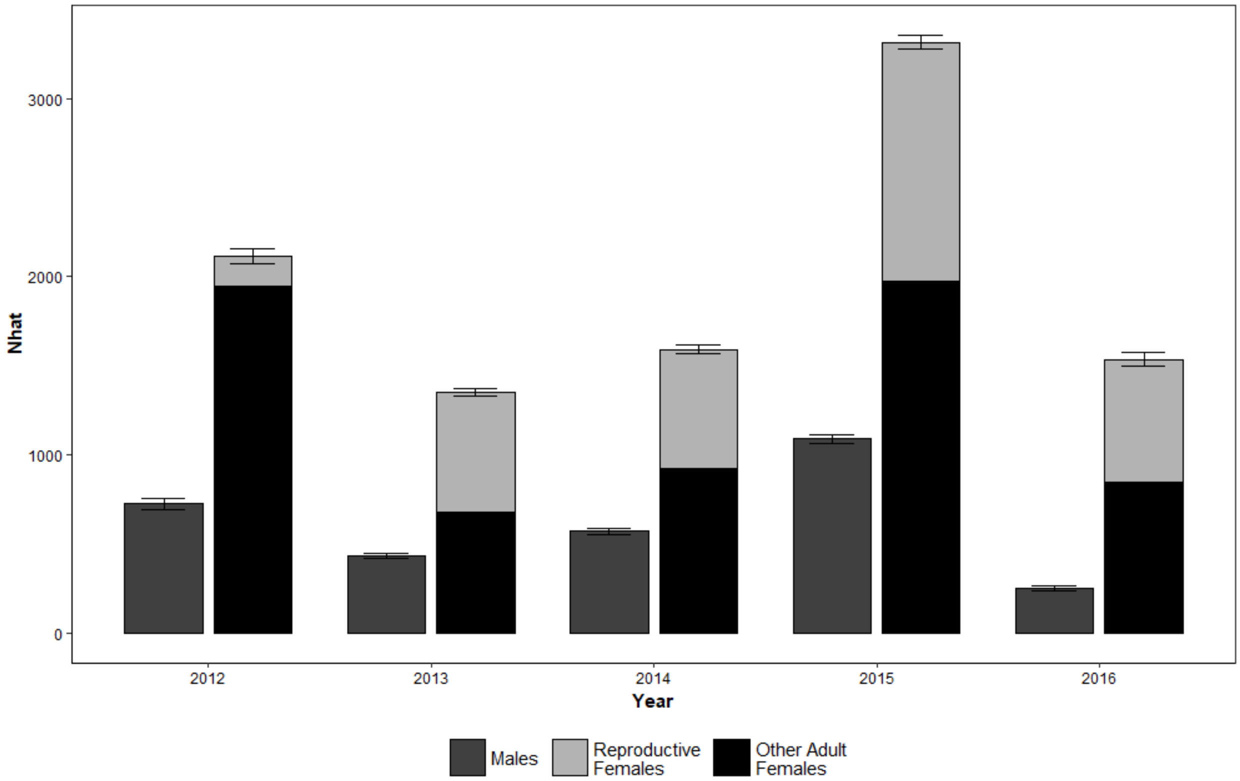

Figure 4.2 – Male and females population sizes (± 95% Confidence Interval) estimated using Huggins closed population models. Females were split into reproductive and non-reproductive according to the observed proportion in the capture history.

The best models always included the effect of either time (sampling occasion) or sex, except in 2015 when sampling effort replaced the time variable (Table S4.4 in Supporting Information). In 2012, capture was higher in July and August when there was a strong positive effect of time (time3 and time4, Table S4.5 and Figure S4.6 in Supporting Information). Although sex had a small effect, capture probability was lower for males than females. Conversely, in 2013, sex had no effect on capture probability and there was a general pattern of decreased capture probability throughout the year. In 2014, there was higher capture probability in July (time4 Table S4.5 and Figure S4.6 in Supporting Information), while sex had no effect on capture probability. In 2015, sampling effort had a weak positive effect on recapture probability, while male recapture probability was higher in this year. Finally, in 2016 male recapture probability was significantly higher and increased throughout the year (Table S4.5 and Figure S4.6 in Supporting Information).

Survival and movement probability

In models assuming transience effects on survival (*S*) and constant state transition probability (*Psi*), candidate variables for explaining the recapture probability (*p*) were time, effort, stratum (bridge) and overall population size each year (Table S4.6 in Supporting

Information). Considering all possible combinations of main effects on *p* (15 models), the best supported model included time, effort and stratum effects, with an AICc model weight of 0.54 (Table S4.7 in Supporting Information). When assuming these effects on *p*, together with constant *Psi*, candidate variables for explaining *S* in addition to transience effects, were time and overall population size (OPopS) (Table S4.6 in Supporting Information). Considering all possible combinations of main effects on *S* (3 models), the best supported model included OPopS effects (in addition to transience effects), with a QAICc model weight of 0.81 (Table S4.7 in Supporting Information). Considering the effects of time, effort and bridge on *p*, and the effects of transients and overall population size on *S*, candidate variables for explaining *Psi* were the bridge and the distance among bridges (Table S4.6 in Supporting Information), resulting in 3 candidate models for explaining inter-bridge movement. QAICc-based model comparison indicated that *Psi* depended on both the bridge of departure and the distance to other bridges, with an overall QAICc model weight of 0.72 (Table S4.7 in Supporting Information). However, the model including together the effects of bridge and distance produced very wide confidence intervals in *Psi* estimates (Table S4.8 in Supporting Information), suggesting problems of model over-fitting. Finally, the model including time, effort and bridge effects on *p*, transience and overall population size effects (OPopS) on *S*, and distance effects on *Psi* [*p(time,effort,stratum), S(trans, OPopS), Psi(dist)*] was taken as the best model explaining the bat demography in our study.

According to our best model, recapture probability (*p*) was time- and bridge-specific, increasing with increasing sampling effort, and being lower in the Sabor and Sardão roosts. State transition probability (*Psi*), representing movement among bridges, decreased with increasing inter-bridge distance. Finally, survival (*S*) was lower for Transients, decreasing with increasing overall population size, and was unaffected by year (Figure 4.3).

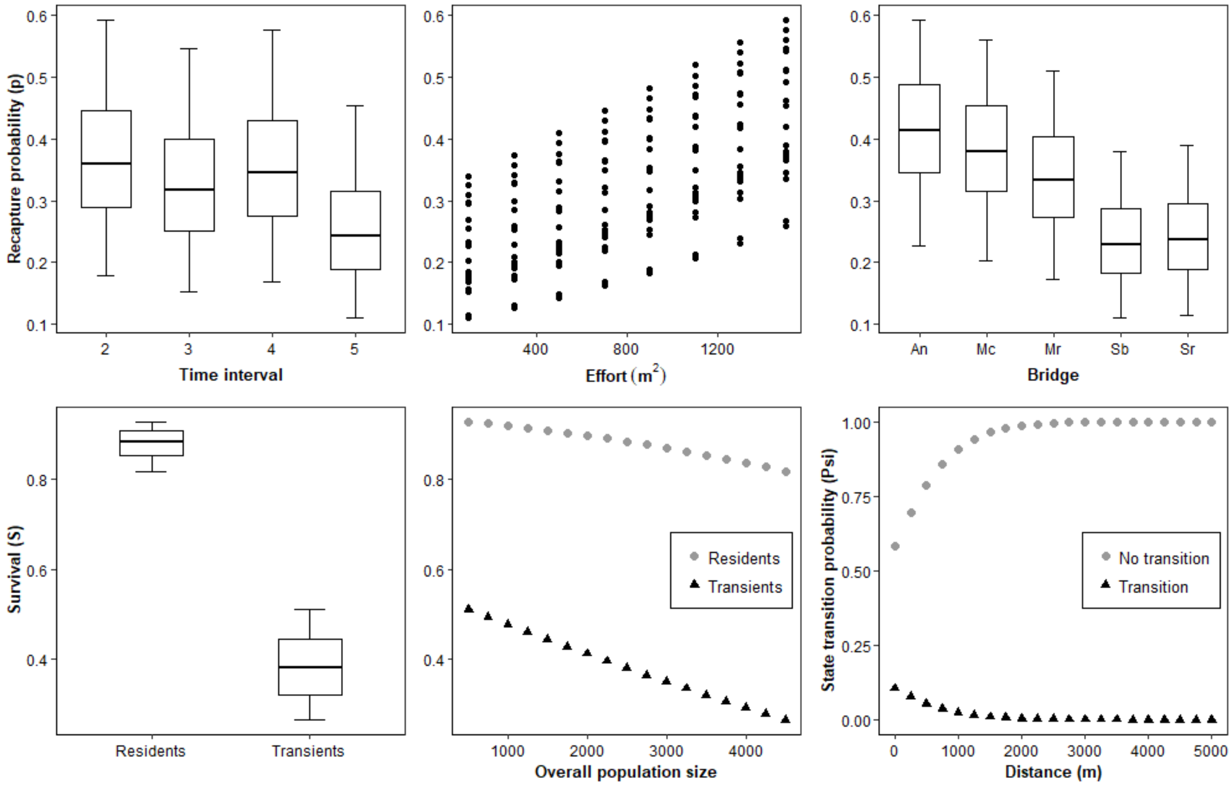

Figure 4.3 – Predicted values according to the best CJS model. for Recapture probability (*p*), Survival (*S*), and State transition probability (*Psi*). Predictors as follow: (*p*) time, effort and bridge; (*S*) transience effects and overall population size (OPopS); (*Psi*) distance between bridges.

4.3.3 Relatedness

When comparing within year relatedness with overall relatedness at each bridge, the general pattern suggests similar or higher relatedness between individuals captured in the same year (Figure 4.4 and Figure S4.7 in Supporting Information). However, the Meirinhos breeding roost (Impact area) had lower than expected relatedness after flooding, while breeding roosts in the control area (Angueira and Moncorvo) did not show any signs of lower relatedness (Figure 4.4 and Figure S4.7 in Supporting Information). In 2012, both bridges at the Impact area (Meirinhos and Sardao) and at the Control area (Angueira) showed significantly lower relatedness (Figure 4.4 and Figure S4.7 in Supporting Information). Sardão and Sabor bridges tended to show less consistent patterns, although observed relatedness was normally within the 95 % CI for expected values (Figure S4.7 in Supporting Information).

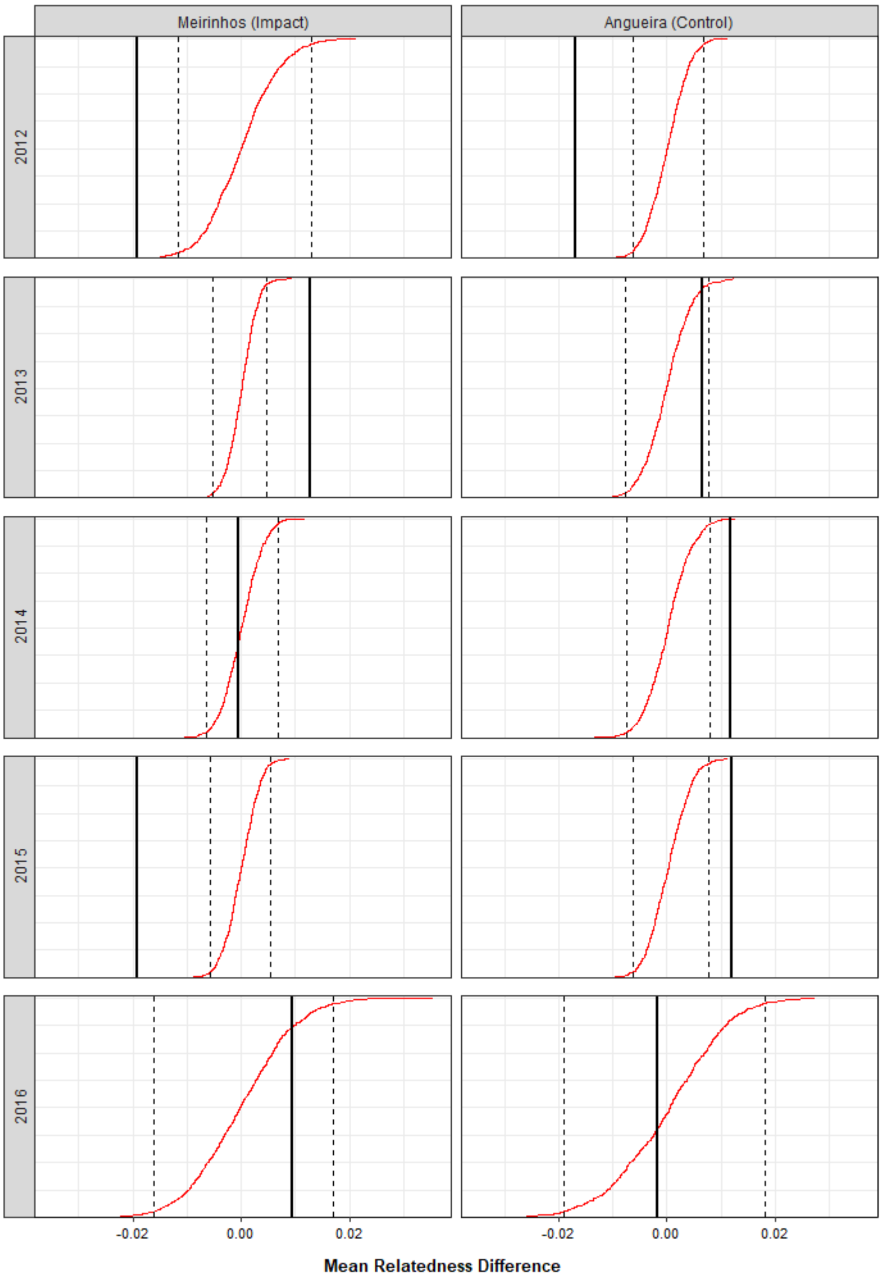

Figure 4.4 – Between group relatedness considering yearly observed relatedness comparing to the remaining years. Black line represents the mean observed relatedness difference between groups, redline and dashed lines represent respectively the cumulative frequency of the same difference and the 5 % confidence limits based on 1000 bootstrapping. Vertical axis ranges (0 – 1) and represents the Cumulative frequency of observations. Plots for the two most important breeding sites at Meirinhos (Impact area) and at the Angueira (Control area). For the remaining roosts, see Figure S4.7 in Supporting Information.

4.4 Discussion

To the best of our knowledge, this is the first study estimating the consequences of landscape alteration caused by reservoirs on the social structure and demography of wild terrestrial animals. Specifically, our study focusing on the bat *T. teniotis* showed that shortly after deforestation and subsequent flooding of the river valley (2015) there was an increase in population size at nearby safe roosts unaffected by landscape disturbance. Relatedness among individuals declined in roosts closer to the flooded area, but not in roosts farther upstream. Despite these effects, apparent survival did not change over the study period. One year after flooding (2016), population estimates returned to numbers similar to 2013-2014, but relatedness in safe roosts located in the impact area was still lower than expected. Together these results suggest an influx and subsequent establishment of new individuals, probably due to the loss of roosting linked to the flooding event, which lead to short-term changes in *T. teniotis* populations at roosts located closer to the flooded area.

4.4.1 **Limitations and potential shortcomings**

Our study had some limitations and potential shortcomings, but we believe that they did not affect our key results in any significant way. First, bridges were very long and we were not able to sample all possible roosting area. Although this limits the information and conclusion in unsampled areas, because we focused our sampling on bridge sections showing high occupation rates, it is then safe to assume that we captured the general patterns of the population roosting in the bridges. For instance, our results clearly show an increase in population size after flooding and at the most, this will be an underestimation. Regarding the long-term impacts of the dam on the local populations of *T. teniotis,* we point out that we would need to sample more years after flooding. In spite of this, the robust and unique dataset allowed us to confidently measure the immediate effects of the flooding. Finally, small sample sizes can lead to biased relatedness estimates, although this is only truly critical when sample size is lower than 10 individuals (Wang, 2017). Such small sample sizes tend to result in an underestimation of relatedness and in such circumstances caution should be taken when interpreting the results. In this study, sample sizes were only lower than 10 individuals in two specific occasions at one of the bridges (Sabor), and could actually explain the somewhat unexpected result of lower relatedness observed in this bridge in 2016. However, Sabor was a non-breeding roost and had low contribution to our conclusions and overall patterns.

4.4.2 **Effects of flooding on demography**

The increase in population size estimated in 2015 was the likely result of the arrival of new individuals, due primarily to the almost certain loss of roosts after the flooding of crevices in cliffs and ravines. It cannot be ruled out, however, that these changes were at least partly due to the loss of foraging habitats, as this river valley was previously found to be important for foraging bats, particularly during the dry summer season (Amorim et al., 2018). Notwithstanding, loss of foraging habitats was likely to be less important for *T. teniotis*, because this is a fast and high-flying species covering long distances to forage (Marques et al., 2004, O'Mara, *in prep*). Furthermore, *T. teniotis* females are known to feed heavily on large moths that migrate at high altitude during the night (Mata et al., 2016), thus making them less dependent on local foraging habitats. Such behaviour suggests that *T. teniotis* may be less affected than other bat species by the loss of foraging habitats associated with river damming. However, because, previous studies suggest a vertical segregation between sexes, with males preying on species flying at lower altitudes (Mata et al., 2016), there might have been differential responses to habitat loss between sexes.

Considering the geographic proximity to the submerged area, it seems reasonable to assume that in 2015 the newly arrived individuals used the bridges as alternative roosting sites. Some bat species are known to discriminate between social calls of conspecifics when searching for new roosts (Schöner et al., 2010), meaning that these individuals could be initially attracted to nearby already occupied roosts, such as the bridges crossing the flooded area. This hypothesis is in line with the higher capture probability observed from June to August 2015. These months correspond to a peak in the aggregation of individuals, especially socially active breeding females (Amorim et al., 2015), increasing their attractiveness potential. Because our population size estimates refer to the 'super-population' of individuals associated to the sampled bridge roots during each year, it is difficult to explain the population declines in 2016 to numbers similar to those estimated for 2013 and 2014. However, it is possible that newcomers used the bridges as stepping stones, exploring them during a short period (< 1year), while trying to find a new roosting site (Rebelo & Rainho, 2009). Because there is a physical limit to the number of bats that can use these bridge roosts, some individuals might have been forced to move outside the study area, thus becoming inaccessible for capture in 2016. The lower recapture probability found in 2016 and the high apparent survival among residents further supports this hypothesis, and is in line with studies carried out in a range of species, which have shown that long-lived species maximize adult survival during stressful periods (Linden & Møller, 1989; Hanssen et al., 2005).

Despite the relatively short time period during which bat numbers increased at bridge roosts after the flooding, this increase may have negatively affected bats, as apparent survival tended to decrease with increasing population size. Additionally, other demographic consequences might also be at play. For instance, Borkin et al. (2011) suggested a link between colony size reduction and roost loss in long-tailed bats (*Chalinolobus tuberculatus*), while Brigham & Fenton (1986) found that big brown bats (*Eptesicus fuscus*) had reduced reproductive success if forced to move to an alternative roost. While we did expect a reduction on the overall proportion of reproductive females at roosts located at the impact area in 2015, this did not seem to be the case. Interestingly, the proportion of reproductive females at the

Sabor roost (Impact area) has even increased after flooding (2015 and 2016), an indication that not only this roost has suitable conditions for breeding, but also that the newly established individuals may have already bred there. Nonetheless, we cannot discard the possibility of a delayed effect of habitat flooding along the Baixo Sabor valley on the reproductive activity in bridge roosts. This idea is suggested by the decrease on the proportion of reproductive females observed at Meirinhos (Impact area) in 2016, which could reflect a cumulative regional negative effect of the dam on bat reproduction in colonies that have been already established in the region.

Although we do not have information prior to 2012, population size estimates were also higher in that year. The winter of 2011-2012 was extremely dry and *T. teniotis* failed to reproduce within the study area (Amorim et al., 2015). Reproductive females show specific roosting requirements, hence it is reasonable to assume that under stressful conditions non-reproductive females will show higher willingness to search and move to new roosts. In face of the dry conditions observed in 2012, individuals might have moved to roosts located along the valleys (including both rock and bridge cervices) where water availability is higher throughout the year (Amorim et al., 2018), leading to increased population estimates in that year. This hypothesis is also supported by the higher capture probability observed in July and August, which correspond to the driest summer months in our study area and is likely to reflect a higher number of individuals arriving to roosts located at the valleys.

4.4.3 **Effects of flooding on social structure**

As predicted, after 2015 the putative influx of newcomers influenced social structure at roosts located at the impact area, but not at bridges farther from the flooded area. In addition, after the dam filling, the effects on social structure were not similar between all three bridges at the impact area, suggesting a differential response according to usage type (breeding vs. non-breeding). Relatedness was significantly lower than expected by chance at the Meirinhos breeding roost after flooding (2015). The probability of individuals moving between bridges was very low and was not affected by sex. Together with the higher recapture probability at breeding roosts, this suggests that roost fidelity is likely to be stronger here than in non-breeding roosts. If breeding roosts have higher social stability, the influx of individuals should then be lower. As described above, individuals escaping inundated roosts might have been attracted to nearby roosts already occupied, but this does not necessarily mean they actually roosted there. On the other hand, not all roosts are suitable for breeding (Racey, 1982) and, if availability is limited, intraspecific competition is likely to arise with the newly arrived individuals moving, or forcing others to move, to roosts with less pressure. In the second year after flooding (2016) observed relatedness in roosts at the impact area increased again, reaching expected values at Meirinhos breeding roost, despite decreasing at Sabor. Data on the later suggests that prior to flooding this was not an important breeding site and that occupation was rather low. Overall, this would mean that if Sabor roost was under lower pressure, then a rearrangement of the colonies located in the impact area could lead to a larger number of individuals moving to this roost, hence contributing to the lower relatedness observed in 2016. Inconsistent patterns found in some roosts might also be the result of small sample size, although this was only truly critical at Sabor bridge in the years of 2012 and 2016 (Table S4.9 in Supporting Information), when sample size was lower than 10 individuals (Wang, 2017).

In 2012, relatedness was also lower than expected in most of the roosts. This may be a consequence of the severe drought in 2011-2012 (Amorim et al., 2015), and the possibility of individuals moving to roosts located near rivers. Interestingly, apart from Sabor (impact area), for which relatedness sample size in 2012 was very low (N = 7), only Moncorvo (control area) did not show signs of lower relatedness than expected in this year. Among the five roosts monitored, Moncorvo was the only one located in a valley with no water bodies underneath, hence less attractive in face of a drought event. Besides, it also shows some unique features, such as being close to a village, harbouring large *Pipistrellus* spp. colonies, and having much shorter bridge length and lower height.

4.4.4 **Conservation implications**

This study provides evidences that roost loss caused by a hydroelectric dam lead to immediate effects on the demography and social structure of the European free-tailed bat in safe roost over the area directly affected by landscape disturbance. Ultimately, the impacts of human mediated activities on a population arise from changes to the demographic parameters, and they should be investigated with long-term population monitoring, including mark-recapture studies designed to assess population turnover, immigration and reproductive activity (Henry et al., 2007). While our study provides valuable insights on the immediate social and demographic responses to rapid habitat disturbance caused by hydroelectric dam flooding, the long-term consequences for the persistence of local populations is uncertain. According to our results, it is possible that permanent roost loss caused by dam filling will translate in a decrease in the number of bats using this landscape. The limited carrying capacity of remaining roosts across the river valley to accommodate all the population formerly present might increase intraspecific competition, especially among reproductive females, since not all roosts are suitable for breeding. Recruitment failure can then reduce subsequent breeding population (Cairns, 1992), and if juveniles disperse outside the study area, as it seems to be the case, this could affect populations at a broader geographic scale. Proper assessment of the long-term consequences requires demographic studies conducted over longer time periods. Because such studies are costly, simulation-based modelling could provide a possible experimental approach to predict the social and demographic consequences of rapid and permanent habitat loss related to hydropower development (e.g. Boyce et al., 2006; Bennett et al., 2009). In spite of this, due consideration should be given to offset the impacts of roost loss not only on the population directly affected, but also the populations in surrounding areas. One possibility might be to create artificial roosts specifically designed for crevice-dwelling in the surroundings areas that will be subject to roost loss. This should be part of the mitigation measures and implemented before the destruction of existing roosts, allowing enough time for bats to discover and occupy them.

Finally, we highlight that the observed effects must be considered in a broader perspective, due to the combined effects of roost loss with other stressors such as extreme climate events, as the drought experienced during our study (Amorim et al., 2015). In fact, the combined pressures of landscape alteration and climate change may result in species declines occurring faster than otherwise predicted (Selwood et al., 2015). However, only through long-term monitoring of the regional population and by increasing spatial coverage, we will be able to understand the true consequences of these events. Future studies should focus on the effects of these pressures in first year survival, juvenile dispersal, and long-term reproductive success. This understanding of species traits will help managers to cope with the challenges of conservation in the context of disturbance events.

Acknowledgments

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Supporting Information

Methods S4.1 – Microsatellite genotyping

A total of 12 di and 2 tetra-nucleotides polymorphic markers (Amorim et al., *in press*) were genotyped for 1407 individuals in two multiplex panels with seven markers each. PCR fragments were fluorescent labelled following Schuelke (2000) but with FAM, VIC, NED, and PET dyes. A pig tail (GTTT) was added to the 5' end of the primer reverse in order to reduce stutter and drive the reaction to the ''plusA'' band (Brownstein et al., 1996). For additional details on microsatellite primers see Table S4.2.

The PCR reactions were carried in volumes of 10 µL, comprising of 5 µL of Multiplex PCR Master Mix (QIAGEN), 1 µL of primer mix was used per reaction, and 1 µL of DNA extract. Cycling conditions for COI, ATP6, CytB, and D-loop used initial denaturing at 95 °C for 15 min, followed by 40 cycles of denaturing at 94 °C for 30 s, annealing at 59 °C for 45 s and extension at 72 °C for 45 s, with a final extension at 72 °C for 10 min. For COI-mini and D-loop-mini the cycling conditions were the same except the annealing temperature that was 52 ºC and the number of cycles was increased to 45. profile was divided in four main steps: denaturation at 95 ºC for 15 min; 13 cycles with denaturation at 95 ºC for 30 s, annealing at 58 ºC for 90 s with a touchdown of 0.5 ºC per cycle and extension at 72 ºC for 45 s; 27 cycles with denaturation at 95 ºC for 30 s, annealing at 52 ºC for 60 s and extension at 72 ºC for 45 s; and a final extension at 60 ºC for 30 min. PCR products were later separated by capillary electrophoresis on the same automatic sequencer ABI3130xl Genetic Analyzer (AB Applied Biosystems). Fragments were scored using GENEMMAPER V4.0 (Applied Biosystems) and checked independently by two people.

Table S4.1 – Sampling effort as mist-net area per sampling occasion (date) and roosts. (I) Roosts located at the impact area; (C) Roosts located at the control Area.

Meirinhos (I)		Sabor (I)		Sardao (I)		Angueira (C)		Moncorvo (C)	
Occasion	Effort (m^2)	Occasion	Effort (m^2)	Occasion	Effort (m^2)	Occasion	Effort (m^2)	Occasion	Effort (m^2)
2012									
22/05/2012	62.4	11/05/2012	124.8	09/05/2012	124.8	08/05/2012	124.8	12/05/2012	124.8
29/06/2012	166.4	26/06/2012	166.4	18/06/2012	124.8	19/06/2012	124.8	01/07/2012	124.8
20/07/2012	187.2	24/07/2012	124.8	16/07/2012	124.8	23/07/2012	124.8	17/07/2012	124.8
23/08/2012	171.6	18/08/2012	171.6	17/08/2012	124.8	22/08/2012	171.6	21/08/2012	124.8
10/09/2012	109.2	13/09/2012	109.2	21/09/2012	109.2	12/09/2012	109.2	03/10/2012	156.0
02/10/2012	156.0	12/10/2012	109.2	09/10/2012	109.2	04/10/2012	156.0		
					187.2	07/10/2012	171.6		
2013									
11/10/2012	109.2	22/04/2013	187.2	16/04/2013	187.2	24/04/2013	187.2	26/04/2013	124.8
18/04/2013	187.2	10/05/2013	46.8	07/05/2013	31.2	15/05/2013	187.2	13/05/2013	93.6
20/04/2013	187.2	12/06/2013	187.2	12/05/2013	187.2	24/06/2013	187.2	11/07/2013	62.4
09/05/2013	187.2	18/07/2013	93.6	16/06/2013	140.4	03/08/2013	187.2	05/09/2013	124.8
20/06/2013	187.2	14/08/2013	124.8	08/07/2013	46.8	10/10/2013	187.2		
16/07/2013	187.2	08/10/2013	187.2	01/08/2013	140.4				
03/09/2013	187.2			12/08/2013	187.2				
22/09/2013	187.2			15/09/2013	187.2				
16/10/2013	187.2			17/10/2013	187.2				
2014									
16/04/2014	187.2	20/04/2014	187.2	23/04/2014	156	21/04/2014	187.2	23/05/2014	124.8
26/05/2014	187.2	25/05/2014	156	24/05/2014	156	26/06/2014	187.2	14/07/2014	124.8
16/06/2014	187.2	26/06/2014	187.2	18/06/2014	156	14/08/2014	187.2	11/09/2014	62.4
26/07/2014	187.2	24/07/2014	187.2	16/07/2014	109.2	10/10/2014	234		
20/08/2014	187.2	18/08/2014	187.2	12/08/2014	187.2				
15/09/2014	187.2	06/10/2014	187.2	17/09/2014	187.2				
08/10/2014	187.2			16/10/2014	187.2				
2015									
22/04/2015	187.2	27/04/2015	140.4	20/04/2015	187.2	29/04/2015	187.2	13/05/2015	93.6
11/05/2015	187.2	20/05/2015	187.2	18/05/2015	187.2	21/06/2015	187.2	29/07/2015	124.8
28/06/2015	187.2	25/06/2015	187.2	23/06/2015	187.2	26/08/2015	234		
21/07/2015	187.2	23/07/2015	187.2	27/07/2015	187.2	15/10/2015	156		
11/08/2015	187.2	13/08/2015	187.2	24/08/2015	187.2				
14/09/2015	187.2	16/09/2015	187.2	21/09/2015	156				
07/10/2015	187.2	09/10/2015	187.2	13/10/2015	187.2				
2016									
22/04/2016	187.2	29/04/2016	140.4	27/04/2016	140.4	26/04/2016	187.2		
25/04/2016	187.2	02/05/2016	156.0	03/05/2016	93.6	28/04/2016	140.4		
02/06/2016	187.2	05/06/2016	187.2	07/06/2016	234.0	06/06/2016	187.2		
12/06/2016	140.4	13/06/2016	234.0	09/06/2016	346.5	08/06/2016	187.2		
19/09/2016	374.4	21/09/2016	321.4	12/09/2016		15/09/2016	312		
		21/09/2016	65.7						

Table S4.2 – Microsatellite loci and primer sequences used in the study, including information on motif, concentration of forward (F) primer and tail, allele size range, observed (Hobs), expected (Hexp) heterozygosity, P values for deviation from Hardy-Weinberg equilibrium (P(HW)), and estimated frequency of null alleles. Concentration of reverse primer was 10x the forward primer.

Primer name	motif	Sequence (5'-3'), F: 10 μM, R: 100 μM	Tail	Concentration (pM)	
				Primer	Tail
TAD1	ac	F: GGCTACCTGAGCTTCTTAGTCTTC R: TGTTGCTTTCTAGGCTGTTGC	FAM	0.11	4.00
TAD5	tatc	F: ATTCCTGGTCAGTGCATCGT R: TCACTGACACAGATGATAATAGAGACA		0.19	
TAD6	ac	F: TTCTTAACCACACTGGCCCT R: TGCAAGCCAAACATAAGTCG		0.11	
TAD23	ctat	F: GAGCACAGTTCTCCTGATTGG R: TTCAGTCCTAAACTAGGATCAAAGAC	VIC	0.08	0.80
TAD2	ac	F: TCATCGGTATCCTCAAAGAAATG R: GCATTGATGGAGACAGAAATATAAA	NED	3.20	33.20
TAD4	ag	F: ACCTTTGATTACGCAGCACC R: AATTTTGAGAAGTTTGAATGCCA		0.12	
TAD7	ac	F: AAAAGCAGATGGTCAGCAGG R: TGCCAGGCCTAAGATGCTAC	PET	0.12	1.20
TAD13	atct	F: TTTCTCTTTTATGGGAAATCCA R: CCTCAGAAGTAGCCGCAAGT	FAM	0.08	1.60
TAD19	ca	F: TGTTCAATTACACTGTTACATGCG R: CAACCTTGGTCAATTTTAAAGC		0.08	
TAD9	tg	F: CCCTTCTGCCAGTCCAAATA R: CATTGCCACCAAATTCCTCT	VIC	0.08	1.60
TAD18	gaag	F: CCTCAGCTATCTTGGGATTGTC R: AAGCACCACCGATCTGAATC		0.08	
TAD26	tg	F: TTGAAACACCACATTCAGTCG R: AGTTGAATAAGTTAATTGAAATAAGCC	NED	0.2	2.00
TAD12	ag	F: CGCTAATCTCTGCTAATCTCTGG R: GCCTTACCTATACTTCGCTTGG	PET	0.12	3.60
TAD24	ag	F: GATTGTTCCATTGTTATTCCTATTTG R: TGACTGTGGGCAGGTTGTTA		0.24	

Table S4.3 – Yearly estimated population size (Nhat) per sex (F – Females, M –Males). The number of captured individuals (N caught) and recaptures (N recaptures) is also presented. Unc. SE – unconditional standard error; LCL – Lower confidence limit; UCL – Upper confidence limit.

Year	Group	N caught	N recapture	Nhat	Unc. SE	LCL	UCL
All individuals							
2012	F	300	19	2113.72	420.78	2073.96	2154.37
	M	90	3	724.76	230.55	695.69	755.22
2013	F	440	61	1349.73	123.04	1328.25	1371.73
	M	149	24	436.60	51.63	2073.96	2154.37
2014	F	443	55	1589.98	161.84	695.69	755.22
	M	166	26	571.30	72.29	1328.25	1371.73

Year	Group	N caught	N recapture	Nhat	Unc. SE	LCL	UCL
All individuals							
2015	F	585	44	3315.07	421.49	422.85	451.03
	M	248	30	1090.85	156.59	1565.32	1615.19
2016	F	268	17	1487.08	321.47	554.97	588.31
	M	92	13	274.26	57.58	3275.12	3355.60

Table S4.4 – Results of the selection of the best Huggins models for each year ranked according to AICc. Models in bold have DeltaAICc lower than two and were selected for model averaging. (*p*) denotes capture probability and (*c*) recapture probability, note however that variables were shared between both parameter (see Methods).

model	k	AICc	ΔAICc	AICcWt	Deviance
2012					
_p_(~time), _c_(.)	**6**	**1582.97**	**0.00**	**0.49**	**3519.07**
_p_(~time + sex), _c_(.)	**7**	**1584.39**	**1.41**	**0.24**	**3518.47**
p(~time + effort), *c*(.)	7	1584.98	2.01	0.18	3519.07
p(~time + effort + sex), *c*(.)	8	1586.40	3.43	0.09	3518.47
p(~effort), *c*(.)	2	1620.49	37.52	0.00	3564.62
p(~effort + sex), *c*(.)	3	1621.90	38.93	0.00	3564.02
p(~1), *c*(.)	1	1625.48	42.50	0.00	3571.61
p(~sex), *c*(.)	2	1626.88	43.91	0.00	3571.01
2013					
_p_(~time), _c_(.)	**7**	**3037.49**	**0.00**	**0.48**	**5863.33**
_p_(~time + sex), _c_(.)	**8**	**3038.83**	**1.34**	**0.25**	**5862.67**
p(~time + effort), *c*(.)	8	3039.50	2.01	0.18	5863.33
p(~time + effort + sex), *c*(.)	9	3040.84	3.35	0.09	5862.67
p(~effort), *c*(.)	2	3070.23	32.74	0.00	5906.10
p(~effort + sex), c(.)	3	3071.57	34.08	0.00	5905.43
p(~1), *c*(.)	1	3093.25	55.76	0.00	5931.12
p(~sex), *c*(.)	2	3094.59	57.10	0.00	5930.46
2014					
_p_(~time), _c_(.)	**7**	**3024.95**	**0.00**	**0.50**	**6036.94**
_p_(~time + sex), _c_(.)	**8**	**3026.46**	**1.50**	**0.23**	**6036.43**
p(~time + effort), *c*(.)	8	3026.96	2.01	0.18	6036.94
p(~time + effort + sex), *c*(.)	9	3028.46	3.51	0.09	6036.43
p(~1), *c*(.)	1	3083.21	58.26	0.00	6107.22
p(~sex), *c*(.)	2	3084.71	59.76	0.00	6106.72
p(~effort), *c*(.)	2	3085.01	60.05	0.00	6107.01
p(~effort + sex), *c*(.)	3	3086.51	61.56	0.00	6106.51
2015					
_p_(~effort + sex), _c_(.)	**3**	**3886.73**	**0.00**	**0.57**	**8738.00**
_p_(~effort), _c_(.)	**2**	**3888.22**	**1.49**	**0.27**	**8741.49**
p(~time + sex), *c*(.)	8	3890.68	3.95	0.08	8731.92
p(~time), *c*(.)	7	3892.16	5.43	0.04	8735.41
p(~time + effort + sex), *c*(.)	9	3892.69	5.95	0.03	8731.92
p(~time + effort), *c*(.)	8	3894.17	7.44	0.01	8735.41
p(~sex), *c*(.)	2	3940.00	53.27	0.00	8793.27

p(~1), *c*(.)	1	3941.48	54.75	0.00	8796.75
2016					
***p*(~time + sex), *c*(.)**	**5**	**1082.83**	**0.00**	**0.63**	**3140.71**
p(~time + effort + sex), *c*(.)	6	1084.84	2.02	0.23	3140.71
p(~time), *c*(.)	4	1086.51	3.69	0.10	3146.41
p(~time + effort), *c*(.)	5	1088.53	5.70	0.04	3146.41
p(~effort + sex), *c*(.)	3	1116.37	33.54	0.00	3178.28
p(~effort), *c*(.)	2	1120.05	37.22	0.00	3183.96
p(~sex), *c*(.)	2	1235.37	152.54	0.00	3299.28
p(~1), *c*(.)	1	1238.99	156.16	0.00	3304.91

Table S4.5 – Coefficient estimates (means, standard errors, and 95% confidence intervals) obtained for the best closed population models obtained for each year (Table S4.4). These models were further used to calculate average capture probability across time (Figure S4.6)

	Estimate	SE	LCL	UCL
2012				
***p*(~time), *c*(.)**				
Intercept	-4.37	0.26	-4.88	-3.87
time2	0.50	0.22	0.07	0.92
time3	1.00	0.20	0.61	1.39
time4	1.15	0.20	0.77	1.54
time5	0.53	0.22	0.11	0.95
time6	0.58	0.21	0.16	1.00
***p*(~time + sex), *c*(.)**				
Intercept	-4.29	0.27	-4.83	-3.76
time2	0.50	0.22	0.07	0.92
time3	1.00	0.20	0.61	1.39
time4	1.15	0.20	0.77	1.54
time5	0.53	0.22	0.11	0.95
time6	0.58	0.21	0.16	1.00
sex (Male)	-0.40	0.55	-1.48	0.67
2013				
***p*(~time), *c*(.)**				
Intercept	-2.46	0.13	-2.71	-2.21
time2	-0.01	0.12	-0.25	0.24
time3	-0.46	0.14	-0.73	-0.19
time4	-1.04	0.17	-1.37	-0.72
time5	-0.53	0.14	-0.81	-0.25
time6	-0.29	0.13	-0.55	-0.03
time7	-0.61	0.14	-0.89	-0.32
***p*(~time + sex), *c*(.)**				
Intercept	-2.51	0.14	-2.79	-2.23
time2	-0.01	0.12	-0.25	0.24
time3	-0.46	0.14	-0.73	-0.19
time4	-1.04	0.17	-1.37	-0.72

time5	-0.53	0.14	-0.81	-0.25
time6	-0.29	0.13	-0.55	-0.03
time7	-0.61	0.14	-0.89	-0.32
sex (Male)	0.18	0.22	-0.24	0.60
2014				
***p*(~time), *c*(.)**				
Intercept	-3.02	0.14	-3.30	-2.75
time2	0.00	0.14	-0.28	0.28
time3	-0.08	0.15	-0.36	0.21
time4	0.60	0.13	0.34	0.85
time5	-0.42	0.16	-0.73	-0.10
time6	-0.18	0.15	-0.48	0.11
time7	-0.26	0.15	-0.56	0.04
***p*(~time + sex), *c*(.)**				
Intercept	-3.07	0.16	-3.38	-2.76
time2	0.00	0.14	-0.28	0.28
time3	-0.08	0.15	-0.36	0.21
time4	0.60	0.13	0.34	0.85
time5	-0.42	0.16	-0.73	-0.10
time6	-0.18	0.15	-0.48	0.11
time7	-0.26	0.15	-0.56	0.04
sex (Male)	0.17	0.23	-0.29	0.62
2015				
***p*(~effort + sex), *c*(.)**				
Intercept	-9.37	0.80	-10.94	-7.80
effort	0.01	0.00	0.01	0.01
sex (Male)	0.44	0.23	-0.01	0.89
***p*(~effort), *c*(.)**				
Intercept	-9.22	0.80	-10.78	-7.66
effort	0.01	0.00	0.01	0.01
2016				
***p*(~time + sex), *c*(.)**				
Intercept	-3.44	0.27	-3.97	-2.92
time2	-1.00	0.24	-1.46	-0.53
time3	0.63	0.16	0.32	0.94
time4	1.09	0.15	0.80	1.38
sex (Male)	0.90	0.37	0.18	1.62

Table S4.6 – Identification of candidate variables to be used in CJS submodels of bats roosting in five bridges in NE Portugal between 2012 and 2016. Potentially relevant covariates (in bold) were carried out in subsequent steps of model building and selection (see Methods), although the results shown for Submodel II and Submodel III only include the best models identified in the previous steps. Regarding population size measurements, since these were highly correlated, only one was used in subsequent analyses (in this case, given that the three measures provided similar results, the overall estimate of population size (OPopS) was considered). Submodel I – identification of candidate variables for bat recapture probability (*p*) with transient effect (trans) on survival (*S*) and constant movement probability (*Psi*); Submodel II – identification of candidate variables for bat apparent survival (*S*) including the transient effect (trans), while considering the best submodel for the recapture probability (*p*) and constant movement probability (*Psi*) and Submodel III – identification of candidate variables for bat movement probability (*Psi*) considering the best submodels found for the recapture probability (*p*), and survival (*S*). Submodels selected and ranked based on Quasi-Akaike Information Criteria corrected for small samples (QAICc). k – number of parameters in the model; ΔQAICc – difference between model QAICc and the QAICc of the best model. Stratum – Bridge, OPopS – Overall Population Size, FPopS – Female Population Size; MPopS – Male Population Size; dist – distance between bridges

Covariate tested	k	QAICc	ΔQAIC
Submodel I (*p*)			
p(~ 1), *S*(~ trans), *Psi*(~ 1)	5	3585.65	0.00
***p*(~ time), *S*(~ trans), *Psi*(~ 1)**	**8**	**3538.86**	**-46.79**
***p*(~ effort), *S*(~ trans), *Psi*(~ 1)**	**6**	**3580.31**	**-5.34**
p(~ sex), *S*(~ trans), *Psi*(~ 1)	6	3586.56	0.91
***p*(~ stratum), *S*(~ trans), *Psi*(~ 1)**	**9**	**3576.50**	**-9.15**
***p*(~ OPopS), *S*(~ trans), *Psi*(~ 1)**	**6**	**3557.97**	**-27.68**
p(~ FPopS), *S*(~ trans), *Psi*(~ 1)	6	3557.74	-27.91
p(~ MPopS), *S*(~ trans), *Psi*(~ 1)	6	3558.43	-27.22
Submodel II (*S*)			
p(~ time + effort + stratum), *S*(~ trans), *Psi*(~ 1)	13	3530.19	0.00
***p*(~ time + effort + stratum), *S*(~ trans + time), *Psi*(~ 1)**	**16**	**3528.36**	**-1.83**
p(~ time + effort + stratum), *S*(~ trans + sex), *Psi*(~ 1)	14	3530.89	0.70
p(~ time + effort + stratum), *S*(~ trans + stratum), *Psi*(~ 1)	17	3532.92	2.73
***p*(~ time + effort + stratum), *S*(~ trans + OPopS), *Psi*(~ 1)**	**14**	**3524.89**	**-5.30**
p(~ time + effort + stratum), *S*(~ trans + FPopS), *Psi*(~ 1)	14	3525.25	-4.94
p(~ time + effort + stratum), *S*(~ trans + MPopS), *Psi*(~ 1)	14	3524.46	-5.73
Submodel III (*Psi*)			
p(~ time + effort + stratum), *S*(~ trans + OPopS), *Psi*(~ 1)	14	3524.89	0.00
p(~ time + effort + stratum), *S*(~ trans + OPopS), *Psi*(~ time)	17	3530.74	5.85
p(~ time + effort + stratum), *S*(~ trans + OPopS), *Psi*(~ sex)	15	3526.7	1.81
***p*(~ time + effort + stratum), *S*(~ trans + OPopS), *Psi*(~ stratum)**	**18**	**3506.17**	**-18.72**
***p*(~ time + effort + stratum), *S*(~ trans + OPopS), *Psi*(~ dist)**	**15**	**3483.32**	**-41.57**
p(~ time + effort + stratum), *S*(~ trans + OPopS), *Psi*(~ OPopS)	15	3664.37	139.48
p(~ time + effort + stratum), *S*(~ trans + OPopS), *Psi*(~ FPopS)	15	3615.16	90.27
p(~ time + effort + stratum), *S*(~ trans + OPopS), *Psi*(~ MPopS)	15	3527.77	2.88

Table S4.7 – Results of the selection of the best CJS submodels. Submodel I – bat recapture probability (*p*) with transient effect (trans) on survival (*S*) and constant movement probability (*Psi*); Submodel II – bat apparent survival (*S*) including the transient effect (trans), while considering the best submodel for the recapture probability (*p*) and Submodel III – bat movement probability (*Psi*) considering the best submodels found for the recapture probability (*p*), and survival (*S*). Submodels selected and ranked based on Quasi-Akaike Information Criteria corrected for small samples (QAICc). k – number of parameters in the model; ΔQAICc – difference between model QAICc and the QAICc of the best model; QAICcWt – QAICc-based model weights. Bold highlights the models taken to the next steps of the analyses. Stratum – Bridge; OPopS – Overall Population Size; dist – distance between bridges.

Model	**k**	**QAICc**	**ΔQAICc**	**QAICcWt**
Submodel I (*p*)				
***p*(~ time + effort + stratum), *S*(~ trans), *Psi*(~ 1)**	**13**	**3530.19**	**0.00**	**0.54**
p(~ time + effort + stratum + OPopS), *S*(~ trans), *Psi*(~ 1)	14	3532.22	2.02	0.20
p(~ time + stratum), *S*(~ trans), *Psi*(~ 1)	12	3532.52	2.32	0.17
p(~ time + stratum + OpopS), *S*(~ trans), *Psi*(~ 1)	13	3534.54	4.34	0.06
p(~ time + effort), *S*(~ trans), *Psi*(~ 1)	9	3537.73	7.53	0.01
p(~ time), *S*(trans), *Psi*(~ 1)	8	3538.86	8.67	0.01
p(~ effort + stratum + OPopS), *S*(~ trans), *Psi*(~ 1)	11	3539.04	8.85	0.01
p(~ time + effort + OPopS), *S*(~ trans), *Psi*(~ 1)	10	3539.74	9.55	0.00
p(~ time + OPopS), *S*(~ trans), *Psi*(~ 1)	9	3540.87	10.68	0.00
p(~ stratum + OPopS), *S*(~ trans), *Psi*(~ 1)	10	3549.33	19.14	0.00
p(~ effort + stratum), *S*(~ trans), *Psi*(~ 1)	10	3551.86	21.66	0.00
p(~ effort + OPopS), *S*(~ trans), *Psi*(~ 1)	7	3556.75	26.56	0.00
p(~ OPopS), *S*(~ trans), *Psi*(~ 1)	6	3557.97	27.77	0.00
p(~ stratum), *S*(~ trans), *Psi*(~ 1)	9	3576.50	46.30	0.00
p(~ effort), *S*(~ trans), *Psi*(~ 1)	6	3580.31	50.12	0.00
Submodel II (*S*)				
***p*(~ time + effort + stratum), *S*(~ trans + OPopS), *Psi*(~ 1)**	**14**	**3524.89**	**0.00**	**0.81**
p(~ time + effort + stratum), *S*(~ trans + time), *Psi*(~ 1)	16	3528.36	3.48	0.14
p(~ time + effort + stratum), *S*(~ trans + time + OPopS), *Psi*(~ 1)	17	3530.39	5.50	0.05
Submodel III (*Psi*)				
p(~ time + effort + stratum), *S*(~ trans + OPopS), *Psi*(~ dist + stratum) *	19	3481.39	0.00	0.72
***p*(~ time + effort + stratum), *S*(~ trans + OPopS), *Psi*(~ dist) ǂ**	**15**	**3483.32**	**1.94**	**0.28**
p(~ time + effort + stratum), *S*(~ trans + OPopS), *Psi*(~ stratum)	18	3506.17	24.79	0.00

* Potential over-parameterization problems
ǂ Best model explaining the demography of European free-tailed bats in our study

Table S4.8 – Coefficient estimates (means, standard errors, and 95% confidence intervals) obtained from model *p(time,stratum), S(trans,time,stratum), Psi(dist, stratum)*, with a QAICc of 3481.39. Wide confidence intervals in *Psi* estimates by bridge are indicative of model overfitting, and therefore, the model including only the effects of inter-bridge distance on *Psi* [i.e. *p(time,stratum), S(trans,time,stratum), Psi(dist)*], with a QAICc of 3483.32 was taken as the best model (see Results).

	Estimate	**SE**	**LCL**	**UCL**
Recapture Probability (*p*)				
Intercept	-0.16	0.21	-0.57	0.25
time3	-0.20	0.19	-0.58	0.18
time4	-0.06	0.19	-4.48	0.32
time5	-0.57	0.27	-1.09	-0.05
Effort	0.32	0.16	0.02	0.63
Bridge B	-0.26	0.22	-0.70	0.17
Bridge C	-0.17	0.32	-0.79	0.46
Bridge D	-0.81	0.24	-1.29	-0.34
Bridge E	-0.88	0.26	-1.40	-0.37
Survival (S)				
Intercept	2.04	0.45	1.14	2.93
AgeClass-transients	-2.51	0.45	-3.39	-1.62
OPopS	-0.32	0.11	-0.53	-0.10
Movement probability (*Psi*)				
Intercept	-11.92	50.83	-112.00	87.72
Bridge B	6.17	50.85	-93.50	105.84
Bridge C	-8.07	1595.38	-3140.00	3118.88
Bridge D	6.01	50.85	-93.70	105.68
Bridge E	4.13	50.86	-95.50	103.81
Distance	-2.49	0.83	-4.13	-0.86

Table S4.9 – Sample size for the analysis of between group relatedness differences. Within –number of samples from the year, All – number of samples from the remaining years. Impact refers to roosts located at the impact area, where All is the combination of the three roosts (Meirinhos, Sardao, Sabor), and Control refers to roosts located at the control area.

	All (Impact)		**Meirinhos (Impact)**		**Sabor (Impact)**		**Sardao (Impactl)**		**Angueira (Control)**		**Moncorvo (Control)**	
Year	**Within**	**All**	**Within**	**All**	**Within**	**All**	**Within**	**All**	**Within**	**All**	**Within**	**All**
2012	50	387	33	187	7	68	10	140	65	116	12	34
2013	157	280	112	108	13	62	32	118	53	128	33	13
2014	124	313	67	153	21	54	38	112	57	124	15	31
2015	205	232	77	143	36	39	92	58	64	117	10	36
2016	41	396	24	196	6	69	11	139	21	160	NA	NA

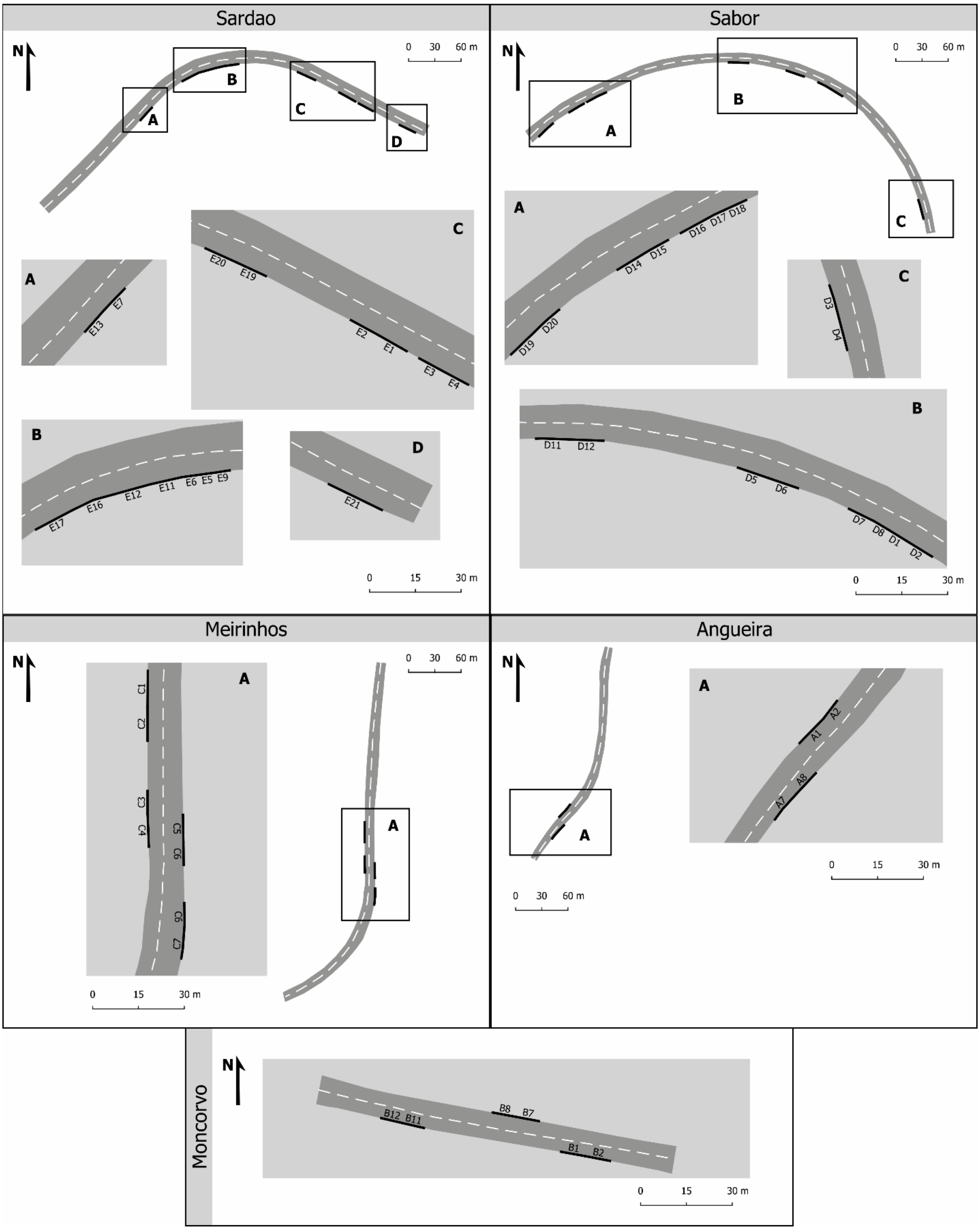

Figure S4.1 – Schematic representation of roosting bridges, showing sampled areas within each bridge roost. Insets zoom in the sampled areas, showing mist-net sections sampled on a regular basis.

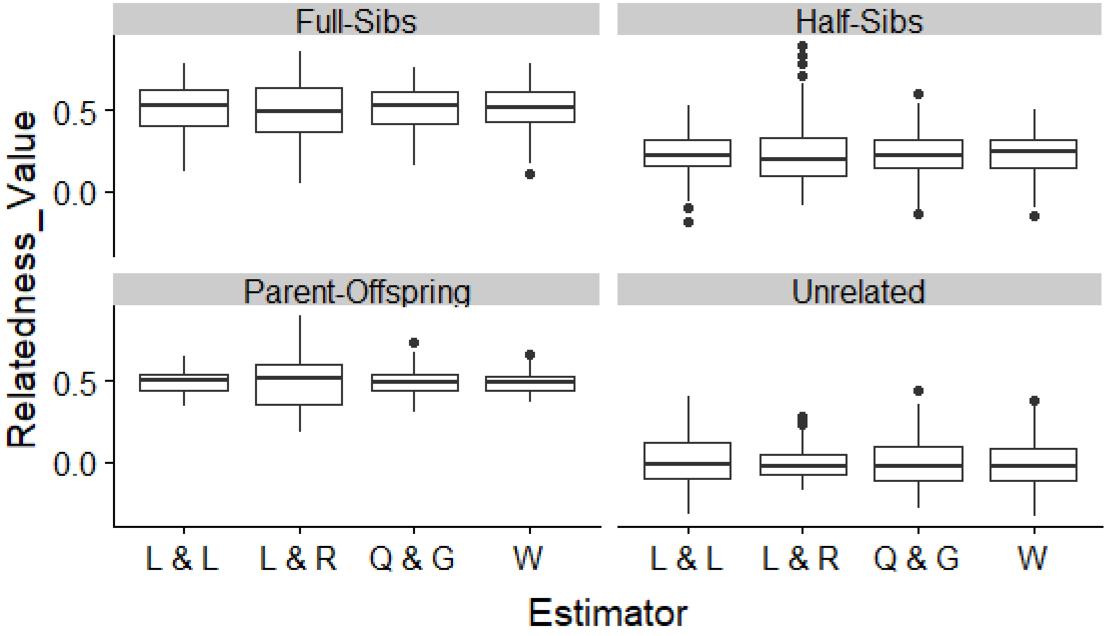

Figure S4.2 – Relatedness estimates for 500 simulated individuals with known relatedness considering four different estimators (L&L – Lynch and Li, L&R – Lynch and Ritland, Q&G – Queller and Goodnight, W – Wang). Correlation coefficient (Pearson's) between Observed & Expected values as follow: W = 0.856; Q&G = 0.852; L&L = 0.851; L&R = 0783.

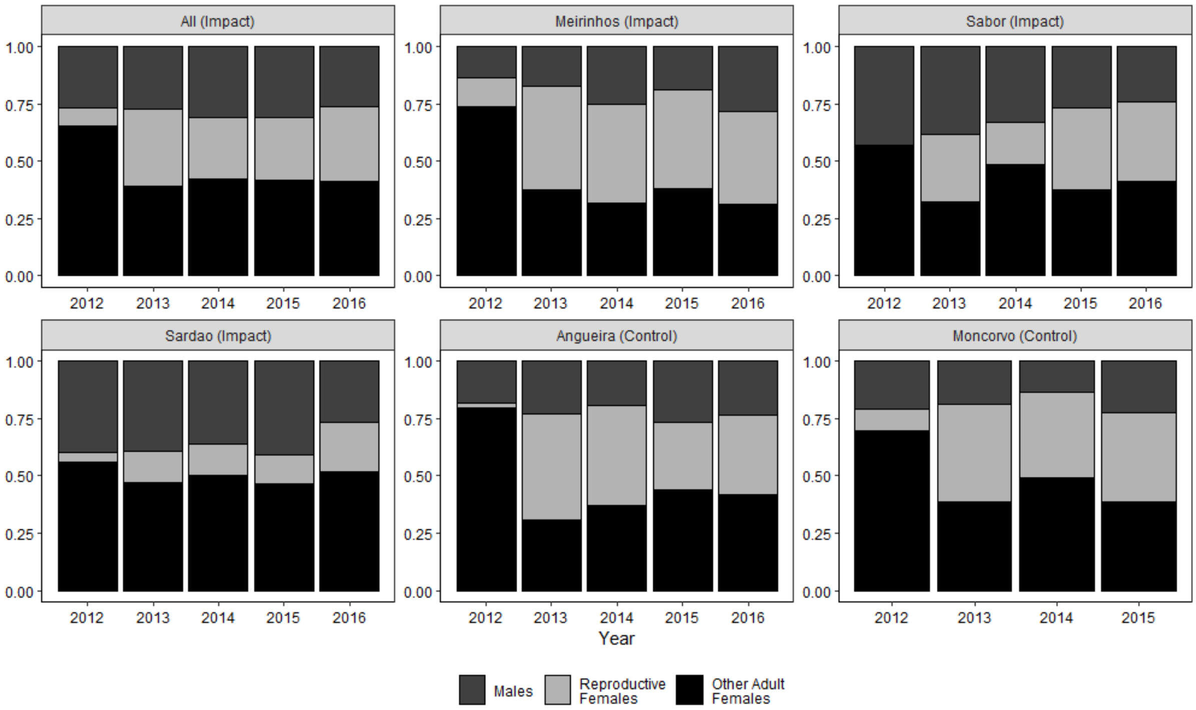

Figure S4.3 – Yearly proportion of Males, Reproductive Females and Other Adult Females. Impact refers to roosts located at the impact area, where All is the combination of the three roosts (Meirinhos, Sardao, Sabor), and Control refers to roosts located at the control area.

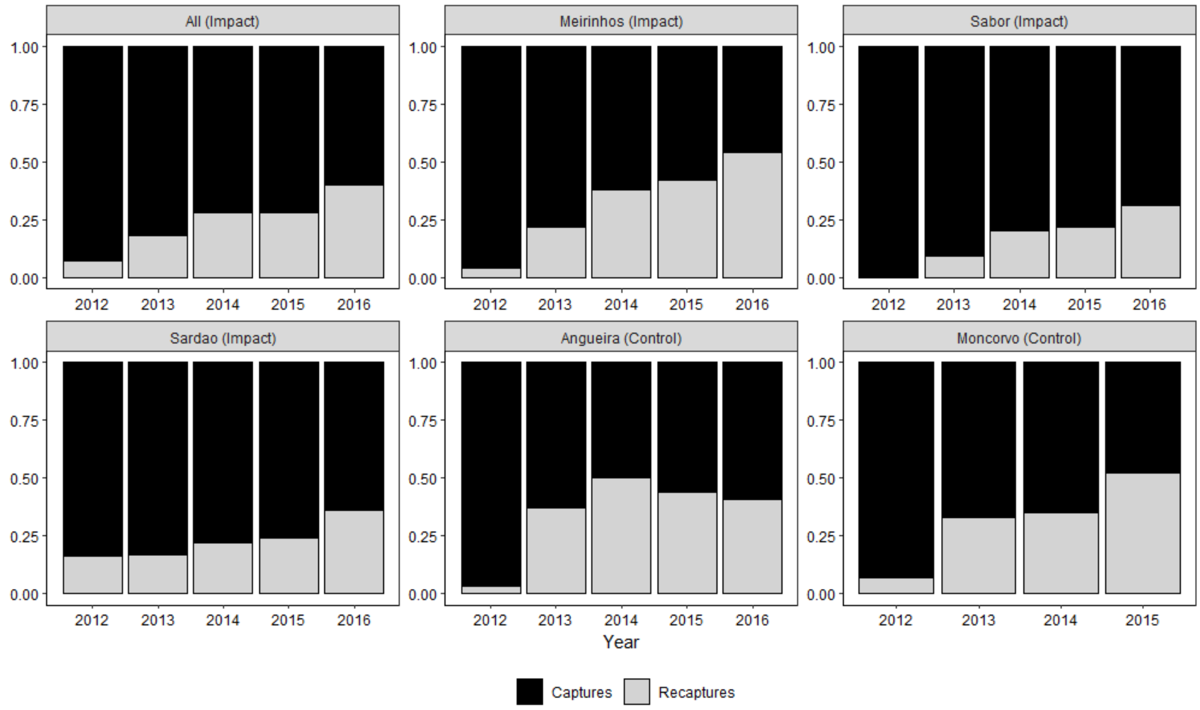

Figure S4.4 – Yearly proportion of Captures and Recaptures. Impact refers to roosts located at the impact area, where All is the combination of the three roosts (Meirinhos, Sardao, Sabor), and Control refers to roosts located at the control area.

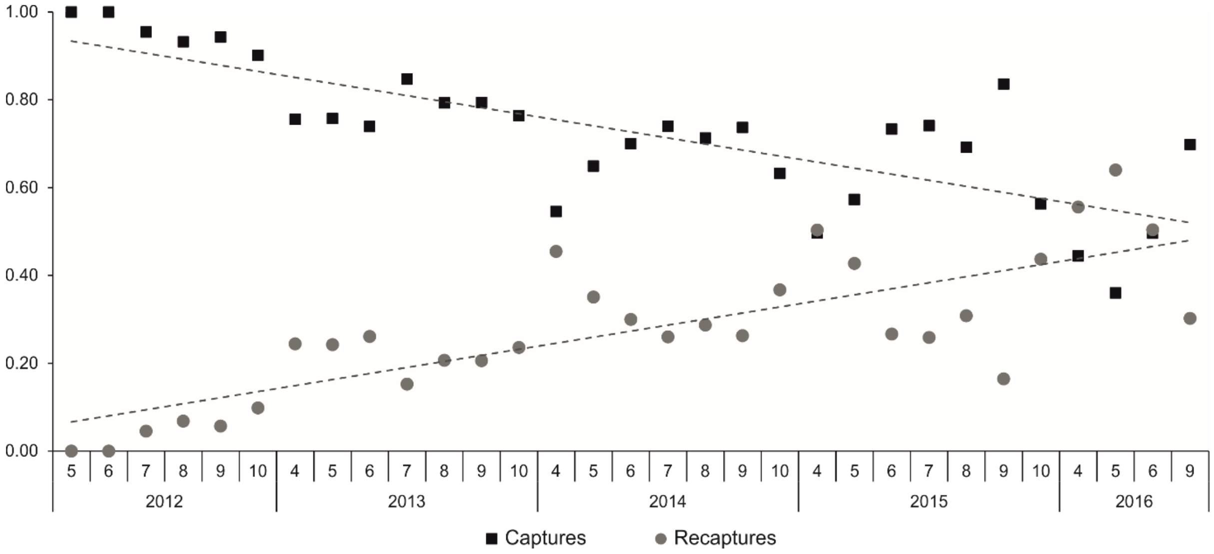

Figure S4.5 – Capture-recapture proportion over the whole study time, showing an increasing trend on the proportion of recaptures. A linear regression was fitted (R = 0.776, p = 0.002).

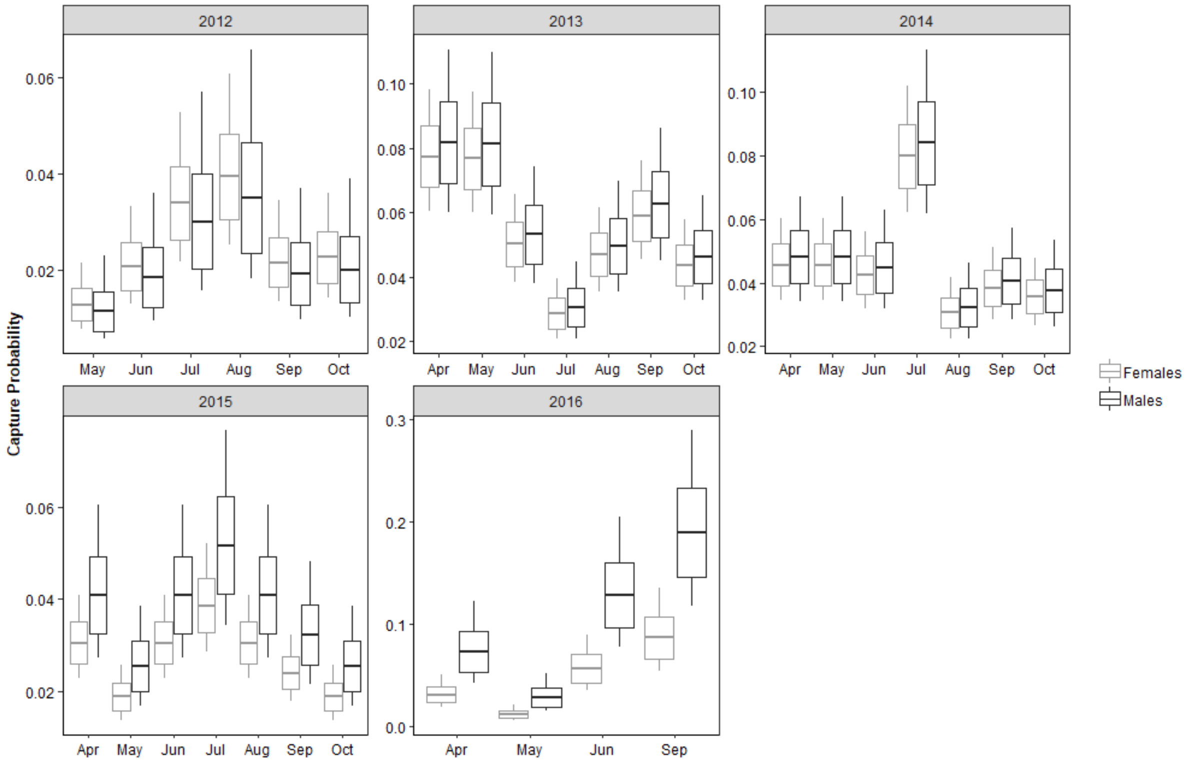

Figure S4.6 – Capture probability after model averaging by group (Females and Males) and per year. Sampling occasions are shown as months in the 'x' axis.

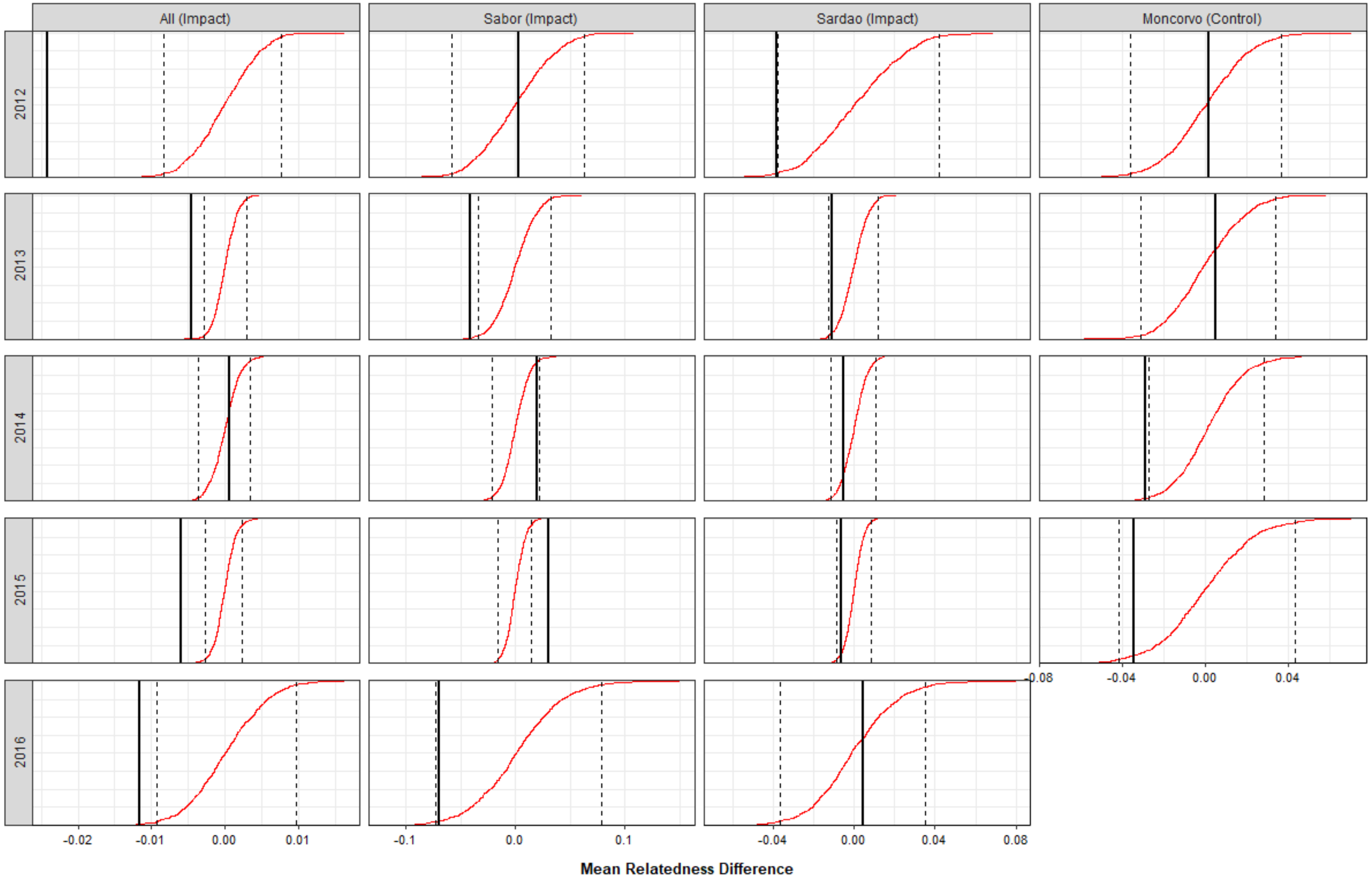

Figure S4.7 – Between group relatedness considering yearly observed relatedness comparing to all the remaining years. Black line represents the mean observed relatedness difference between groups, redline and dashed lines represent respectively the cumulative frequency of the same difference and the 5 % confidence limits based on 1000 bootstrapping. Impact refers to roosts located at the impact area, where All is the combination of the three roosts (Meirinhos, Sardao, Sabor), and Control refers to roosts located at the control area,

Chapter 5

Effects of a drought episode on the reproductive success of European free-tailed bats, *Tadarida teniotis*

Francisco Amorim, Vanessa A. Mata, Pedro Beja and Hugo Rebelo

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Keywords: body condition; normalized difference vegetation index; precipitation; reproductive output; sex-ratio

Abstract

Increases in the frequency and severity of droughts are expected in the Mediterranean region under ongoing climatic change, but their potential effects on bat populations still remain largely unstudied. Here we address this issue, by using data from a monitoring program of *Tadarida teniotis* to assess the reproductive consequences of a severe drought event. Unusually dry conditions occurred in the first of the two years under study (2012-2013), when the annual precipitation was the lowest on record during three decades, and the Normalized Difference Vegetation Index (NDVI) was well below the long term median, particularly during the pre-breeding season. A total of 1304 individuals were captured between May-October 2012 and 2013, and their body condition index (BCI), gender, age, and reproductive status were assessed. In both years, the BCI of adults increased throughout the year, reaching its maximum in October. BCI in May was significantly lower in 2012 than 2013, but converged thereafter to similar values. The sex-ratio varied throughout the year, but the proportion of females was consistently higher in both years. The number of pregnant or lactating females and the proportion of juveniles were significantly lower in 2012 than in 2013. Overall, our results suggest that the drought event largely impaired the reproduction of *T. teniotis*, though it only affected body condition early in the pre-breeding season. Possibly, dry conditions in 2012 resulted in reduced food resources during pre-breeding, limiting individuals' ability to restore their body condition after the winter and before breeding season. As a consequence, most individuals did not produce offspring, probably using the food resources available during the dry breeding season to restore body condition before winter. This is in line with the view that long-lived species reduce investment in reproduction to maximise adult survival during stressful periods, and suggest that multiyear droughts may result in population declines due to recurrent reproductive failure.

5.1 Introduction

There is currently a global anthropogenic decline in biological diversity, and in the goods and services it provides to humankind (Tittensor *et al.*, 2014). A factor likely to become one of the major threats to biodiversity is climate change and the associated increases in the frequency of extreme events such as hurricanes, floods, heat waves and droughts (IPCC, 2012). Droughts may be particularly relevant because they contribute to decrease ecosystem productivity, increase mortality and reduce fecundity rates at the population level (Archaux & Wolters, 2006), thereby affecting a wide range of taxa (Ledger et al., 2012).

Given its pervasive effects, droughts are considered a major threat to conservation in biodiversity hotspots worldwide (Myers et al., 2000; Milly et al., 2005), with Mediterranean areas featuring at the top of the most vulnerable (Dai, 2011). This might be regarded as unexpected, however, because Mediterranean climates are naturally characterised by predictably dry and hot summer periods (Blondel *et al.*, 2010). It might thus be assumed that species associated to this biogeographical region should be adapted to cope with the occurrence of droughts. Notwithstanding, there is evidence that the frequency of severe droughts is increasing in the Mediterranean basin, with 10 out of the 12 driest winters since 1902 occurring during the last 20 years (Hoerling *et al.*, 2012). This new and more severe drought regime may have negative consequences, as suggested for Mediterranean stream fish (Magalhães *et al.*, 2007).

Information on the effects of droughts is lacking for Mediterranean bats. This information is needed, because the Mediterranean basin harbours a particularly speciose bat fauna in the European context, including some endemics (Dietz et al., 2009). Moreover, some of these species will likely suffer major range shifts due to climate change (Rebelo *et al.*, 2010), which will result in species losses at the regional level (Amorim *et al.*, 2014). Droughts may exacerbate these negative effects, because peak dry conditions occur in summer during the periods of pregnancy and lactation of many species, when females experience greater metabolic requirements, and thus have a high demand for water and food (Kunz et al., 1995; Mclean & Speakman, 1999; Adams & Hayes, 2008; Frick et al., 2010). As a consequence, the reproductive output of Mediterranean bats may strongly decline during unusually dry years, as suggested for a few other bat species studied in arid and temperate regions elsewhere (Adams & Hayes, 2008; Adams, 2010; Frick et al., 2010).

Here we aimed to assess the consequences of a severe drought event on Mediterranean bat reproduction, using data from a monitoring program of the European free-tailed bat, *Tadarida teniotis* (Rafinesque, 1814). *T. teniotis* is a medium-large crevice-dweller that mainly occurs in the Palearctic (Dietz et al., 2009), where it is strongly associated with the Mediterranean region (Horáček et al., 2000; Rebelo et al., 2010). It is locally abundant, naturally roosting in cliffs, but often occurring also in man-made structures such as bridges, which somehow mimic the rupicolous environment (Amorim *et al.*, 2013; Russo & Ancillotto, 2014). A monitoring program of the species started in northeast Portugal in 2012, corresponding to an unusually dry year that provided the opportunity to assess the short term effects of a severe drought on a Mediterranean adapted species. Based on comparisons between the dry (2012) and an average year (2013), we aimed to assess the effects of this drought on (a) the body condition of individuals, (b) the age structure and sex-ratio, and (c) the reproductive output of the population. The results highlight the need for long-term monitoring and will help to calibrate monitoring programs to detect and measure potential fluctuations on the breeding success of natural populations.

5.2 Methods and materials

5.2.1 **Study area**

The study area was located in north-eastern Portugal (N41º09'-42º00', W7º15'-6º15') in the Sabor river valley (Figure 5.1). Climate is transitional between meso- and supra-mediterranean, with cold winters (average temperature of the coldest month < 6ºC) and dry summers (total annual precipitation <600 mm, of which < 5% in July-August), which are particularly hot in some valleys where monthly average temperatures exceed 21ºC (Monteiro-Henriques, 2010). Topography is characterized by plateaus with average altitudes of 700–800m a.s.l., and the vegetation is mainly meso-mediterranean with evergreen oaks (*Quercus suber*, *Q. rotundifolia*) dominating the native woodlands (Hoelzer, 2003). Valleys are deep and narrow, and watercourses can have steep slopes and a highly variable hydrological regime, with many of them drying out seasonally while others persist year-round. The orography of the area provides optimal conditions for rupicolous fauna and flora.

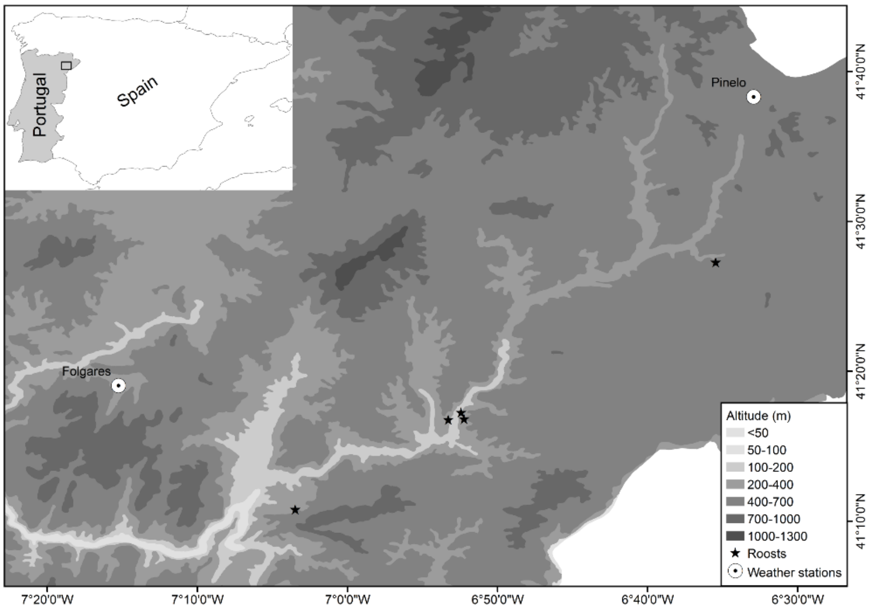

Figure 5.1– Location of *Tadarida teniotis* roosts in bridges monitored in NE Portugal in 2012-2013, and that of weather stations used to describe precipitation patterns in 1983-2013.

Monitoring focused on *T. teniotis* roosts found in five bridges (Figure 5.1), where the number of individuals was large and captures were much easier than in natural roosts in cliff crevices. These man-made structures offer several roosting possibilities for bats, mainly crevices of different types, but also box girders that mimic cave roosts. All the bridges are of modern construction (the oldest was built in 1992) and are part of the Portuguese main road network. The length of the studied bridges ranged from 60-600m, and height from 10-110m.

5.2.2 **Fieldwork**

The study was based on monthly live-trapping of *T. teniotis* throughout the breeding season, from May to October 2012 and 2013. Captures were carried out from dusk till dawn in a total of 57 nights, corresponding to an average of 4.8±1.1 (3-7) nights per month. Captures were made with a trap specifically designed to increase trapping efficiency of *T. teniotis* roosting in bridge crevices, consisting of a steel frame for mist nets that can be secured to the bridge rails or walls (Amorim & Rebelo, 2011). This system can be easily assembled by two persons and the capturing area is adjustable by moving vertically up to two mist-nets. Length is also adjustable and, in our case, up to three sections of 12 meters were successfully assembled. An average of 78.1±16.8m^2 of mist-nets were assembled per night, resulting in the capture of 1034 *T. teniotis* individuals, of which 565 were captured in 2012, and 739 in 2013 (Table 5.1).

Table 5.1 – Number of *Tadarida teniotis* individuals captured in NE Portugal during the years of 2012 and 2013, according to age, gender, and reproductive status.

Age	**Gender**	**Reproductive status**	**2012**	**2013**	**Total**
Adult			546	589	1135
	Female		376	446	822
		Pregnant	17	90	107
		Lactating	7	144	151
		Non-reproducing	352	212	564
	Male		170	143	313
		Active	0	5	5
		Not-active	170	138	308
Juvenile			19	150	169
	Female		12	77	89
	Male		7	73	80
Total			565	739	1304

Captured bats were kept in individual cotton bags until they were processed. Individuals were sexed, aged, weighed and measured for forearm length. A digital scale (100±0.01g) and a calliper (150±0.01mm) were used to measure weight and forearm length, respectively. Age and reproductive status were assessed following Kunz & Parsons (2009). We used the presence of dried semen around the vulva as an indication of mating in females, while in males we used the size of testes and the presence of a well-developed gular gland, often with visible secretions and lacking hair around it (Supporting Information, Figure S5.1). To the best of our knowledge, this gland has never been described in this species (Ibáñez & Arlettaz, 2013). We did not attempt to determine oestrus due to the difficulty in using morphological evidence (Kunz & Parsons, 2009). Pregnancy was assessed by palpation, distention and size of the abdomen, though early pregnancy was difficult to diagnose. Evidences for lactation included the presence of milk and the enlargement of nipples.

The presence of unfused epiphyses in young bats was used to separate them from adults. All individuals captured before September were considered adults, because young of the year were only observed from that month onwards, and individuals born in the previous year (sub-adults) were no longer distinguishable. During October some individuals could no longer be classified as young solely by epiphyses observation, and so in young males we used as additional characteristics the presence of a small non-secreting gular gland and the smaller size of testes. Young females were usually distinguishable by their small nipples and smaller size.

5.2.3 **Environmental data**

Precipitation patterns in 2012-2013 in relation to the long-term regime were assessed using monthly and annual precipitation data for the period 1983-2013 (source http://snirh.apambiente.pt/), based on hydrological years (from October 1 to September 30), instead of calendar years. We used data from one weather station located at the south-western (Folgares) and other at the north-eastern (Pinelo) limits of the study area (Figure 5.1), covering both meso- and supra-mediterranean bioclimatic regions. Because the two stations showed much the same temporal patterns, we averaged data to characterise the precipitation regime in study area. To measure drought severity we used deviations of annual precipitation from the corresponding long-term median and 25% quartile (Magalhães *et al.*, 2007). An additional parameter was computed as the run-sums of the negative deviations from the median, with positive values reset to zero (Yevjevich et al., 1967), hereafter referred as water deficit. This has the advantage over simple deficits of accounting for eventual cumulative inter-annual effects of low rainfall on the shortage of water (Yevjevich *et al.*, 1967).

Biological productivity during the study period was estimated from the Normalized Difference Vegetation Index (NDVI), which is known to relate with net primary productivity (Goward et al., 1985; Box et al., 1989; Running et al., 2004). The NVDI is generally related to climatic variables (Ichii et al., 2002; Gong & Shi, 2003), in particular precipitation (Wang et al., 2003; Jamali et al., 2011), supporting its use as a proxy for the effects of climate on biological productivity. Data on NDVI were downloaded from http://ivfl-info.boku.ac.at/ (Vuolo et al., 2012) for the available period of 2001-2013 with a spatial resolution of 250x250m. Hydrologic years were also considered for NDVI to be comparable with precipitation data. We used the maximum-value composite procedure (MVC) (Holben, 1986), meaning that each month series (composed of three NDVI rasters) was examined on a pixel-by-pixel basis and only the highest value was retained, thus a MVC image was produced for every month. This approach allows to overcome the lack or biased information due to cloud coverage, sun angle, water vapour, aerosols and directional surface reflectance (Holben, 1986). MVC values obtained following this procedure were then used to calculate the annual NDVI median. We used deviations of annual NDVI from the corresponding long-term median and 25% quartile to determine when the low levels of vegetation productivity occurred.

Both precipitation and NDVI median values were calculated for five different seasons that were set according to *T. teniotis* phenology in our study area (F. Amorim, unpublished data): 1) pre-breeding (February-April); 2) pregnancy (May-June); 3) lactation (July-August); 4) post-lactation (September-October), and 5) wintering (November-January).

5.2.4 **Data analysis**

To test differences between the drought and the regular year in individual fitness, sex ratio, age composition, and reproductive output, the following indicators were compared using generalized linear models (GLM): body condition index (BCI=bodyweight(g)/forearm(mm)) (Zahn et al., 2007; Rigby et al., 2012); sex ratio; and proportion of juveniles and reproductive adults. For the BCI models, a Gaussian distribution was considered, and year (coded as 0 for 2012 and 1 for 2013), date of capture (number of days since the 1st of May), and gender (coded as 0 for females and 1 for males), were used as explanatory variables. For the sex-ratio, a binomial distribution was considered with a logit link function, and year and date of capture were used as explanatory variables. For the proportion of juveniles and reproductive adults, a binomial distribution with a logit link function was also considered, and only year was used as an explanatory variable. In model building we followed the information theoretic approach of Burnham & Anderson (2002), which compares the relative support of a suite of candidate models using Akaike's information criterion (AIC) and the corresponding Akaike weights. Candidate models were built using all possible combinations of variables and their interaction terms. For each dependent variable we then computed an average model based on the 95% confidence set of candidate models (minimum number of models whose Akaike weight sum up to 0.95), and estimated the selection probability of each explanatory variable as a measure of its relative importance in the model. Uncertainty in parameter estimates were assessed through 95% confidence intervals (95%CI), considering as equivocal the meaning of coefficients with 95% CI overlapping zero (Burnham & Anderson, 2002). All analyses were carried out with the software R (R Core Team, 2018), using the MuMIn package for model selection and averaging (Barton, 2016).

5.3 Results

5.3.1 **Environmental data**

Annual precipitation in the dry year 2012 was the second lowest on record since 1983 (Figure 5.2). Values in this year were well-below the 25% quartile for the period 1983-2013, and the cumulative water deficit was among the highest on record (Figure 5.2). Dry conditions in 2012 were particularly severe during the pre-breeding season, when precipitation was 51mm, whereas the long term median was 139mm (Figure 5.2). In contrast, precipitation in 2013 during the same season was 222mm, well-above the long term median (Figure 5.2). In the remaining seasons, precipitation in 2012 was closer to that in 2013, and comparable to that observed in other dry years in the period 1983-2013, particularly during the pregnancy and lactation periods (Supporting Information, Figure S5.2).

In 2012 the annual NDVI was the lowest on record during 2001-2013, while it was above the median in 2013 (Figure 5.2). Seasonal NDVIs were also always lower than the median in 2012, with particularly low values in the pre-breeding season respectively) (Figure 5.2) and, to a lesser extent, the pregnancy and lactation seasons (Supporting Information, Figure S5.3). Indeed, the NDVI observed in the pre-breeding season of 2012 was by far the lowest on record. In 2013, seasonal NDVIs were either close to (post-lactation) or above the long-term median. The annual NDVI and precipitation were correlated (Spearman Rank Correlations: $R_s = 0.52$; $p<0.05$).

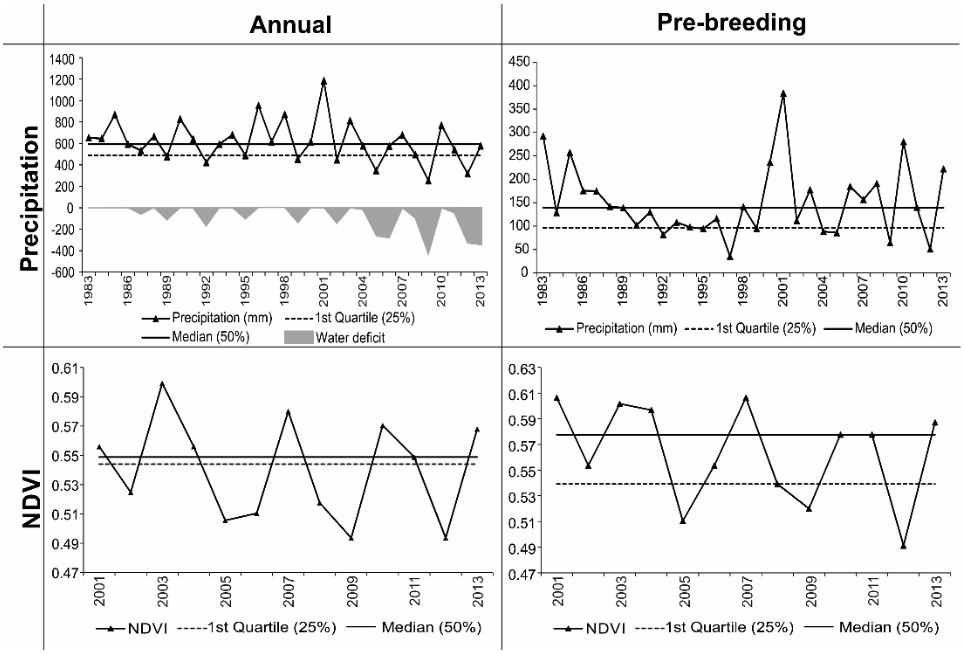

Figure 5.2 – Temporal variation in precipitation (1983-2013) and in the Normalized Difference Vegetation Index (NDVI) (2001-2013) in the study area, considering both the values for the hydrologic year (October 1 – September 30) and for the pre-breeding season (February-April) values. In each panel we show the long-term median and 25% quartile of values. For the annual precipitation we show the water deficit, as the run-sums of the negative deviations from the median with positive values reset to zero.

5.3.2 **Body condition**

There were four models in the 95% confidence set of GLM models for body condition, all of which included as explanatory variables the date of capture, year, gender, and the interaction term between date and year (Table 5.2). In the average model, all variables except gender had 95% confidence intervals of coefficient estimates that did not overlap zero, providing support for their effect on body condition (Table 5.3). Overall, this model suggested that body condition increased each year from May to October, though the patterns differed between years and, to a lesser extent, between females and males (Figure 5.3). In females, body condition in May was much lower in 2012 than in 2013, but thereafter the values of both years converged progressively (Figure 5.3). Males showed broadly the same patterns, though body condition in May differed less between years and it varied less over time than in females (Figure 5.3). At the end of the breeding season (September/October) female body condition was higher in 2012 than in 2013, while in males the values were similar in both years (Figure 5.3).

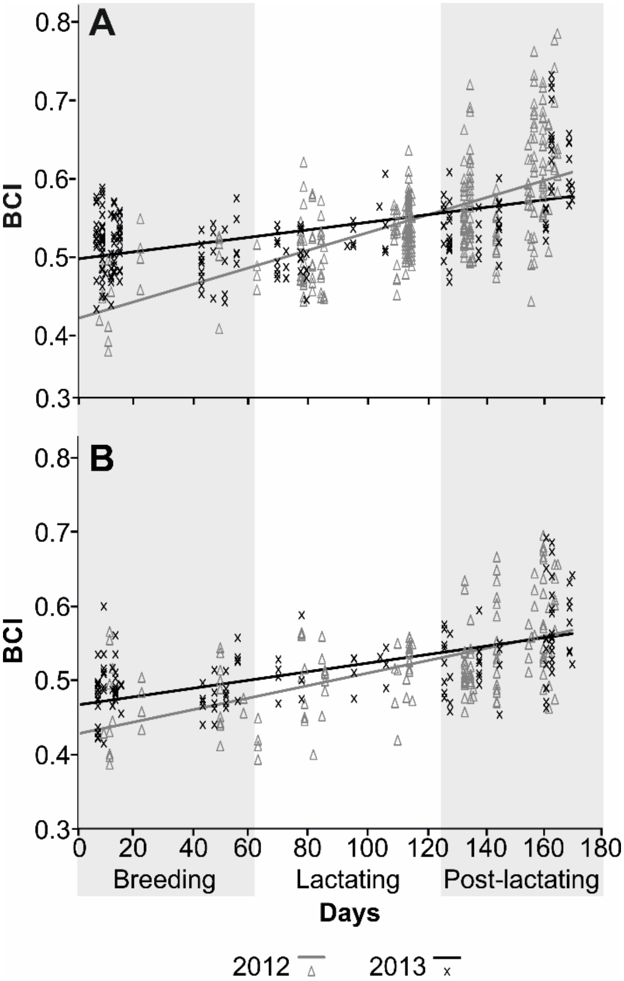

Figure 5.3 – Predicted curves derived from average GLM models describing variation over time in body condition of *Tadarida teniotis* during the breeding seasons of 2012 and 2013, for both females (A) and males (B). Symbols represent the observed data. Day 1 = 1st of May.

5.3.3 Sex ratio

The sex ratio varied slightly throughout the year, though the proportion of females was always higher than that of males (Figure 5.4A). The 95% confidence set of models included three plausible models with explanatory variables (Table 5.2), consistently including year as the variable with highest selection probability (Table 5.3). The null model was also included in the confidence set of models, but its Akaike weight (0.055) was very low (Table 5.2). In the average model, the year was the only explanatory variable with a 95% confidence interval of coefficient estimates that did not overlap zero (Table 5.3), providing support for the presence of a higher proportion of females in 2013 than in 2012.

Table 5.2 – Summary results of information-theoretic model selection for the effects of explanatory variables (and all their interactions) on the body condition index (BCI) and adult sex-ratio of *Tadarida teniotis* sampled in NE Portugal (2012-2013). For each dependent variable we show the 95% confidence set of best-ranked regression models, and for each one we provide: k - number of variables included in the model, logLik - maximized log-likelihood value, Δ_i - delta Akaike information criteria (AIC), wi - Akaike weight, wi+ - cumulative sum of Akaike weights.

Candidate models		**k**	**logLik**	Δ_i	w_i	w_i+
Body Condition Index						
1	Date + Gender + Year + Date*Gender + Date*Year + Gender*Year + Date*Gender*Year	9	1225.4	0	0.702	0.702
2	Date + Gender + Year + Date*Gender + Date*Year	7	1221.8	3.17	0.144	0.846
3	Date + Gender + Year + Date*Year	6	1220.1	4.72	0.066	0.912
4	Date + Gender + Year + Date*Gender + Date*Year + Gender*Year	8	1221.9	5.04	0.056	0.968
Sex-ratio						
1	Year	2	-140.2	0	0.572	0.572
2	Date + Year	3	-140.1	1.72	0.242	0.814
3	Date + Year + Date*Year	4	-140.1	3.68	0.091	0.905
4	Null	1	-143.6	4.67	0.055	0.960

Table 5.3 – Summary statistics of average models relating explanatory variables to body condition index (BCI), adult sex-ratio, proportion of reproductively active females, and proportion of juveniles for *Tadarida teniotis* sample in 2012-2013 in NE Portugal. For each variable and model we indicate the coefficient estimate (Coefficient), the Standard error (Std. Error), the 95% confidence interval of coefficient estimates (95%CI), the variable selection probability (Selection Probability), and the number of models containing the variable in the 95% set of models (N models). CIs that do not overlap zero are highlighted with an asterisk.

Coefficient	**Estimate**	**Std. Error**	**95% Confidence Interval**	**Selection probability**	**N models**
BCI					
(Intercept)	4.03E-01	1.16E-02	[3.80E-01, 4.26E-01] *		
Date	1.15E-03	9.50E-05	[9.68E-04, 1.34E-03] *	1.00	4
Gender	2.47E-02	2.03E-02	[-1.51E-02, 6.44E-02]	1.00	4
Year	7.99E-02	1.35E-02	[5.33E-02, 1.06E-01] *	1.00	4
Date*Gender	-3.34E-04	1.54E-04	[-6.35E-04, -3.18E-05] *	0.93	3
Date*Year	-6.60E-04	1.22E-04	[-8.99E-04, -4.21E-04] *	1.00	4
Gender*Year	-4.08E-02	2.01E-02	[-8.04E-02, -1.42E-03] *	0.78	2
Date*Gender*Year	4.04E-04	1.53E-04	[1.04E-04, 7.03E-04] *	0.72	1
Sex-ratio					
(Intercept)	8.35E-01	1.51E-01	[5.34E-01, 1.14E00]		
Date	-8.22E-04	1.64E-03	[-4.10E-03, 2.46E-03]	0.35	2
Year	3.31E-01	1.64E-01	[2.56E-03, 6.60E-01] *	0.94	3
Date*Year	5.69E-04	2.79E-03	[-5.03E-03, 6.7E-03]	0.09	1
Proportion of Reproductive Females					
(Intercept)	-2.69E00	2.11E-01	[-6.37E00, -4.67E00] *		
Year	2.78E00	2.31E-01	[2.35E00, 3.26E00] *	1.00	1
Proportion of Juveniles					

(Intercept)	-4.64E03	5.27E02	[-5.73E03, -3.65E03] *		
Year	2.30E00	2.62E-01	[1.81E00, 2.85E00] *	1.00	1

5.3.4 **Reproductive output**

The GLM models provided strong support for a higher proportion of reproductive females and juveniles in 2013 than in 2012 (Table 5.3). The overall proportion of adult females showing either pregnancy or lactation was much higher in 2013 (52.5%) than in 2012 (6.4%), and this difference was apparent in every month except May (Figure 5.4). The proportion of juveniles captured in September and October was also much higher in 2013 (10.3% and 38.3%) than in 2012 (6% and 2.3%). Adult males were excluded from analyses due to the low captures of reproductively active individuals.

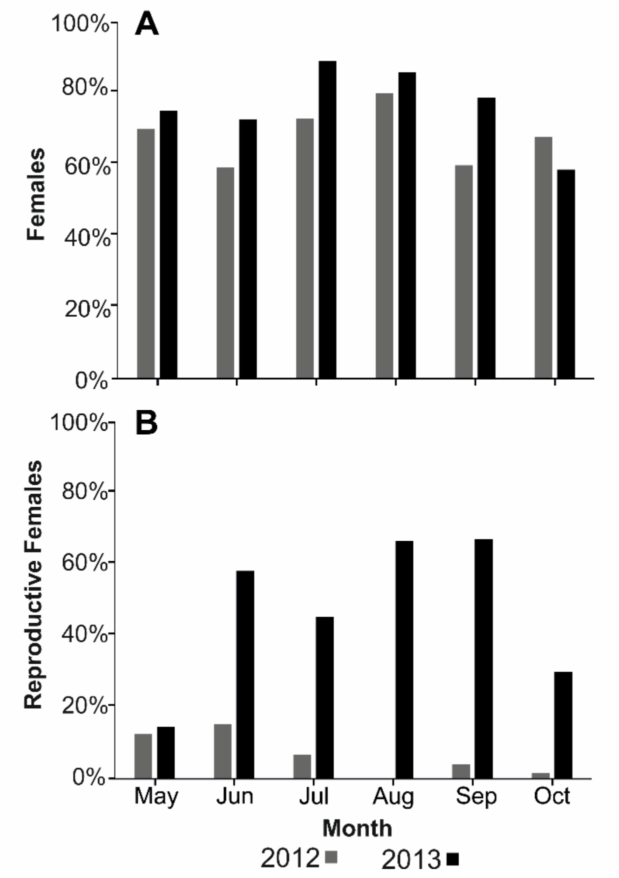

Figure 5.4– Variation between May and October of 2012 and 2013, of the (A) Proportion of adult females; and (B) Average proportion of reproductively active adult females of *Tadarida teniotis*.

5.4 Discussion

Our results support the view that droughts may have a significant effect on the reproductive success of Mediterranean bats, in line with the results of studies carried out in arid and temperate zones elsewhere (Adams, 2010; Frick et al., 2010; Lučan et al., 2013). During 2012, the study area suffered a severe drought and over that period the monitored colonies of *T. teniotis* had a much lower reproductive success than in a regular year, probably as a consequence of low body condition of individuals in the months prior to the breeding season. The drought decreased overall biological productivity (as measured by the NDVI), probably resulting in lower prey availability (Frampton et al., 2000). This likely resulted in undernourishment and lower body condition of local bat populations, and hence females probably did not meet the energetic demands to carry a successful pregnancy. Overall, results suggest that low biological productivity during a dry year resulted in low body condition of breeding females, which in turn resulted in reduced reproductive output. The consequences of such effects for population viability are still uncertain, though it is likely that population declines may result from the increasing frequency and severity of droughts expected in the Mediterranean region due to climate change (Dai, 2011). The patterns observed in this study for a Mediterranean bat species are in contrast with those for other, more northern populations, where water deficit is usually not a constraint, and so bats are probably more affected by excessive precipitation, with high levels of rainfall in late Spring and early Summer leading to lower reproductive outputs (Lučan et al., 2013).

5.4.1 **Limitations and potential shortcomings**

This study was based on a two-year monitoring dataset of *T teniotis* colonies and encompassed a single drought event, which limits the generality of the inferences that can be drawn from our results. In particular, it cannot be ruled out that differences in population parameters observed between 2012 and 2013 were driven by factors other than the occurrence of a drought event, though we believe this is unlikely. First, it is worth noting that 2012 was among the driest years on record during about three decades, which may be expected to have major consequences at the ecosystem and population levels (e.g., Tilman & El Haddi, 1992). Second, these unusually dry conditions were associated with very low values of NDVI, which can be taken to indicate a much reduced primary productivity (e.g., Goward *et al.*, 1985; Box *et al.*, 1989; Running *et al.*, 2004), which in turn tends to be associated with low availability of insect prey (Bailey *et al.*, 2004). Third, a large marking effort with PIT tags suggests that adult *T. teniotis* are faithful to the breeding sites across years (F. Amorim, unpublished data), and thus it is unlikely that reduced reproductive output in 2012 was an artefact of individuals breeding in nearby, unsampled colonies (Amorim *et al.*, 2013; F. Amorim, unpublished data). Finally, the main patterns observed in this study are consistent with the effects of droughts on bats reported in arid and temperate regions elsewhere (Adams, 2010; Frick *et al.*, 2010), thus providing support to our inferences. Despite this reasoning, however, a full appreciation of the effects of unusually dry conditions on Mediterranean bats would require a longer time series and more drought events.

5.4.2 **Effects of the drought on *T. teniotis***

The low biological productivity and prey availability resulting from drought episodes are likely to cause a poor physical condition in breeding females that may limit the reproductive success. In these circumstances, females might have been unable to meet the physical condition to carry successful pregnancies. This view is supported by a range of studies showing that breeding female bats have to meet particularly high metabolic costs. For instance, Mclean & Speakman (1999) found that average food consumption of *Plecotus auritus* females was highest in lactating females, while Kunz *et al.*, (1995) showed that food intake of female *Tadarida brasiliensis* increases from mid to late pregnancy, stabilizing or decreasing during late pregnancy, and increasing again during early to mid-lactation. Therefore, under harsh climatic conditions pregnancy might be hampered due to associated high metabolic costs, providing a likely mechanism for the observed reduction in breeding output during the drought event.

Despite the reduction in reproductive output during the dry year, there appeared to be much less marked effects of the drought on the body condition of individuals. Nevertheless, body condition was lower in the pre-breeding season (May) of 2012 than that of 2013, which was coincident with the period when precipitation and the NDVI were lowest in relation to the long term median. In the following months, however, the body condition of individuals became similar in the two years, though the drought persisted in 2012 during the pregnancy and lactating seasons. Reasons for these results are uncertain but it is possible that individuals failing to reproduce during the dry year used the available food resources to restore their body condition, thereby increasing their chances of survival during the next wintering season. In temperate ecosystems, where water deficit is usually not a constraint, bats reproductive success seems to be mostly affected by excessive cold and rainfall in late spring and early summer (Burles et al., 2009; Lučan et al., 2013). As a result parturition can be delayed, so that juveniles fledge when prey availability is higher (Racey & Swift, 1981; Burles et al., 2009; Lučan et al., 2009). However in Mediterranean ecosystems if precipitation is low during winter and early spring it is unlikely that levels of rainfall will then compensate the low biological productivity observed during that period, and only in the subsequent year and if climatic conditions are met, bats will be able to successfully reproduce. Our view is in line with studies carried out in a range of species, which have shown that long-lived species usually reduce investment in reproduction to maximise adult survival during stressful periods (Linden & Møller, 1989; Hanssen et al., 2005).

5.4.3 **Conservation implications**

Although the effects of droughts described in this study were short term, they point out the possibility of negative effects on the long term population viability of *T teniotis*, due to the increasing frequency of severe droughts expected in the Mediterranean region under climate change (Dai, 2011). In these circumstances, multiyear droughts may result in population declines because of recurrent reproductive failure. This mechanism may affect other Mediterranean bat species, and it was already suggested to influence Mediterranean stream fish (Magalhães *et al*., 2007). The study thus adds to the growing evidence that even species adapted to the very hot and dry summer conditions characteristic of the Mediterranean region may be negatively affected by droughts. Confirming these hypothesis would require the development of long-term population monitoring programs, which are also needed for the effective development of conservation measures by allowing to determine population trends and changes in the structure of biotic communities, in line with environmental change, anthropogenic disturbance, or targeted management actions (Lindenmayer & Likens, 2009; Amorim et al., 2014).

To this end, monitoring programs should include estimates of the body condition of individuals in the pre-breeding season, which might provide an early warning for oncoming reproductive failures. This may trigger conservation action, such as for instance the provision of artificial sources of water (Razgour et al., 2010). On a more long-term basis, the construction of ponds or small reservoirs near important bat colonies may provide a reliable source of food resources during dry periods (Razgour et al., 2010). In general, however, there is limited understanding on the most effective conservation actions for bats under drought conditions, and this should be subject to further research.

Acknowledgments

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Supporting Information

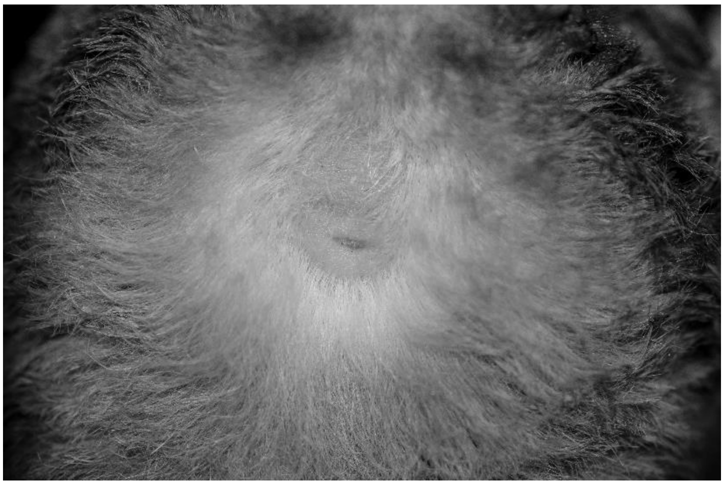

Figure S5.1 – Photograph illustrating a gular gland of a *Tadarida teniotis* adult male individual captured in late April.

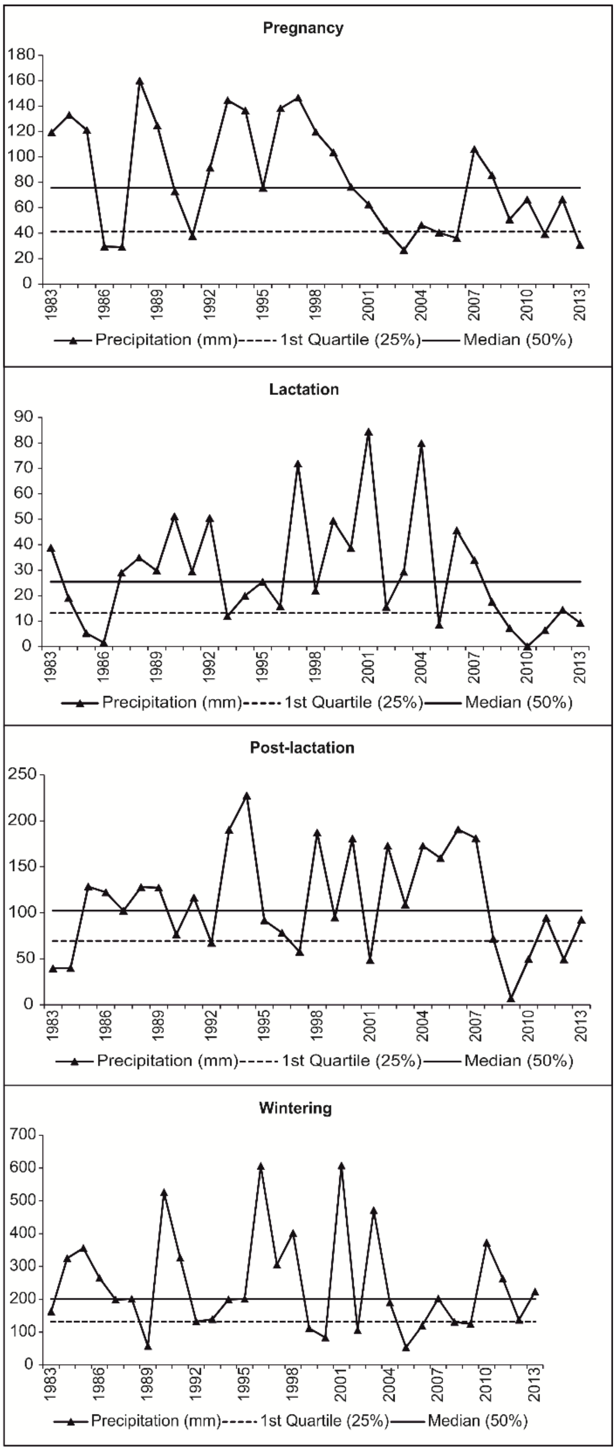

Figure S5.2 – Temporal variation (1983-2013) of the precipitation in the pregnancy, lactation, post-lactation and wintering periods. In each panel we show the long-term median and the 25% quartile of precipitation values.

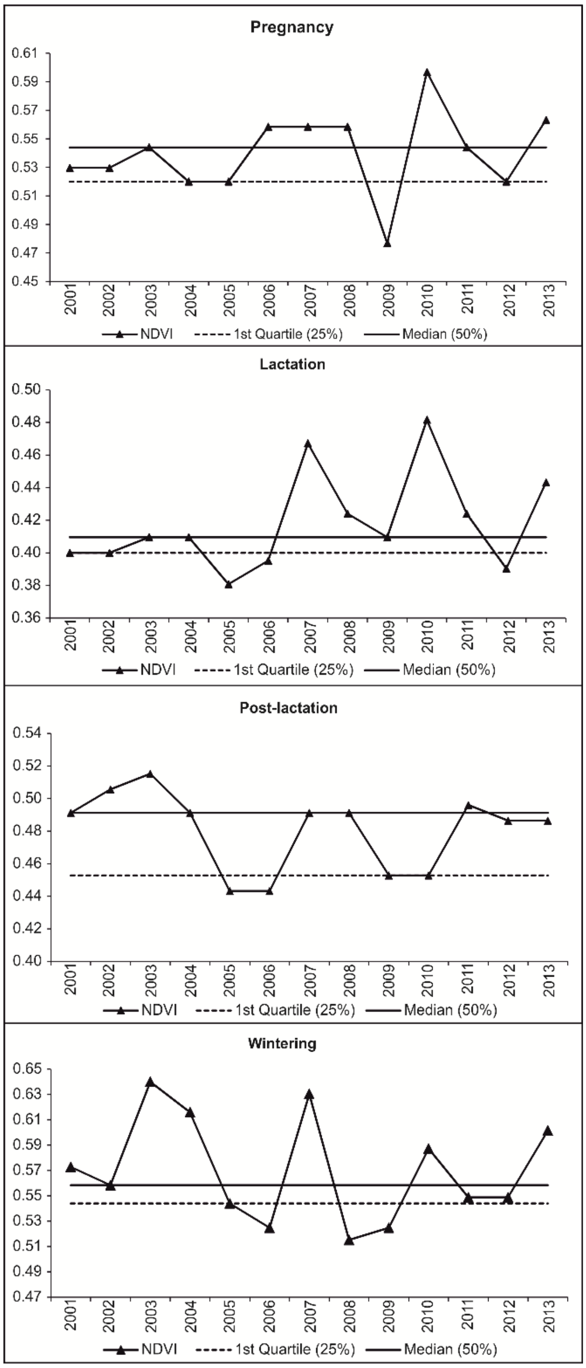

Figure S5.3 – Temporal variation (2001-2013) of Normalized Difference Vegetation Index (NDVI) in the pregnancy, lactation, post-lactation and wintering seasons. In each panel we show the long-term median and 25% quartile of NDVI values.

Chapter 6

General Discussion

“Men may dam it and say that they have made a lake, but it will still be a river. It will keep its nature and bide its time, like a caged animal alert for the slightest opening. In time, it will have its way; the dam, like the ancient cliffs, will be carried away piecemeal in the currents.”

Berry W. (1970). The Unforeseen Wilderness. *The Hudson Review*, 23(4), 633.

6.1 In a nutshell

This thesis presents compelling evidence on how the impacts of different stressors of riverine habitats affect bats at multiple levels, including species richness and abundance, population size, survival, reproduction and social structure. The value of water availability in such systems is thoroughly discussed, as well as the implications of disturbances such as severe droughts and the profound alteration of riverine habitats by hydroelectric infrastructures. Overall, the case studies presented describe the spatiotemporal changes and the demographic implications of both natural and human mediated alterations of riverine habitats, with implications for bat conservation and management. It is shown that bat species inhabiting semi-arid regions are able to cope with marked seasonal changes in resource availability, and that they can even survive extreme events such as droughts or roost loss at a regional scale. Despite bat apparent ability to cope with these disturbances, these can have demographic costs (e.g. reproductive success, disruption of social systems) that are still not completely understood. In the end, even resilient species such as *T. teniotis*, may face severe population reductions, and eventually local extinctions because of increasing frequency of extreme events and cumulative threats acting at a larger spatial scale. This chapter presents the key results from these studies, their conservation implications, general management guidelines targeted at bat populations living in semi-arid regions in the Mediterranean and elsewhere, and future research prospects.

6.2 Major findings

6.2.1 **Importance of riverine habitats for bats**

In line with a large body of literature on bat habitat use in arid and semi-arid regions (see section 1.3), Chapter 2 (Amorim et al., 2018) identifies large rivers and streams in the study area as key features for species richness and activity, the novelty being the inclusion of the seasonality effect on such preferences. As predicted, bats in a semi-arid landscape were able to track the spatiotemporal resource variation determined by the progressive reduction in water availability during the summer drought, leading to a seasonal pattern in habitat preferences. The results show that bats rely heavily on permanent water bodies and riparian habitats at the end of summer, while in spring there is no strong association to any specific habitat feature, probably due to higher water availability across the landscapes. The reason for bats tracking the receding waters is unknown, but it is probably linked to prey availability and drinking (see section 1.3). Insect prey distribution and abundance is affected by water availability (Hawkins & Porter, 2003; Bailey et al., 2004) and primary productive (Frampton et al., 2000), the latter being mostly restricted to areas where soil moisture persist throughout the summer drought (Chapter 5). Thus, during the dry season main rivers and streams can offer one of the very few drinking and feeding opportunities for bats (Russo & Jones, 2003; Tuttle et al., 2006; Rainho, 2007; Adams & Hayes, 2008; Greif & Siemers, 2010). Bats resource tracking in the context of semi-arid regions can also be related to breeding. For instance, pregnancy in most bat species living in the Mediterranean occurs during spring, a period when not only resources will be more abundant but also when females may range more widely and forage for longer periods than during lactation (Henry et al., 2002; Encarnação et al., 2004; Daniel et al., 2010; Vincent et al., 2011). Contrary, during lactation (late spring and early summer) shorter foraging bouts and higher water intake (Adams & Hayes, 2008) is likely to force females to concentrate in areas closer to the roosts and where both prey and water availability are higher (i.e. rivers and streams).

Understanding habitat associations over the breeding season is highly valuable, and Chapter 5 (Amorim et al., 2015) provides insights on how spatiotemporal variation in resource availability might affect reproduction. In 2012, reproductive success of European free-tailed bat was largely impaired by a severe drought affecting the study area. The extreme dry conditions led to an overall decrease in biological productivity (as measured by the NDVI), probably resulting in lower prey availability (Frampton et al., 2000). Thus, females might have been unable to meet the physical condition to carry successful pregnancies. Despite the reduction in reproductive output during the dry year, there appeared to be much less marked effects on the body condition of individuals. It is possible that individuals failing to reproduce used the available food resources to restore their body condition, thereby increasing their chances of survival during the next wintering season. These results suggest that, similar to other long-lived species, bats might reduce investment in reproduction to maximise adult survival during stressful periods (Linden & Møller, 1989; Hanssen et al., 2005).

6.2.2 How climate change affect bats in riverine habitats

A considerable part of the findings in this thesis is related to, or could be exacerbated by, climate change. As I show in Chapter 2, as summer progresses bats in semi-arid systems will become more dependent on the few habitats where water remains available. During the Mediterranean natural summer drought, bats will be mostly restricted to freshwater habitats (Chapter 2). Such dependence is expected to extend to other seasons since the current trends of climate change are likely to reduce water flows in semi-arid regions such as the Mediterranean (Milly et al., 2005). Early seasonal drying of riverbeds as well as decreased soil moisture will result in lower food and drinking water availability overlapping with critical periods such as pregnancy and lactation. Chapter 5 provides data on historical precipitation data for the period of 1983-2013 and shows that the three driest years in the study area were recorded from 2005 onwards (2005, 2009 and 2012). The severe drought observed in 2012 impaired the reproductive success of *T. teniotis*, and although we do not have data for the years of 2005 and 2009, it is reasonable to assume that reproductive success was also compromised.

The findings from Chapter 4 and 5 suggest that *T. teniotis* populations are able to survive disturbance events and extreme dry conditions. If this is the case, the question is to what spatial and temporal extents they would be able to do so. Chapter 3 (Amorim et al., *in press*), addresses the evolutionary history of the species to understand if, and where, it survived the LGM, a cold and, more important, extremely dry period. Despite being a species with tropical affinities that is nowadays associated to the semi-arid Mediterranean, inferences of demographic and evolutionary history indicate that it was able to survive the LGM in two main Western Palearctic refugia, one in the Italian Peninsula and another further east in the Anatolian/Middle East region. The results further suggest that during this period the species may have been extinct throughout the rest of southern Europe. Range contraction during the LGM is not surprising, since a large area of the Western Palearctic was covered in ice sheets and permafrost, and temperatures were 10-20 ºC cooler than today (Kageyama et al., 2006), thus not only water availability was much lower but also the environment carrying capacity was presumably reduced (Frenzel et al., 1992). After the LGM, *T. teniotis* expanded to its present range while population growth was observed following the Iberian colonization. The historical perspective of population contraction and local extinctions observed for *T. teniotis* enhances the importance of addressing the consequences of extreme dry conditions predicted from climate change.

6.2.3 **How anthropogenic changes of riverine habitats affect bats?**

To my best knowledge, Chapter 4 (Amorim et al., *in prep*) provides the first evidence on the consequences of landscape alteration caused by reservoirs on the social structure and demography of wild terrestrial animals. Shortly after the deforestation and subsequent flooding of the river valley (2015), *T. teniotis* population size increased substantially in bridge roosts that were unaffected by landscape disturbance. At the same time, relatedness among individuals declined in roosts closer to the flooded area, but not in roosts farther away.

Considering the geographic proximity between the submerged area and the bridge roosts, it seems reasonable to assume that individuals losing their roosts after the flooding of crevices in cliffs and ravines moved to these bridges as alternative roosting sites. Thus, the increase in population size was the likely result of the arrival of new individuals. Just one year after disturbance population size declined to numbers similar to those before the habitat alteration which could mean that newcomers used already occupied roosts as stepping stones while trying to find a new roosting site (Rebelo & Rainho, 2009). On the other hand, some individuals might have been forced to move outside the sampling area. Adult apparent survival was high even following habitat alteration, further confirming that bats maximize adult survival during stressful periods (Linden & Møller, 1989; Hanssen et al., 2005). Social structure, measured as relatedness among individuals, was also affected by the putative influx of newcomers, but this effect was only observed in safe roosts over the impact area.

Similar effects on both demography and social structure were also observed in 2012. As shown in Chapter 5, the winter of 2011-2012 was extremely dry and *T. teniotis* failed to reproduce within the study area. In face of the dry conditions, individuals might have moved to roosts located along the valleys (including both rock and bridge cervices) where water availability is higher throughout the year (Chapter 2), leading to increased population estimates at the monitored roost in that year.

6.3 Conservation implications

6.3.1 **Bat conservation in riverine habitats of semi-arid regions**

As thoroughly discussed throughout this thesis, bats living in semi-arid regions face many challenges related primarily to water scarcity and habitat alteration. Species in semi-arid regions are adapted to cope with water scarcity, by for instance skipping reproduction to maximize adult survival during dry periods (Chapter 5). However, the cumulative effect of multiple dry years is likely to result in recruitment failure, reducing subsequent breeding population (Cairns, 1992). When juveniles disperse from their natal site, as it seems to be the case of *T. teniotis* (Chapter 4), regional recruitment failure can potentially affect populations at a broader geographic scale. As shown in Chapter 3, *T. teniotis* populations experienced severe demographic and range contractions during the long and dry period of the LGM and since semi-arid regions currently face increased aridity, it is then likely that bat populations living therein could experience similar threats.

Anthropogenic alteration of riverine habitats represents an additional challenge for bat conservation. Chapter 2 provides evidence on the importance of the Sabor valley as foraging ground for the local bat community, particularly during the dry summer season. Although I do not have any information about habitat use after the river impoundment, I would expect the extensive loss of foraging habitat to negatively affect the local bat community. Loss of foraging habitat will reduce carrying capacity at a regional level, and even though bats are able to track spatiotemporal variations in resource distribution (Chapter 2), the need to cover longer distances from their roosts will result in higher energy consumption. This effect will be further exacerbated by the reduction in overall water availability due to climate change and can be especially relevant for breeding females. During late pregnancy females cover shorter distances from roosts due to the increase energetic costs of flying, while foraging bouts during lactation are shorter because females need to return to their roosts multiple times to feed their pups (Henry et al., 2002). Thus, successful pregnancy, as well as, female and juvenile survival is likely to decrease if breeding female bats are forced to fly further away from their roosts and through longer periods to find food and water. This effect will be even more dramatic in the context of warming climate, due to the combined effect of increased evaporative water loss and lower water availability (Adams, 2010).

To reduce the distance travelled, bats inhabiting semi-arid regions might move to roosts closer to water and together with the reduction of regional roost availability due to the predicted increase in river impoundments (i.e. hydropower) this can bring drastic consequences to local populations (Chapter 4). Social links between individuals may be broken with the arrival of new individuals that could disrupt social structure at already occupied roosts. Such effects may hamper the benefits of social stability, such as improved foraging efficiency, increased reproductive success and reduced predation (Altringham & Senior, 2006), with potential effects for long-term population persistence. Moreover, because there is a limit to the number of individuals that can occupy a roost, and at the same time not all roosts offer the conditions needed to successful breeding (Kerth et al., 2001; Lausen & Barclay, 2003), individuals could be forced to move to poor quality roosts or roosts far apart from their previous location. Both events can negatively affect local populations by either leading to lower reproductive success or reducing local populations. The search for a new roost will also bring additionally energetic costs potentially affecting adult survival and reproductive success (Brigham & Fenton, 1986).

6.3.2 **Management guidelines**

Water provision for wildlife through artificial water bodies is particularly relevant in the context of arid and semi-arid systems (Krausman et al., 2006), and many studies have shown how small artificial water ponds are frequently used by bats (e.g. Russo et al., 2012; Lisón & Calvo, 2014; López-González et al., 2015). Creating small artificial permanent water bodies will provide drinking water through all the season, including the critical reproduction and lactation periods. During natural seasonal droughts, they will compensate water scarcity and this will be particularly relevant in exceptional dry years. By creating small artificial water bodies near roosts, bats will decrease the distance travelled in search for water, thus reducing water loss through evapotranspiration. This measure will be even more relevant considering that bats need to travel greater distances during warmer periods, when water scarcity peaks. Placing such structures near breeding roosts will allow closer access to water during pregnancy and lactation bringing increased benefits for species conservation. Implementing a network of small artificial permanent water bodies across the landscape can significantly contribute for species persistence in arid and semi-arid regions, where water stress is predicted to increase.

Additionally, a network of well-designed ponds can provide a series of favourable habitats for invertebrate species that are scattered across the landscape according to their ecological requirements (Céréghino et al., 2007) and offers an extra feeding source for insectivorous species. Having these artificial ponds close to the roosts will bring increased benefits for bats, since it will allow reducing the energy costs of tracking prey across the landscape. Planning a network of small artificial water bodies at a regional scale beforehand can prevent the abandonment of breeding roosts that are otherwise critical for species safeguard. Due to the AHBS dam, a reduction on the abundance and diversity of aquatic insects at a local scale may also occur (see Section 1.2.1 for more details). Thus, small artificial water bodies that mimic natural processes has the adding benefit to restore freshwater biodiversity at landscape level (Chester & Robson, 2013; Biggs et al., 2017).

Results in this thesis support that roost loss among crevice-dwellers probably occurred in the study area due to flooding of the river valley, producing an immediate effect over local populations (Chapter 4). Whenever human activities lead to roost loss, creating artificial roosts is among the most widely used mitigation measure (Mering & Chambers, 2014), although very few studies addressed its’ efficacy (Brittingham & Williams, 2000; Lourenço & Palmeirim, 2004). Notwithstanding, the use of road bridges as roosts has been reported all over the world (Keeley & Tuttle, 1999; Ferrara & Leberg, 2005; Amorim et al., 2013), and they seem to provide roosting opportunities for species showing very different requirements (Figure 6.1), including large colonies of crevice-dweller species (this thesis; Amorim et al., 2013). Dams often lead to the flooding of parts of the road network, in such circumstances the connections need to be re-established and frequently new bridges have to be built to overcome the flooded areas, especially in regions with rugged terrain (i.e. with cliffs and ravines). Whenever river impoundments imply the construction of new bridges, the engineering project should include bat friendly options. To my best knowledge, there are few studies focusing on the features that promote the use of bridges by bats, although some species seem to prefer warmer parts of the bridge that are also distant from the edge (Ferrara & Leberg, 2005), while occupation will dependent on whether the bridge was occupied in the previous year (Bennett et al., 2008). However, roost occupation is likely to be very site specific and will depend on the species using bridges in the affected area. That said the new bridges should at least try to mimic those already occupied.

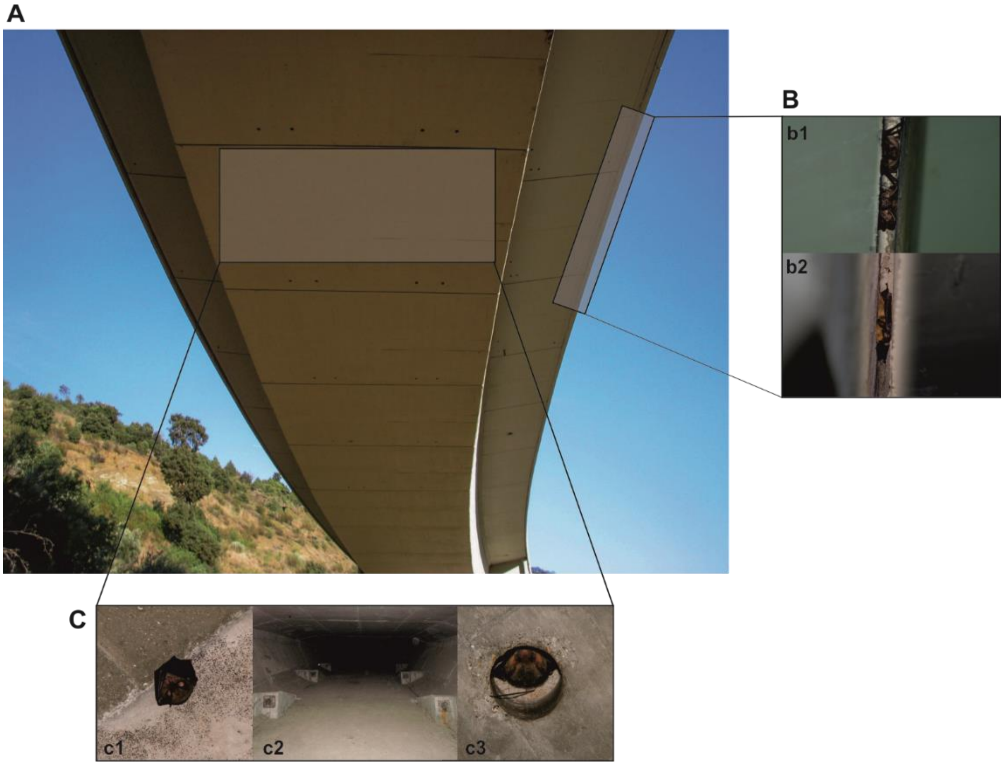

Figure 6.1 – (A) Example of different roosting opportunities provided by road bridges. (B) Crevices used by *Tadarida teniotis* (b1) and *Eptesicus serotinus* (b2). (C) Box girder used by cave-dwellers such as *Rhinolophus ferrumequinum* (c1) and *Myotis myotis* (c3); inside view of the box girder is also shown (c2).

As discussed in Chapter 4, it is possible that individuals losing their roosts are especially attracted to already occupied roosts. Installing artificial roosts in occupied bridges will increase roost availability while reducing the pressure over the resident colonies. Although there is very little experience about artificial roosts for crevice-dweller bat species in Europe, Keeley & Tuttle (1999) provide some examples that have been used in USA bridges for *T. brasiliensis*, the congeneric species of *T. teniotis*. Likewise, large bat houses have been installed in the USA providing alternative roosts to *T. brasiliensis*, some of which showing high occupation (e.g. University of Florida Bat House www.floridamuseum.ufl.edu/bats/). Although we should first invest money and efforts in solutions that have already proved their effectiveness in the specific context (e.g. bridges), the installation of similar bat boxes close to areas where loss of roosts is predicted to occur might prove useful. Finally, we should allow time for the individuals to find these new alternative roosts and regardless of the solution adopted, it should be implemented way before the loss of roosts occurs.

Box 6.1 – Summary of management guidelines to promote the conservation of bat species living in semi-arid regions and facing multiple climate change and anthropogenic threats.

Create a network of small artificial water bodies

- Provides extra drinking and foraging opportunities for bats all year-round and will compensate seasonal water scarcity
- If placed near roosts allows decreasing the distance travelled and reduces evaporative water loss, especially relevant for breeding females
- Promote invertebrate abundance and diversity and provides an extra food source

Minimize the impacts of the hydopower

- Small artificial water bodies that mimic natural processes can help to restore the loss of freshwater biodiversity at a landscape level

Increase roost availability for crevice dwellers before roost loss event

- Construction of new bridges should include bat friendly options that mimic the features of already occupied bridges
- Installing artificial roosts in already occupied bridges
- Installing large bat boxes (e.g. University of Florida Bat House www.floridamuseum.ufl.edu/bats/) close to areas where loss of crevice-dwellers roosts are predicted to occur

6.4 Future research

Understanding the underlying mechanisms leading to biodiversity loss in riverine habitats will be highly relevant to promote the persistence of species. Chapter 2 shows that during seasonal dry periods bats are highly dependent on water bodies and riparian habitats. Although this is likely to reflect the abundance of prey close to water bodies or the need to drink, I was not able to establish a direct link between these mechanisms. Investigating seasonal changes in bats diet alongside with insect prey availability will provide a clear picture on seasonal food webs, and will help to understand the different energetic requirements throughout the season. Moreover, collecting information on the drinking frequency should also be possible through well designed experiments using acoustic sampling, since bats produce specific echolocation calls when drinking (Griffiths, 2013; Russo et al., 2016). The use of miniaturized GPS will allow clarifying the distance travelled in each season and how bats may track resources across the landscape. Together this information will inform management actions, for instance on the home range to consider when installing small artificial water bodies, as well as which prey insects to promote.

As discussed in the previous section, there is very little information on the features promoting the use of bridges by bats, especially in Europe. Due consideration should be given to the characteristics promoting the use of bridges by different species. For instance, studies on the thermal conditions found in different bridges, or even within bridges, solar exposure, crevice size, preferred construction materials, parts of the bridge more often selected and surrounding habitats, will make a valuable contribution to design effective guidelines for future construction of bat-friendly bridges. Research on additional structures that can increase bridge roosting availability will also be a plus, while knowledge on species physiology (e.g. stress) and energetics can further contribute to an effective management of the populations roosting in bridges.

Future studies should also address the limitations faced by reproductive females of species living in semi-arid environments, especially during water stressed periods. It seems clear that water scarcity leads to reproductive failure without necessarily compromising female fitness and survival (Chapter 5, Adams, 2010), and it is well known that breeding females have higher energetic demands (Anthony & Kunz, 1977; Kurta et al., 1990; Dietz & Kalko, 2006). However, increased frequency of dry years can lead to successive reproductive failure and extirpation of local populations. Thus, priority must be given to understand inter-annual differences in diet composition between reproductive and non-reproductive females, as well as how these differences relate to climatic conditions.

Finally, Chapter 4 and 5 show the demographic effects of both extreme climatic events and landscape alteration acting in the same population over short time intervals. The cumulative impacts of both disturbance events can result in species declines occurring faster than otherwise predicted (Selwood et al., 2015). Only through long-term monitoring of the regional population and by increasing spatial coverage, we will be able to understand the true consequences of these events. Ultimately, the impacts of human mediated activities on a population arise from changes to the demographic parameters and should be investigated with long-term population monitoring, to assess population turnover, immigration and reproductive activity (Henry et al., 2007). Regarding bats, future studies should focus on the effects of these pressures in first year survival, juvenile dispersal, and long-term reproductive success.

Biodiversity conservation in riverine habitats of semi-arid regions is a daunting challenge, but understanding changes on species demographic traits in face of its’ main threats is definitely an excellent starting point.

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Appendix A

Paper Proofs

Following the water? Landscape-scale temporal changes in bat spatial distribution in relation to Mediterranean summer drought

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ORIGINAL RESEARCH

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Following the water? Landscape-scale temporal changes in bat spatial distribution in relation to Mediterranean summer drought

Francisco Amorim[1,2,3] | Inês Jorge[3] | Pedro Beja[1,2] | Hugo Rebelo[1,2,4]

[1] CIBIO-InBIO, Research Center in Biodiversity and Genetic Resources, University of Porto, Porto, Portugal

[2] CEABN-InBIO, Centre for Applied Ecology "Prof. Baeta Neves", Institute of Agronomy, University of Lisbon, Lisbon, Portugal

[3] Faculty of Sciences, University of Porto, Porto, Portugal

[4] School of Biological Sciences, University of Bristol, Bristol, UK

Correspondence
Francisco Amorim, CIBIO-InBIO, Research Center in Biodiversity and Genetic Resources, University of Porto, Porto, Portugal.
Email: famorim@cibio.up.pt

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Abstract

Understanding how the spatial distribution of ecological resources shapes species' diversity and abundance in human-modified landscapes is a central theme in conservation biology. However, studies often disregard that such patterns may vary over time, thereby potentially missing critical environmental constraints to species persistence. This may be particularly important in highly mobile species such as bats, which are able to track temporal variations in spatial resource distribution. Here we test the hypothesis that bats in Mediterranean landscapes are strongly affected by the progressive reduction in water availability during the seasonal summer drought. We analyzed the effects of landscape composition and structure on bat diversity and activity, during pregnancy, lactation, and postlactation periods, and identified the most influential variables within and across periods. Water bodies showed the strongest positive effect on bats, followed by riparian habitats and areas with steeper (>30%) slopes. However, while during pregnancy, there were only small landscape effects, these increased during lactation and postlactation, highlighting a progressively stronger association with water habitats during the summer drought. The spatial projection of habitat models showed that the landscape distribution of bat diversity and activity hotspots changed markedly over time. During pregnancy, the spatial pattern of hotspot distribution was weakly defined, while during lactation and particularly postlactation, there was a concentration of hotspots along permanently flowing watercourses. Our study highlights that permanently flowing watercourses are critical for bat conservation in Mediterranean landscapes, calling for measures to counteract their ongoing degradation due in particular to climate change, water abstraction and damming. More generally, our study underlines the importance of considering the temporal dimension in habitat selection studies, without which there is the risk of overlooking the importance of habitats that are key for species persistence only at certain times of the year.

KEYWORDS
acoustic monitoring, habitat use, landscape management, resource tracking, species diversity, water scarcity

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Evolutionary history of the European free-tailed-bat, a tropical affinity species spanning across the Mediterranean Basin

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ORIGINAL ARTICLE

WILEY

Evolutionary history of the European free-tailed bat, a tropical affinity species spanning across the Mediterranean Basin

Francisco Amorim[1,2] | Orly Razgour[3] | Vanessa Mata[1,2] | Susana Lopes[1] | Raquel Godinho[1,2,4] | Carlos Ibáñez[5] | Javier Juste[5] | Stephen J. Rossiter[6] | Pedro Beja[1,7] | Hugo Rebelo[1,7]

[1]CIBIO-InBIO, Research Center in Biodiversity and Genetic Resources, University of Porto, Vairão, Portugal
[2]Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Porto, Portugal
[3]Biological Sciences, University of Southampton, Southampton, UK
[4]Department of Zoology, University of Johannesburg, South Africa
[5]Estación Biológica de Doñana (CSIC), Sevilla, Spain
[6]School of Biological & Chemical Sciences, Queen Mary University of London, London, UK
[7]CIBIO-InBIO, Research Center in Biodiversity and Genetic Resources, Institute of Agronomy, University of Lisbon, Lisbon, Portugal

Correspondence
Francisco Amorim, CIBIO-InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Campus de Vairão, Rua Padre Armando Quintas, no 7, 4485-661 Vairão, Portugal.
Email: famorim@cibio.up.pt

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Abstract
The Mediterranean Basin is a global biodiversity hotspot, hosting a number of native species belonging to families that are found almost exclusively in tropical climates. Yet, whether or not these taxa were able to survive in the Mediterranean region during the Quaternary climatic oscillations remains unknown. Focusing on the European free-tailed bat (*Tadarida teniotis*), we aimed to (a) identify potential ancient populations and glacial refugia; (b) determine the post-glacial colonization routes across the Mediterranean; and (c) evaluate current population structure and demography. Mitochondrial and nuclear markers were used to understand *T. teniotis* evolutionary and demographic history. We show that *T. teniotis* is likely restricted to the Western Palearctic, with mitochondrial phylogeny suggesting a split between an Anatolian/Middle East clade and a European clade. Nuclear data pointed to three genetic populations, one of which is an isolated and highly differentiated group in the Canary Islands, another distributed across Iberia, Morocco, and France, and a third stretching from Italy to the east, with admixture following a pattern of isolation by distance. Evolutionary and demographic reconstruction supports a pre-Last Glacial Maximum (LGM) colonization of Italy and the Anatolian/Middle East, while the remaining populations were colonized from Italy after the Younger Dryas. We also found support for demographic expansion following the Iberian colonization. The results show that during the LGM *T. teniotis* persisted in Mediterranean refugia and has subsequently expanded to its current circum-Mediterranean range. Our findings raise questions regarding the physiological and ecological traits that enabled species with tropical affinities to survive in colder climates.

KEYWORDS
bat, demographic history, Molossidae, phylogeography, population structure

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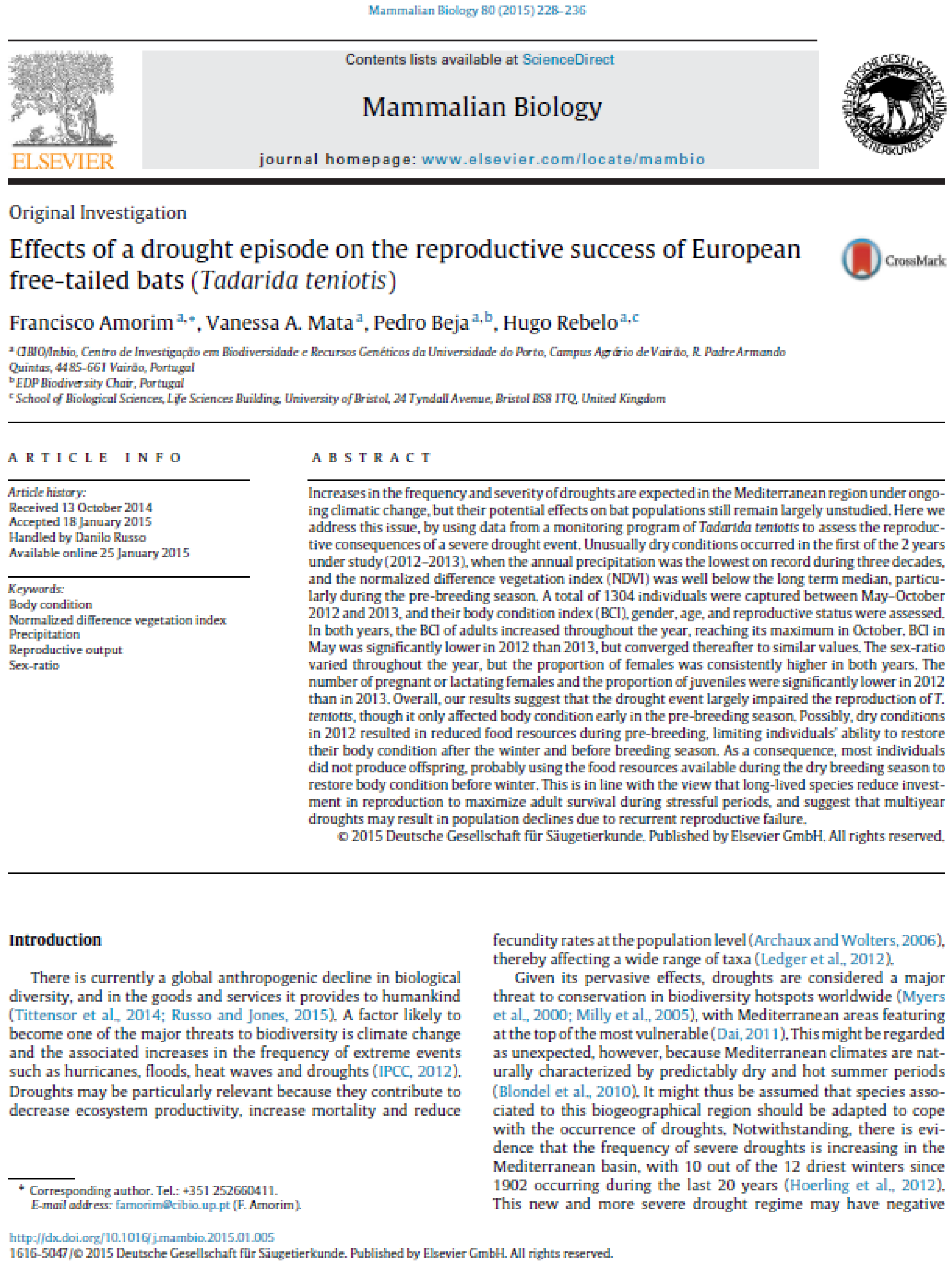
Contributing authors: Orly Razgour (Orly.Razgour@soton.ac.uk); Vanessa Mata (vanessamata@cibio.up.pt); Susana Lopes (susana.lopes@cibio.up.pt); Raquel Godinho (rgodinho@cibio.up.pt); Carlos Ibáñez (ibanez@ebd.csic.es); Javier Juste (juste@ebd.csic.es); Stephen J. Rossiter (s.j.rossiter@qmul.ac.uk); Pedro Beja (pbeja@cibio.up.pt); Hugo Rebelo (hugo_reb@sapo.pt)

Effects of a drought episode on the reproductive success of European free-tailed bats (*Tadarida teniotis*)

Amorim F., Mata V.A., Beja P., & Rebelo H. (2015) Effects of a drought episode on the reproductive success of European free-tailed bats (*Tadarida teniotis*). *Mammalian Biology - Zeitschrift für Säugetierkunde*, **80**(3), 228–236.



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Original Investigation

Effects of a drought episode on the reproductive success of European free-tailed bats (*Tadarida teniotis*)

Francisco Amorim[a,*], Vanessa A. Mata[a], Pedro Beja[a,b], Hugo Rebelo[a,c]

[a] *CIBIO/Inbio, Centro de Investigação em Biodiversidade e Recursos Genéticos da Universidade do Porto, Campus Agrário de Vairão, R. Padre Armando Quintas, 4485-661 Vairão, Portugal*
[b] *EDP Biodiversity Chair, Portugal*
[c] *School of Biological Sciences, Life Sciences Building, University of Bristol, 24 Tyndall Avenue, Bristol BS8 1TQ, United Kingdom*

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Body condition
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ABSTRACT

Increases in the frequency and severity of droughts are expected in the Mediterranean region under ongoing climatic change, but their potential effects on bat populations still remain largely unstudied. Here we address this issue, by using data from a monitoring program of *Tadarida teniotis* to assess the reproductive consequences of a severe drought event. Unusually dry conditions occurred in the first of the 2 years under study (2012–2013), when the annual precipitation was the lowest on record during three decades, and the normalized difference vegetation index (NDVI) was well below the long term median, particularly during the pre-breeding season. A total of 1304 individuals were captured between May–October 2012 and 2013, and their body condition index (BCI), gender, age, and reproductive status were assessed. In both years, the BCI of adults increased throughout the year, reaching its maximum in October. BCI in May was significantly lower in 2012 than 2013, but converged thereafter to similar values. The sex-ratio varied throughout the year, but the proportion of females was consistently higher in both years. The number of pregnant or lactating females and the proportion of juveniles were significantly lower in 2012 than in 2013. Overall, our results suggest that the drought event largely impaired the reproduction of *T. teniotis*, though it only affected body condition early in the pre-breeding season. Possibly, dry conditions in 2012 resulted in reduced food resources during pre-breeding, limiting individuals' ability to restore their body condition after the winter and before breeding season. As a consequence, most individuals did not produce offspring, probably using the food resources available during the dry breeding season to restore body condition before winter. This is in line with the view that long-lived species reduce investment in reproduction to maximize adult survival during stressful periods, and suggest that multiyear droughts may result in population declines due to recurrent reproductive failure.

Introduction

There is currently a global anthropogenic decline in biological diversity, and in the goods and services it provides to humankind (Tittensor et al., 2014; Russo and Jones, 2015). A factor likely to become one of the major threats to biodiversity is climate change and the associated increases in the frequency of extreme events such as hurricanes, floods, heat waves and droughts (IPCC, 2012). Droughts may be particularly relevant because they contribute to decrease ecosystem productivity, increase mortality and reduce fecundity rates at the population level (Archaux and Wolters, 2006), thereby affecting a wide range of taxa (Ledger et al., 2012).

Given its pervasive effects, droughts are considered a major threat to conservation in biodiversity hotspots worldwide (Myers et al., 2000; Milly et al., 2005), with Mediterranean areas featuring at the top of the most vulnerable (Dai, 2011). This might be regarded as unexpected, however, because Mediterranean climates are naturally characterized by predictably dry and hot summer periods (Blondel et al., 2010). It might thus be assumed that species associated to this biogeographical region should be adapted to cope with the occurrence of droughts. Notwithstanding, there is evidence that the frequency of severe droughts is increasing in the Mediterranean basin, with 10 out of the 12 driest winters since 1902 occurring during the last 20 years (Hoerling et al., 2012). This new and more severe drought regime may have negative

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[*] Corresponding author. Tel.: +351 252660411.
E-mail address: famorim@cibio.up.pt (F. Amorim).

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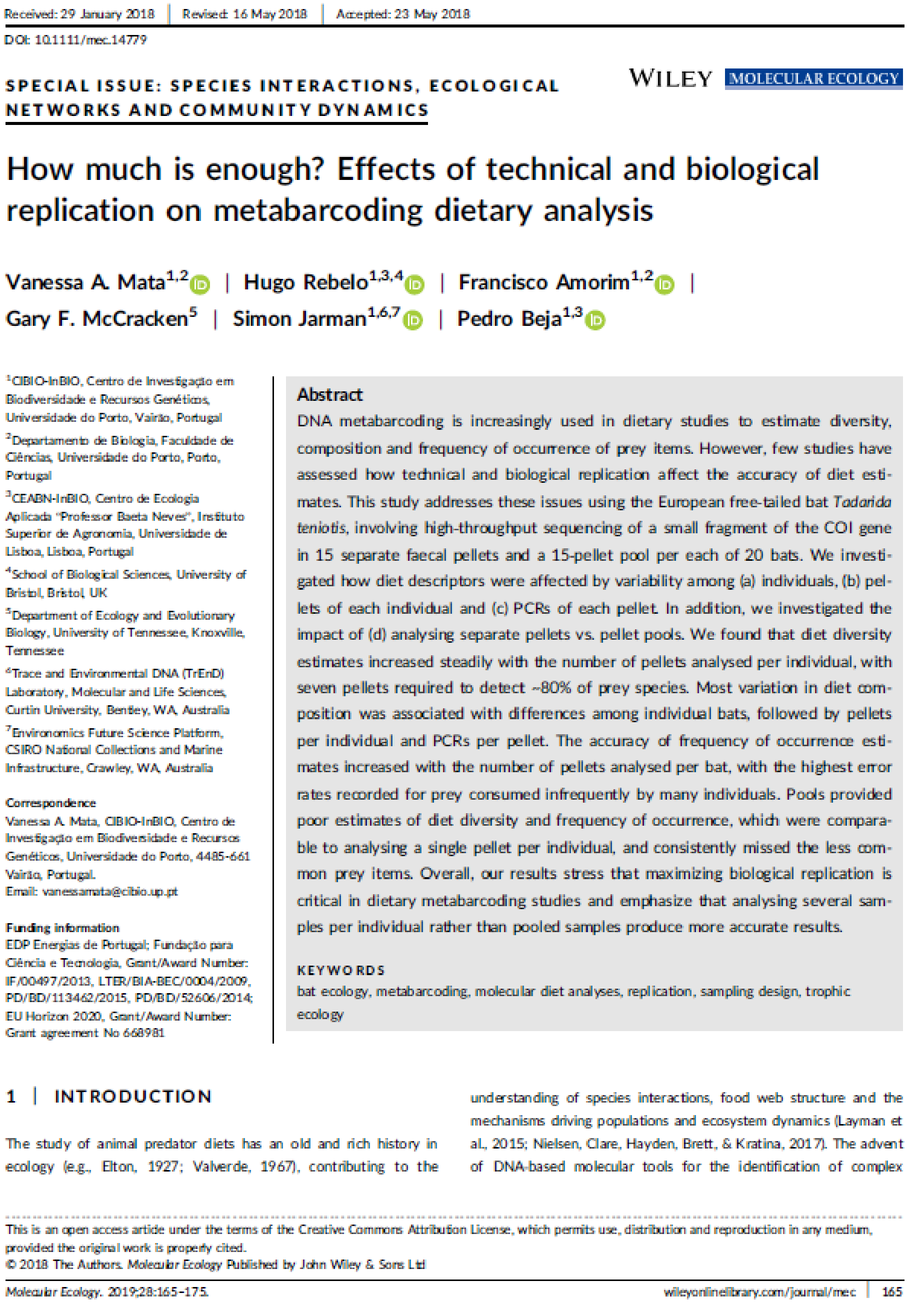
Appendix B

Other papers published during the PhD

How much is enough? Effects of technical and biological replication on metabarcoding dietary analysis

Mata V.A., Rebelo H., Amorim F., McCracken G.F., Jarman S., & Beja P. (2019) How much is enough? Effects of technical and biological replication on metabarcoding dietary analysis. *Molecular Ecology*, **28**(2), 165–175.



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SPECIAL ISSUE: SPECIES INTERACTIONS, ECOLOGICAL NETWORKS AND COMMUNITY DYNAMICS

WILEY MOLECULAR ECOLOGY

How much is enough? Effects of technical and biological replication on metabarcoding dietary analysis

Vanessa A. Mata[1,2] | Hugo Rebelo[1,3,4] | Francisco Amorim[1,2] | Gary F. McCracken[5] | Simon Jarman[1,6,7] | Pedro Beja[1,3]

[1]CIBIO-InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Vairão, Portugal
[2]Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Porto, Portugal
[3]CEABN-InBIO, Centro de Ecologia Aplicada "Professor Baeta Neves", Instituto Superior de Agronomia, Universidade de Lisboa, Lisboa, Portugal
[4]School of Biological Sciences, University of Bristol, Bristol, UK
[5]Department of Ecology and Evolutionary Biology, University of Tennessee, Knoxville, Tennessee
[6]Trace and Environmental DNA (TrEnD) Laboratory, Molecular and Life Sciences, Curtin University, Bentley, WA, Australia
[7]Environomics Future Science Platform, CSIRO National Collections and Marine Infrastructure, Crawley, WA, Australia

Correspondence
Vanessa A. Mata, CIBIO-InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, 4485-661 Vairão, Portugal.
Email: vanessamata@cibio.up.pt

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Abstract

DNA metabarcoding is increasingly used in dietary studies to estimate diversity, composition and frequency of occurrence of prey items. However, few studies have assessed how technical and biological replication affect the accuracy of diet estimates. This study addresses these issues using the European free-tailed bat *Tadarida teniotis*, involving high-throughput sequencing of a small fragment of the COI gene in 15 separate faecal pellets and a 15-pellet pool per each of 20 bats. We investigated how diet descriptors were affected by variability among (a) individuals, (b) pellets of each individual and (c) PCRs of each pellet. In addition, we investigated the impact of (d) analysing separate pellets vs. pellet pools. We found that diet diversity estimates increased steadily with the number of pellets analysed per individual, with seven pellets required to detect ~80% of prey species. Most variation in diet composition was associated with differences among individual bats, followed by pellets per individual and PCRs per pellet. The accuracy of frequency of occurrence estimates increased with the number of pellets analysed per bat, with the highest error rates recorded for prey consumed infrequently by many individuals. Pools provided poor estimates of diet diversity and frequency of occurrence, which were comparable to analysing a single pellet per individual, and consistently missed the less common prey items. Overall, our results stress that maximizing biological replication is critical in dietary metabarcoding studies and emphasize that analysing several samples per individual rather than pooled samples produce more accurate results.

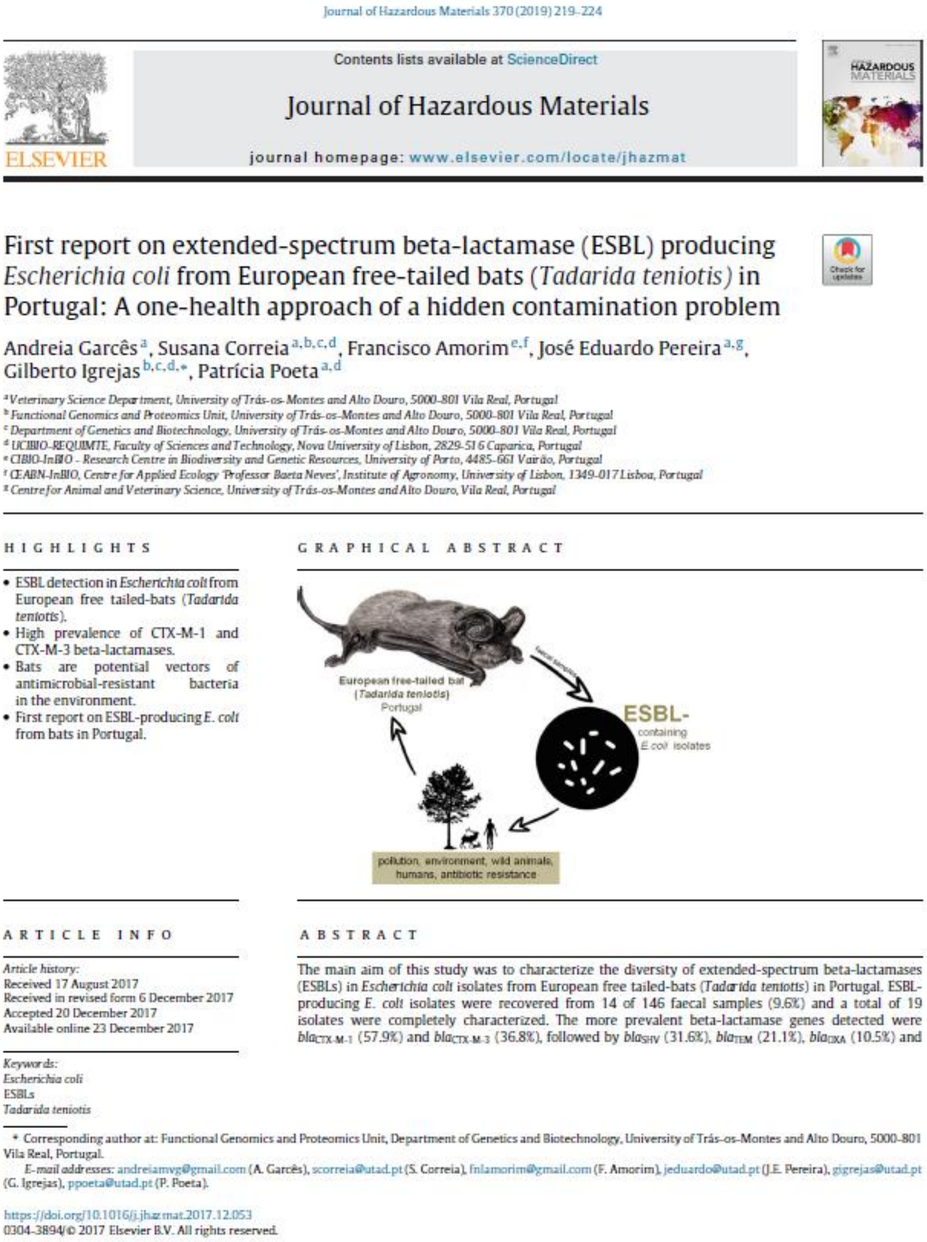
KEYWORDS
bat ecology, metabarcoding, molecular diet analyses, replication, sampling design, trophic ecology

1 | INTRODUCTION

The study of animal predator diets has an old and rich history in ecology (e.g., Elton, 1927; Valverde, 1967), contributing to the understanding of species interactions, food web structure and the mechanisms driving populations and ecosystem dynamics (Layman et al., 2015; Nielsen, Clare, Hayden, Brett, & Kratina, 2017). The advent of DNA-based molecular tools for the identification of complex

First report on extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* from European free-tailed bats (*Tadarida teniotis*) in Portugal: A one-health approach of a hidden contamination problem

Garcês A., Correia S., Amorim F., Pereira J.E., Igrejas G., & Poeta P. (2019) First report on extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* from European free-tailed bats (Tadarida teniotis) in Portugal: A one-health approach of a hidden contamination problem. *Journal of Hazardous Materials*, **370**, 219–224.



Journal of Hazardous Materials 370 (2019) 219–224

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First report on extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* from European free-tailed bats (*Tadarida teniotis*) in Portugal: A one-health approach of a hidden contamination problem

Andreia Garcês[a], Susana Correia[a,b,c,d], Francisco Amorim[e,f], José Eduardo Pereira[a,g], Gilberto Igrejas[b,c,d,*], Patrícia Poeta[a,d]

[a] *Veterinary Science Department, University of Trás-os-Montes and Alto Douro, 5000-801 Vila Real, Portugal*
[b] *Functional Genomics and Proteomics Unit, University of Trás-os-Montes and Alto Douro, 5000-801 Vila Real, Portugal*
[c] *Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, 5000-801 Vila Real, Portugal*
[d] *UCIBIO-REQUIMTE, Faculty of Sciences and Technology, Nova University of Lisbon, 2829-516 Caparica, Portugal*
[e] *CIBIO-InBIO - Research Centre in Biodiversity and Genetic Resources, University of Porto, 4485-661 Vairão, Portugal*
[f] *CEABN-InBIO, Centre for Applied Ecology 'Professor Baeta Neves', Institute of Agronomy, University of Lisbon, 1349-017 Lisboa, Portugal*
[g] *Centre for Animal and Veterinary Science, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal*

HIGHLIGHTS

- ESBL detection in *Escherichia coli* from European free tailed-bats (*Tadarida teniotis*).
- High prevalence of CTX-M-1 and CTX-M-3 beta-lactamases.
- Bats are potential vectors of antimicrobial-resistant bacteria in the environment.
- First report on ESBL-producing *E. coli* from bats in Portugal.

GRAPHICAL ABSTRACT

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ABSTRACT

The main aim of this study was to characterize the diversity of extended-spectrum beta-lactamases (ESBLs) in *Escherichia coli* isolates from European free tailed-bats (*Tadarida teniotis*) in Portugal. ESBL-producing *E. coli* isolates were recovered from 14 of 146 faecal samples (9.6%) and a total of 19 isolates were completely characterized. The more prevalent beta-lactamase genes detected were $bla_{\text{CTX-M-1}}$ (57.9%) and $bla_{\text{CTX-M-3}}$ (36.8%), followed by bla_{SHV} (31.6%), bla_{TEM} (21.1%), bla_{OXA} (10.5%) and

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* Corresponding author at: Functional Genomics and Proteomics Unit, Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, 5000-801 Vila Real, Portugal.

E-mail addresses: andreiamvg@gmail.com (A. Garcês), scorreia@utad.pt (S. Correia), fnlamorim@gmail.com (F. Amorim), jeduardo@utad.pt (J.E. Pereira), gigrejas@utad.pt (G. Igrejas), ppoeta@utad.pt (P. Poeta).

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First complete mitochondrial genomes of molossid bats (Chiroptera: Molossidae)

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MITOCHONDRIAL DNA PART B: RESOURCES, 2017
VOL. 2, NO. 1, 152–154
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MITO COMMUNICATION

OPEN ACCESS

First complete mitochondrial genomes of molossid bats (Chiroptera: Molossidae)

Vanessa A. Mata[a,b], Francisco Amorim[a,b], Antonio Guillén-Servent[c], Pedro Beja[a,d] and Hugo Rebelo[a,d,e]

[a]CIBIO-InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Vairão, Portugal; [b]Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Porto, Portugal; [c]Instituto de Ecología, Xalapa, México; [d]CEABN-InBIO, Centro de Ecologia Aplicada 'Professor Baeta Neves', Instituto Superior de Agronomia, Universidade de Lisboa, Lisboa, Portugal; [e]School of Biological Sciences, University of Bristol, Bristol, UK

ABSTRACT
Bats represent around one-fourth of the world's mammals and their taxonomy is still controversial. Molossids are one of the most diverse bat families with a wide knowledge gap. In this study, we report the first complete mitochondrial genomes of three molossid bats: the European free-tailed bat *Tadarida teniotis*, the La Touche's free-tailed bat *Tadarida latouchei*, and the Wrinkle-lipped free-tailed bat *Chaerephon plicatus*. The mitogenomes are 16,869 and 16,784 bp long for *T. teniotis* and *T. latouchei*, respectively, while in *C. plicatus* it is at least 16,216 bp although the control region was not fully recovered due to its higher divergence from *T. teniotis*. The genomes show conserved synteny with other mammalian mitogenomes, containing 13 protein-coding genes, 2 ribosomal RNA genes, 22 transfer RNA genes, and 1 control region (d-loop). All protein-coding genes start with the ATG start codon, except for ND2, ND3, and ND5 which begin with ATA or ATT. Eleven protein-coding genes terminated in a canonical stop codon, TAA or TAG, two contain incomplete stop codons, T or TA. Cytochrome b terminates in the mitochondria-specific stop codon AGA. These mitogenomes provide a valuable resource for future studies of Molossidae and other bat and mammal species.

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KEYWORDS
Molossidae; mitogenome; *Tadarida teniotis*; *Tadarida latouchei*; *Chaerephon plicatus*

Molossidae is the fourth largest bat family (Mammalia, Chiroptera), with ~100 species divided into 17 genera (IUCN 2017). Although molossids are distributed throughout tropical and subtropical regions of the world, the range of most species is poorly known. Many species are similar in appearance and difficult to capture due to their fast and high-flying behaviours (Vaughan 1966). This has led to an under-representation of this family in museum collections and several taxonomic inconsistencies (Ammerman et al. 2012). In the Eurasian region there are at least three recognized species of free-tailed bats: *Tadarida teniotis* complex (composed of *Tadarida teniotis*, *Tadarida insignis*, *Tadarida latouchei*, and *Tadarida coecata*), *Tadarida aegyptiaca* and *Chaerephon plicatus* (Hutson et al. 2001). The *teniotis* species group has been subject to great debate, with some authors considering *insignis*, *latouchei* and *coecata* as subspecies of *teniotis*, while more recently others consider *insignis* and *latouchei* as full species, and *coecata* as a subspecies of *insignis* (Simmons 2005). Morphological analysis of *teniotis*, *insignis*, and *latouchei*, have found consistent differences, suggesting the full species status for each group, and therefore the restriction of *teniotis* to the west of India, and *insignis* to further east (Funakoshi & Kunisaki 2000). However, to date this classification has lacked any molecular support. In fact, there are no available mitogenomes for any species of the family Molossidae. Our results provide useful reference for future phylogenetic and phylogeographic studies.

Genomic DNA was extracted from *T. teniotis* (Portugal, 41.287 N 6.873 W), *T. latouchei* (Laos, 20.153 N 103.407 E, voucher ROM MAM 118321), and *C. plicatus* (Laos, 20.723 N 101.138 E, voucher ROM MAM 118373) tissue samples using QIAamp DNA *Micro Kit* (QIAGEN). Due to the degraded state of *T. latouchei* and *C. plicatus* samples, a mitochondrial capture-based protocol was used. Specific primers for *T. teniotis* were designed in order to amplify 2 overlapping fragments of ~10kb (MtF13 (5′-TGCATTACACATCCGACACA-3′) with MtR12 (5′-GGCTTTGAAGGTCCTTGGTC-3′), and MtF12 (5′-CGGCTAACATACGCTACATCC-3′) with MtR13 (5′-GCCTATGAAGGCAGTGGCTA-3′) using Takara LA *Taq* polymerase. Custom primers had to be designed due to several problems in the amplification process related to nuclear copies of mitochondrial genes. Complete mitochondrial mitogenomes of each sample were then captured following the protocol by Maricic et al. (2010) and sequenced with 250 bp paired end reads in Illumina MiSeq (Vairão, Portugal). Due to the high divergence between *T. teniotis* and *C. plicatus*, the control region d-loop of *C. plicatus* mitochondria was not fully recovered. The mitogenomes (Genbank accession no. KY581660/61/62) were assembled *de novo* and annotated using Geneious 9.1.5 (Kearse et al. 2012). A neighbour-joining tree was built with the Tamura–Nei distance to reconstruct the phylogeny of the

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CONTACT Vanessa A. Mata vanessamata@cibio.up.pt CIBIO-InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Vairão 4485-661, Portugal

Female dietary bias towards large migratory moths in the European free-tailed bat (*Tadarida teniotis*)

Mata V.A., Amorim F., Corley M.F. V, McCracken G.F., Rebelo H., & Beja P. (2016) Female dietary bias towards large migratory moths in the European free-tailed bat (*Tadarida teniotis*). *Biology Letters*, **12**(3), 20150988.

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Author for correspondence:
Vanessa A. Mata
e-mail: vanessamata@cibio.up.pt

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THE ROYAL SOCIETY
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Animal behaviour

Female dietary bias towards large migratory moths in the European free-tailed bat (*Tadarida teniotis*)

Vanessa A. Mata[1], Francisco Amorim[1], Martin F. V. Corley[1], Gary F. McCracken[2], Hugo Rebelo[1,3,4] and Pedro Beja[1,4]

[1]CIBIO-InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, 4485-661 Vairão, Portugal
[2]Department of Ecology and Evolutionary Biology, University of Tennessee, Knoxville, TN 37996, USA
[3]School of Biological Sciences, University of Bristol, Bristol BS8 1UG, UK
[4]CEABN-InBIO, Centro de Ecologia Aplicada 'Professor Baeta Neves', Instituto Superior de Agronomia, Universidade de Lisboa, 1349-017 Lisboa, Portugal

VAM, 0000-0003-3005-9030; FA, 0000-0002-7731-9242; GFM, 0000-0002-2493-8103; HR, 0000-0002-7118-4068; PB, 0000-0001-8164-0760

In bats, sexual segregation has been described in relation to differential use of roosting and foraging habitats. It is possible that variation may also exist between genders in the use of different prey types. However, until recently this idea was difficult to test owing to poorly resolved taxonomy of dietary studies. Here, we use high-throughput sequencing to describe gender-related variation in diet composition of the European free-tailed bat (*Tadarida teniotis*), while controlling for effects of age and season. We analysed guano pellets collected from 143 individuals mist-netted from April to October 2012 and 2013, in northeast Portugal. Moths (Lepidoptera; mainly Noctuidae and Geometridae) were by far the most frequently recorded prey, occurring in nearly all samples and accounting for 96 out of 115 prey taxa. There were significant dietary differences between males and females, irrespective of age and season. Compared to males, females tended to consume larger moths and more moths of migratory behaviour (e.g. *Autographa gamma*). Our study provides the first example of gender-related dietary variation in bats, illustrating the value of novel molecular tools for revealing intraspecific variation in food resource use in bats and other insectivores.

1. Introduction

Sexual segregation in resource use is common in vertebrates [1]. Segregation is often associated with morphological and behavioural differences between sexes, which in turn affect a number of ecological and life-history traits such as home range, habitat selection, diet, foraging behaviour and survival rates [1]. Therefore, research on sexual segregation and its underlying causes is important to understand vertebrate ecology, demography and evolution, with implications in wildlife management and conservation.

In bats, most species do not exhibit obvious sexual dimorphism, but segregation between sexes has been described in relation to roosting and foraging habitat use, particularly during the maternity season [1]. In temperate bats, females tend to use warmer roosts for maximizing fetal growth rate and milk production [2], while males tend to choose colder roosts to make use of torpor and maximize energy saving [3]. In some species, females also tend to forage closer to roosts [4–6], as this seems to be more cost-efficient and can lead to lower infant mortality [7], where males seem to be forced to feed away from breeding areas, thereby reducing potential competition with females [8]. As a result, it is possible that segregation may also occur in the use of different prey types [9]. Testing this

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ORIGINAL PAPER

Influence of past agricultural fragmentation to the genetic structure of *Juniperus oxycedrus* in a Mediterranean landscape

Manuel Curto[1,3] · **Marcos Nogueira**[1,2] · **Pedro Beja**[1] · **Francisco Amorim**[1] · **Maximiliane Schümann**[2,3] · **Harald Meimberg**[1,2,3]

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Abstract Habitat fragmentation can have a profound effect on the genetic diversity of forest species. These effects are especially interesting when forests previously fragmented by agriculture start to reconnect due to land abandonment. In this study, we investigate the genetic structure and diversity patterns of *Juniperus oxycedrus* populations from the Sabor river valley in Northeast Portugal. We developed 17 microsatellite markers using pyrosequencing technology as implemented in the 454 platform. As expected, among population differentiation was low with high variability within populations. There was no strong pattern of genetic structure in our analyses (F_{ST}=0.018) suggesting that the individuals analyzed here belong to one population. The genetic structure seems to be equally explained by locality and by tree age. We hypothesize that this is a consequence of the land use history from the region. After the abandonment of cultivated fields, these terrains were probably colonized by individuals from a few older *J. oxycedrus* populations. Thus, the genetic structure pattern found may best be explained by this recent expansion. This expansion may be currently influenced by the construction of two hydroelectric dams that will flood areas with older individuals of the species.

Keywords *Juniperus oxycedrus* · Microsatellites · Genetic diversity · Land abandonment · Habitat fragmentation · Population structure

Introduction

Habitat fragmentation may strongly affect plant population genetics by resulting in decreased effective population sizes (Ellstrand and Elam 1993) and reduced gene flow among populations (Schaal and Leverich 1996; Couvet 2002), thereby potentially causing inbreeding effects and the loss of genetic diversity (Keller and Waller 2002). Ultimately, this can put species survival at risk and is therefore considered one of the major threats to biodiversity (Young et al. 1996). Fragmentation has a higher impact on organisms with low dispersal ability and organisms that are obligate outcrossing. Autogamous plants are expected to be little affected by fragmentation, while exclusively cross pollinated plants (self-incompatible dioecious plants) would be highly sensitive to fragmentation effects (Berge et al. 1998; Larson and Barrett 2000; Lennartsson 2002). In this group, wind pollinated plants are less affected than animal pollinated plants (Berge et al. 1998; Weidema et al. 2000).

The Mediterranean basin is a hotspot of biodiversity, despite the millennial influence of human populations (Cowling et al. 1996; Blondel et al. 2010). Since the introduction of

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✉ Manuel Curto
mcurto@cibio.up.pt

[1] CIBIO, Research Center in Biodiversity and Genetic Resources, InBio Associate Laboratory, University of Porto, Campus Vairão, Vairão 4485-661, Portugal

[2] Chair of Restoration Ecology, Department Ecology and Ecosystem Management, Technical University of Munich, Emil-Ramann-Straße 6, 85354 Freising, Germany

[3] Institute for Nature Conservation Research, University of Natural Resources and Life Sciences (Boku), Vienna 1180, Austria