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REEDBED AND RIPARIAN PASSERINES: LIVING AND MIGRATION IN A CHANGING WORLD

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Ecology) presented to the University of Coimbra,
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Summary

Wetlands have been declining at the fastest rate of any habitat on earth. The conversion of reedbed areas into agricultural land and large-scale reed diebacks have caused these declines. Reedbeds harbour specialized wildlife, provide important ecosystems services and are economically important. Therefore, the conservation of reedbeds and their unique biota is of crucial importance for nature conservation in Europe. Current conservation strategies in Portugal are to designate wetlands or reedbeds as protected areas, but there is very little information about the importance of reedbeds for top predators such as reedbed passerines. In Portugal, reedbeds are important for breeding species, both resident and migratory, and as a stopover for species migrating between Europe and Africa. Migration is often considered the most energetically demanding and stressful period during the migratory life cycle. Stopover sites are important because they provide migratory birds a place to refuel and rest. Because migrating birds spend much time in stopover sites rather than in flight, it is very important to understand migration ecology during stopover periods. Reedbed passerines stopover in Portuguese reedbeds just after (spring migration) or before (autumn migration) individuals cross important ecological barriers, the Atlantic Ocean and the Sahara desert. The use of reedbed stopover sites by these individuals is particularly important, as crossing those barriers can have a strong influence in successfully completing migration. In Chapter I we investigated the stopover ecology of Reed warblers *Acrocephalus scirpaceus,* in Central Portugal over the last decade. During migration Reed warblers use the resources of reedbeds in Central Portugal extensively and can adjust their migration according to favourable weather conditions. We evaluated several environmental parameters as proxies to understand the stopover ecology and their importance in global migration of Reed warblers. We showed that stopover duration in reedbeds of Central Portugal declined significantly over the last decade, which may be a reflection of improving weather conditions, as a result of climate change, in Northern Europe. However, all migrants face trade-offs between refuelling events or carrying higher fuel reserves. These require different physiological patterns which reflect different migratory strategies. In Chapter II we combined several

physiological parameters to evaluate differences in migration ecology of two warbler species, the sedge warbler and the reed warbler, at a stopover site in Central Portugal. We also evaluated differences between these two migratory species and a resident species, the Cetti's warbler *Cettia cetti*. Through the examination of blood parameters we showed that triglycerides can indicate two different migratory statuses: active migration (high triglycerides levels mobilized from adipose tissue) and refuelling at a stopover. Sedge warblers in active migration showed high triglycerides than reed warblers and reed warblers did not showed fattening activity, even when stopping at reedbeds in Central Portugal. Passerines with different energetic conditions can show different behaviours at stopover locations. Portuguese reed beds are more important for Reed warblers than for Sedge warblers during the autumnal migration, because when Sedge warblers arrive at Portuguese reed beds had sufficient fuel to continue their journey without refueling. Sedge warblers only stop at Portuguese reed beds to rest, but reed warblers extensively use the resources and departure only when weather conditions were favorable to resume their migratory journey.

In Portugal, reedbeds are the major breeding habitat for the migratory reed warbler and also Savi's warblers *Locustela luscinioides*, and important habitat for breeding and wintering resident Cetti´s warbler. In relation to the resident Cetti's warbler, previous studies showed that reedbeds are not a major breeding habitat, but there is a large influx of birds to the reedbeds at the end of summer/autumn. Therefore, reedbeds may be particularly important for wintering Cetti's warblers, and riparian habitats may be more important for breeding. Seasonal variations in the differential use of each wetland type should be influenced by food resources and shelter conditions for roosting. In Chapter III we assess the diet, trophic niche and health condition of Cetti's warbler, to examine differences in the abundance of food resources and temperature between the two types of wetlands (reedbeds and riparian habitat). Our results revealed that reedbeds are very important for Cetti's warbler, supporting large numbers of (mainly) juvenile females during the post breeding period. Reedbeds present a higher abundance of higher trophic level food resources and better shelter conditions, and both factors should be important to explain the large numbers of Cetti's warbler dispersing into reedbeds in late summer. Cetti's warbler moving into reedbeds in late summer should come from a wider range of surrounding local sources, which means that connectivity between riparian areas and reedbeds should be important for the conservation of this species.

Survival is other important fitness component playing a central role in animal population dynamics, and understanding the factors that affect it is important in population demography and evolutionary ecology studies. While environmental conditions such as weather and food availability, and density-dependent processes, are key factors shaping survival and hence population demography, individuals may be differently impacted by these factors, depending on their particular attributes within a given population. In Chapter IV we analysed a long term study with Capture-Mark-Recapture dataset on the Cetti's warbler to understand the pattern of mass change during the annual cycle, the role of body mass in explaining individual survival, and the role of sex in explaining individual survival. Cetti's warblers follow the pattern of body mass change during the annual cycle of most passerines, in which adults have a tendency to loose mass during the breeding season and to gain mass in autumn-winter. Body mass was not a good predictor of individual survival, consistent with the fact that winter conditions are probably too mild in central Portugal to be a major determinant of Cetti's warbler population dynamics. Adult survival is more likely to be shaped by ecological and behavioural differences between males and females.

The Breeding, Migration and wintering periods of passerines throughout Europe may suffer a strong impact of environmental changes, which will affect population abundance and migration patterns. In Chapter V we focused in understanding the population dynamics of European long-distance migrants, partial migrants and residents in our two study sites using 10 years of data from constant effort ringing sites. Understanding population patterns of these 3 birds groups in Portuguese Wetlands is particularly relevant due to its strategic geographical position. Using data from two sites differing in habitat is also important in order to provide some control for potential habitat influences on bird numbers. Our results concerning the factors that affecting both long distance and partial migrants indicate that migrant passerines are very sensitive to of climate changes, and warming on Northern latitudes can induce ecological pressures during migration cycle. We need to monitor the populations and climate changes to investigate and understanding these complex ecological interactions.

The migratory birds showed a great fluctuation over these 10 years, and we expected that changes continue affecting the migratory populations in the climate warming context. We also expected that migrants can adjust their migratory timing by either phenotypic plasticity and/or an evolutionary response to avoid the population’s risks. Taken altogether this research highlights the need to protect reedbeds and riparian areas, and promote their connectivity due to protect partial and long-distance migratory birds during their annual cycle.

Key-words: wetlands; migration; climate change; stopover; survival rates; foraging ecology; stable isotopes.

Resumo

As zonas húmidas são áreas que têm sofrido um forte declínio nos últimos anos. Umas das maiores causas desse declínio é a conversão destas áreas para a agricultura. Por serem áreas de grande importância para muitas espécies especializadas é crucial a sua conservação e manutenção na Europa. Em Portugal, estratégias de conservação começaram por designar estas áreas por Zonas de Protecção Especial (ZPEs), mas há, ainda pouca informação sobre a sua importância para passeriformes migradores. A migração é um período muito exigente em termos energéticos e afecta todo o ciclo de vida de uma ave migradora. Para completar a sua migração as aves despendem de locais de paragem migratória (Stopover), locais esses que são essenciais para completarem o seu ciclo migratório, pois permitem que descansem e reponham a energia perdida durante os períodos de voo, fornecendo alimento e protecção. As aves migradoras de caniçal são altamente dependentes dos caniçais portugueses, principalmente depois ou antes de atravessar grandes barreiras ecológicas, como são os casos do Oceano Atlântico e o deserto do Saara. Os caniçais Portugueses devido à sua localização geográfica oferecem a estas aves locais estratégicos importantíssimos e que permitem completarem com sucesso a migração chegando aos locais ou de reprodução (Europa) ou invernada (África sub-Saariana). No Capitulo I avaliamos a ecologia dos Stopovers de Rouxinol-pequeno-dos-caniços *Acrocephalus scirpaceus* através de dados da última década num caniçal do Centro de Portugal. Nós demonstramos que nos caniçais do centro de Portugal os Rouxinóis-pequenos-dos-caniços tem diminuído a duração do Stopover, que pode reflectir as melhores condições no Norte da Europa. Contudo, todas as aves migradoras são confrontadas entre a necessidade de aumentar as reservas de energia e a desvantagem que daí advêm. Vários factores ambientais alteram tanto a velocidade de migração como a condição física, e por isso, estas aves enfrentam um dilema entre a minimização do Stopover e ganho de peso. Estas diferenças podem ser explicadas, também, através de padrões fisiológicos diferentes que se reflectem em diferentes estratégicas migratórias. Com o objectivo de perceber a diferença organizacional da migração desta duas espécies, nós no Capitulo II avaliamos vários parâmetros fisiológicos durante o Stopover. Avaliamos também, os parâmetros

fisiológicos de uma espécie residente o Rouxinol-bravo *Cettia cetti,* para poder comparar as diferenças dos parâmetros fisiológicos entre espécies migradoras e residentes. Os nossos resultados mostraram que os valores de triglicerídeos no sangue são um bom indicador da fase migratória: migração activa e período de ganho de gordura (altos valores de triglicerídeos). As felosas-dos-juncos apresentam, durante a migração activa, valores mais altos de triglicerídeos do que os Rouxinóis-pequenos-dos-caniços, e não fazem reabastecimento muito longos nos caniçais portugueses mesmo quando param. Aves em diferentes condições energéticas durante a paragem nos caniçais portugueses podem apresentar comportamentos muito diferentes.

As zonas húmidas portuguesas são essenciais para migradores de longa distância que se reproduzem na Europa, tais como os Rouxinóis-pequenos-dos-caniços e a Felosa-unicolor *Locustella luscinioides*. São também locais muito importantes para a reprodução e invernada de espécies não migratórias como é o caso do Rouxinol-bravo. Alguns estudos mostraram que o Rouxinol-bravo não se reproduz em caniçais, mas no final do verão princípio de outono há um grande influxo destas aves para os caniçais. Estes locais podem, portanto, ser muito importantes no período pós migratório e as zonas ripícolas serem especialmente importantes na época reprodutora. As variações sazonais que esta espécie apresenta poderão ser explicadas pelas diferenças na abundância de recursos alimentares e de protecção entre estes dois tipos de zonas húmidas, por isso, no Capitulo III avaliamos a dieta, nicho trófico e condição física do Rouxinol-bravo. Os nossos resultados mostraram que os caniçais são de facto muito importantes no período pós reprodutivo, fundamentado pelo grande aumento do número de fêmeas, principalmente jovens. Os caniçais apresentaram maior disponibilidade de recursos alimentares de níveis tróficos superiores e, também, melhores condições de abrigo e protecção que ajudam a explicar o grande número de indivíduos que dispersam no final do verão. Os Rouxinóis-bravos que ocupam os caniçais no final da época reprodutora devem ter origem em zonas ripícolas circundantes, o que significa que é bastante importante conservar e preservar a conectividade destas áreas para conservação desta espécie.

A sobrevivência é outro importante factor de aptidão que pode desempenhar um papel central na dinâmica das populações tais como demografia e ecologia evolutiva. A demografia e a densidade podem ser afectadas directamente por factores como a disponibilidade alimentar ou as condições climáticas que afectam a sobrevivência de alguma forma. No Capitulo IV através da análise de uma série de dados de 10 anos de Rouxinol-bravo utilizamos dados de Captura-Marcação-Recaptura para perceber o padrão anual da variação de peso, explicar a taxa de sobrevivência anual e relacionar o papel do sexo na sobrevivência. Nós mostramos que os Rouxinóis-bravos seguem o padrão anual de variação de massa observado em outras espécies de aves, além de que os indivíduos adultos têm tendência a perder massa durante a época de reprodução e a ganhar massa durante o outono-inverno. No entanto o peso não é um bom parâmetro para avaliar a sobrevivência individual, pois de acordo com os nossos dados os Rouxinóis-bravos em Portugal apresentam valores muito baixos para o peso durante o inverno. A sobrevivência das aves adultas deve estar mais correlacionada com diferenças ecológicas e comportamentais entre machos e fêmeas.

Os períodos de reprodução, migração e invernada dos passeriformes em toda a Europa podem sofrer um grande impacto devido a alterações climáticas, o que pode afectar a abundância e padrões tanto na migração como na reprodução. Para compreender a dinâmica populacional quer de espécies migradoras de longa e curta distância quer das espécies residentes nos nossos locais de estudos avaliamos no Capitulo V uma série de 10 anos de dados de anilhagens regulares. Portugal, como já referimos anteriormente, tem uma localização geográfica privilegiada e pode fornecer dados importantes para a compreensão dos padrões populacionais destes três grupos de aves: migradores de longa distância, migradores de curta distancia e espécies residentes. A utilização de dados de diferentes tipos de habitat foi também importante, para perceber se as tendências globais das espécies não dependem da degradação de um habitat específico. Os nossos resultados mostraram uma diminuição, quer dos migradores parciais quer de migradores de longa distancia, o que pode indiciar a alta sensibilidade dos passeriformes migradores a alterações climáticas, e essencialmente ao aquecimento que se tem vindo a verificar nos últimos anos no norte da Europa. Os nossos resultados mostraram grandes variações nos últimos 10 anos e a previsão é que

as mudanças climáticas no contexto de aquecimento global, continuem a afectar as populações migradoras. Prevemos também que haja algum tipo de plasticidade fenotípica que permita que estas aves ajustem o seu ciclo migratório evitando assim riscos para as suas populações.

Este conjunto de dados ajuda-nos a perceber a grande necessidade de proteger as zonas húmidas bem como promover a sua conectividade a fim de preservar as espécies que delas dependem, desde espécies migradoras a espécies residentes.

Palavras-chave: zonas húmidas; migração; alterações climáticas; paragens migratórias; taxas de sobrevivência; ecologia trófica; isótopos.

Introduction

> *"Not everyone is aware of the diversity of birds around the world, the amazing migrations some take, and the phenomenal range of behaviours, plumages, and songs they exhibit. Some bird species provide practical solutions to problems, such as the need for insect and rodent control. Others disperse seeds, helping to revegetate disturbed areas. Others are pollinators, ensuring that we are graced with flowering plants, trees, and shrubs. Beyond the utilitarian, birds are inspirations for us."*
>
> *International Migratory Bird Day 2014 (http://www.birdday.org/)*

Introduction

Migration ecology

Animals can migrate based on seasonal changes or stages within their lifecycles. They migrate to find better food conditions to reproduce. The largest land animal migration occurs in Africa, between Tanzania and Kenya (Bitanyi et al 2013). This migration includes 2 million animals of wildebeests *Connochaetes taurinus*, zebras *Equus burchellii* and Thomson's gazelle *Eudorcas thomsonii* (Harris et al 2009). This is a circular migration to follow rain periods, which produce lush grasses for these grazing animals. Other popular but not less spectacular migration includes the Pacific and Atlantic salmon. They are anadromous animals, which mean they are born in freshwater streams and travel to oceans, where they mature. After one to two years of living in the ocean, adult salmon begin the difficult journey back through the streams against the current to the place they were born, i.e. their spawning grounds. The journey is very rough and many salmon do not survive (Fleming 1996).

However in this study we care about birds and each year millions of birds migrate to their wintering or breeding grounds as part of their annual cycle. Migration is considered one of the most unpredictable and vulnerable phases of a bird's annual cycle (Sillett and Holmes 2002). It is estimated that every autumn 5 billion of land birds of 187 species migrate from Europe and Asia to Africa. Migration allows year-round activity through the exploitation of seasonal feeding resources elsewhere while living in favourable climates throughout the year (Gill 2007). Bird migrations vary between a few

tens to many thousands of kilometres, but it is the long and difficult journeys that best reveal the capabilities of migratory birds (Newton 2008). Passerines can make non-stop flights of 80–100 hours, enabling them to cover distances of 1500–3000 km. While many migrants are capable of making spectacular nonstop flights over ecological barriers (Moore et al 2005) (e.g. sedge warbler *Acrocephalus schoenobaenus*), and many other birds use stopover sites for resting and refuelling (e.g. reed warbler *Acrocephalus scirpaceus*). For example, most of the European passerines that migrate southwest to Iberia must then turn south or southeast if they are to reach West African wintering areas (Newton 2008). Moreover west European species cross the Sahara Desert immediately after crossing the Mediterranean Sea, a difficult overwater journey. In autumn some species may make this Mediterranean–Saharan flight without a break, or make strategic stopovers. While the final destination of migration, breeding and wintering grounds, are important, critical locations en route for refuelling and resting can be equally, if not more, important for migrants (Fig. 1). Locating quality stopover habitat en route is essential for the successful completion of migration, but much research on the stopover biology of migratory passerines is crucial (e.g., see Mehlman et al 2005, Deutschlander and Beason 2014).

Migration requirements

The time of arrival at the breeding grounds is crucial for migratory birds in order to profit from the summer peak in food availability, thereby optimizing their reproductive output (Both et al 2006). As consistent patterns of earlier arrival to their northern Europe breeding areas have been recognized (Thorup et al 2007), a number of questions arise: What factors drive these changes and which factors do migrants use to determine optimal arrival (Stenseth and Mysterud 2005)? Migration success depends on the availability of high-quality habitats that provide adequate food resources, particularly in areas along migration corridors that support large numbers of birds during migration. The importance of this fact is underlined by the finding that long-distance migratory species are probably the ones with the most serious population declines

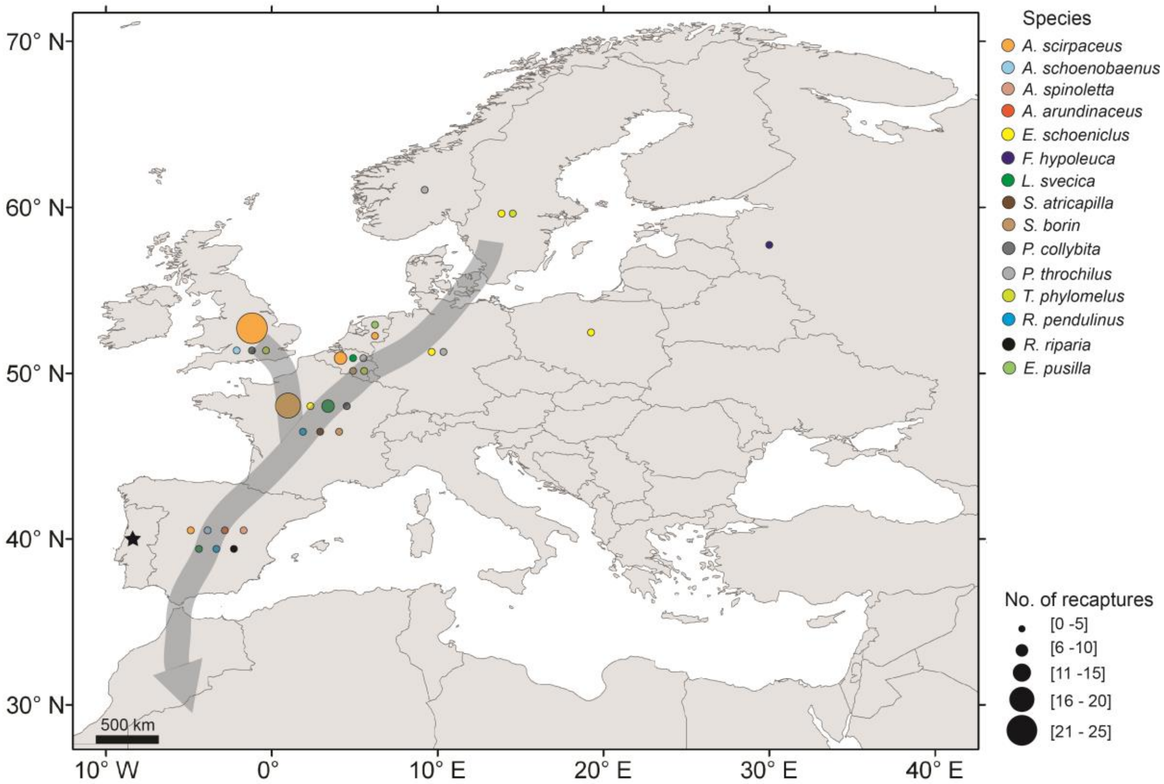

Figure 1: Foreign ring recoveries (colour circles) at Portuguese reedbed (Paul do Taipal) and riparian (Paul da Madriz) habitats and main southward migratory routes (grey shaded arrows) of wetland passerine species.

(Barlein and Schaub 2009). Given the potential consequences of mistakes during migration, there is a high selective pressure for birds to both locate stopover habitat successfully and to refuel effectively during stopover (Moore and Aborn 2000). Plum aphids (*Hyalopterus pruni*) are believed to be the main prey for many passerines migrating through Europe (e.g. *Acrocephalus schoenobaenus*) in late summer and autumn (Bibby and Green 1981, Koskimies and Saurola 1985), but less important in the diet of other migratory passerines (e.g. *Acrocephalus scirpaceus*) (Bibby and Green 1981). It is suggested that the reason for these differences is due to the differences in feeding ecology of species, in particular the spatial distribution of their prey. Understanding how birds locate and use stopover habitat has important management and conservation implications because a migratory bird ’s energetic condition may affect both its probability of survival during migration and its reproductive success once it arrives on the breeding grounds in spring (e.g., Sandberg and Moore 1996, Drent et al 2003). Therefore, the importance of stopover sites as part of the entire range of a

species should be considered into the development of conservation strategies (Moore et al 1995 in Moore et al 2005). Stopover sites are one of the crucial factors for the success of bird migrations (Gill 2007). Portuguese wetlands are very important as stopover sites for long-distance migrants due the its localization before crossing into Africa. Because stopover locations near barriers can help provide migratory birds with the resources they need to cross or circumvent barriers successfully, they are especially worthy of conservation.

Migration physiology

Powered flight have lower costs of transport per unit distance than walking or running (Schmidt Nielsen 1972), consequently the majority of long distance migratory species fly (Alerstam et al 2003; Bowlin et al 2010). For many migratory species, most of their journeys are spent in several stages of supply and not in migratory flights. According to some authors, it is believed that fuelling phases have great influence on the organization of the migratory journey, due to the time and energy expended accumulating fuel reserves and given that the final fuel load will determine the length of the subsequent flights (Alerstam and Lindstrom 1990; Hedenstrom and Alerstam 1997). Much theoretical work has been done to understand how a migrant might regulate its energy reserves, foraging behaviour and length of stopover. Migrants may optimize their behaviour according to an overall migratory strategy (Alerstam and Lindstrom 1990). The migratory disposition characterizes a migrant that is ready for migratory departure. Bird's needs extensive fat stores to reach their destination. Fat is the optimal flight fuel because fat yields twice more energy than an equal amount of carbohydrate and protein. Lipids are ideal for fuelling migration because they are energetically dense, providing higher energy yields (Blem 1980, Jenni and Jenni Eirmann 1998). Stored lipids are also lighter and require less space than glycogen or protein stores of the same weight because lipids are stored anhydrously (Ramenofsky, 1990; Weis Fogh, 1952;). In addition, it can be stored dry without addition of water, and during its oxidation the use of glucose and muscle protein is economical. Protein is also catabolised for fuel in migrants that maintain flight for long periods of time; the resulting amino acids are required as intermediates of the citric acid cycle, and gluconeogenesis (Dohm 1986,

Jenni and Jenni Eiermann 1998). Protein catabolism can used to balance weight distribution for improved flight efficiency, and can act as a source of metabolic water (Gerson and Guglielmo 2011, Jenni and Jenni Eiermann 1998, Piersma 1990). This is particularly important for birds undergoing multiday flights. Moreover different phases of migration require different physiological necessities such as the different fat loads required for different weather conditions.

Climate change on migration

European climate initially warmed in the beginning of the 20th century, and has been warming rapidly since the 1970s (IPCC 2007). Global mean surface temperature increased by 0.76˚C between 1906 and 2005 and the best estimates of climate change models predict a further increase of 0.2 ºC per decade (IPCC 2007). Although some of these changes are part of the natural variability of climate, there is undeniable evidence that some of the changes in climate are due to anthropogenic forcing by amongst other things the increased release of greenhouse gasses (IPCC 2001). Rainfall patterns have also changed, and the incidence of extreme weather events (e.g. heavy precipitation, droughts) has increased (IPCC 2007). Indeed, extreme weather events can severely reduce a population and have long lasting effects (Baillie and Peach 1992, Peach et al 1991, Saino et al 2004a, Watkinson et al 2004). Long-distance migrants are likely to be especially vulnerable to the effects of climate change, as their ability to complete their annual cycle depends on environmental conditions (including weather, habitat quality and food availability) at locations hundreds or thousands of kilometres apart. Climate change has fitness implications for migrants at their breeding grounds, wintering grounds or during migration. The North Atlantic Oscillation (NAO) is a large-scale fluctuation in atmospheric pressure in the Atlantic Ocean that influences the climate and weather in parts of Europe, North America and Africa (Vähätalo et al 2004). The NAO index, the normalized pressure differences between the Azores and Iceland, describes meteorological conditions in winter and spring (Hüppop and Hüppop 2003). Positive values are associated with warm moist winters, while negative values are correlated with cold dry winters in northern Europe (Hurrell 1995). This index has been used in a number of studies to explain the spring arrival in Europe of migrant birds (Ahola et al

2004, Anthes 2004, Marra et al 2005, Sparks et al 2005, Vähätalo et al 2004) as well as laying date (Sanz 2002, Weatherhead 2005). Arriving late at breeding grounds relative to seasonal peaks in food abundance may in turn have negative consequences for reproductive success (Stenseth and Mysterud 2002, Both 2010). Some studies have argued that the NAO affects the spring arrival of all migrant birds, including long-distance Afro-Palaearctic migrants breeding in Europe (Forchhammer et al 2002 Hüppop and Hüppop 2003, Stervander et al 2005), whilst others argue that the NAO only affects short- to medium-distance migrants (Both and Visser 2001, Hubalek 2004, Nott et al 2002, Tryjanowski et al 2002). It is interesting that those studies that found a correlation between the NAO and spring arrival for long-distance migrants were those with study sites in the far North of Europe. This suggests that arrival time for these species is affected by the climate in Europe once the species are already in Europe and at stopovers. Alternatively, arrival time is affected by climate on the non-breeding quarters. Indeed, Cotton (2003) found that winter temperature in Africa was not correlated with winter NAO index, and that arrival dates on the breeding grounds were correlated with temperature in sub-Saharan Africa. Saino et al (2004b) and Gordo et al (2005) also showed that weather (in particular rainfall) on the non-breeding grounds affect the spring arrival of trans-Saharan migrants not the climate on the breeding grounds. Indeed it is postulated that the inter-annual fluctuations of spring arrival may be influenced by plant productivity and hence insect abundance on the African non-breeding grounds (Gordo and Sanz 2008). The ability of migratory species to respond to changes in the timing of food supply will be affected by the ability to change the timing of migration. Short-distance migrants are likely to be able to adjust the timing of spring departure according to local weather conditions, since these are likely to correlate broadly with weather conditions at the destination (Forchhammer et al 2002).

Migration mystery. Why do birds migrate?

Why some birds migrate while others are resident is still one of the most difficult questions in ornithology. Birds migrate primarily to move from areas of low resources to areas of increasing resources. The two main factors being sought are food and nesting locations. However there are even differences among populations of the same migratory species, such as Robins *Erithacus rubecula* or blackcap *Sylvia atricapilla* being one of the extreme examples that the entire range from complete sedentariness to long distance migratoriness can be found (Shirihai et al 2001). In northern latitudes the winter is a season with comparatively scarce food resources for most bird species. Moreover the winter is a time to get ready for the coming breeding season, a preparation that may include defence of a nesting territory and the attraction of breeding partners. For birds that decide to migrate the priority is to stay alive and keep their body condition and perhaps a winter territory, which allows them to arrive at their breeding territories in good time and shape for a successful subsequent nesting season (Newton 2008). For both migratory birds and residents the winter choice may not only influence the survival, but also the next breeding season. For small passerines, surviving the winter represents a fight against starvation, to ensure their genetics in the future. How climate change may influence migratory species has been an important subject of study. Indeed, Berthold (2001) postulated that long-distance migrants may be particularly affected by climate change as migrants rely on spatially separated areas that are often bio-climatically different (e.g. Eurasia and Africa) and that are affected by different processes and drivers of change such as agricultural intensification in Europe and desertification in Africa. The effects of climate change on all these critical areas are unlikely to be uniform and will be exacerbated by other drivers such as habitat loss. Understanding how long, partial and resident birds use their breeding habitats is important for management and conservation implications because a bird’s energetic condition may affect, for example, the survival probability during migration and its reproductive success once it arrives in the breeding grounds in the spring (e.g., Sandberg and Moore 1996, Drent et al 2003).

The Portuguese wetlands and their importance

The European continent is endowed with a great diversity of climates, hydrologies, and human, animal and plant life (Hughes 1995). Consequently, the wetland types reflect this regional diversity. Many wetlands in Europe have been destroyed during the last 100 years by drainage for agriculture, increase in human population and disturbance and pollution (Jones and Hughes 1993). The natural wetland area decreased 80-90% in the Mediterranean Region during the last few centuries (Fasola and Ruiz 1996). Habitat loss and disturbance, mainly as a result of increasing human activity, are among the main causes of these declines (Goss-Custard and Yates 1992, Davidson and Rothwell 1993). The establishment of the Ramsar Convention in 1971 (Hollis and Jones 1991) decrease the Human pressures on the remaining wetlands (but still prevail in most areas); due to increasing conservation awareness. Bird communities of reedbeds are very specialized and include some species with great conservation value in Europe (Tucker and Heath 1994) and the Iberian Peninsula (SEO/BirdLife 1997) as Aquatic warbler *Acrocephalus paludicola* and Savi´s warbler *Locustella luscinioides* which are considered vulnerable in Portugal (Cabral et al 1990). Habitat loss and declines in abundance of some resident bird species and temperate migrants have focused attention on the need for knowledge of their ecological requirements to address year round conservation needs. Birds occupy an extremely diverse range of niches of wetlands systems and are sensitive indicators of environmental conditions (Temple and Wiens 1989, Bryce et al 2002). Ecological conditions required for healthy wildlife populations in riparian habitats include complex vegetation structure that provides birds with nesting sites. Reedbeds and riparian galleries are wetlands of considerable importance for wildlife and provide many ecosystems services to humans (Mönkkönen and Reunanen 1999, Bolger et al 2001). These two wetland types are very productive and provide habitats for many bird species during breeding, migration stopover, juvenile dispersion and wintering seasons (Machtans et al 1996). Many resident and migratory passerine species use both habitats during part or their entire life cycle, therefore riparian and reedbed habitats offer the opportunity for detailed studies of habitat requirements of individual bird species associated with them (Table I). Cetti´s warblers, a small non-migratory brown bush warbler common in southern and central Europe, using extensively both habitats (Bonham and Robertson 1975, Bibby 1984), and is a good

model species to evaluate the differential importance of both types of wetlands for insectivorous passerines throughout their annual cycle.

Table 1: Total of captures and recaptures of birds for both study areas (Paul do Taipal and Paul da Madriz). The number of recoveries is the number of recaptured birds in our specific study area with foreign ring.

Species	Common name	Status	Reedbed (Paul do Taipal)		Riparian (Paul da Madriz)		Country (number of ring recoveries)
			Cap	Ret	Capt	Ret	
Acrocephalus arundinaceus	Great reed warbler	Long distant migrant	91	43	5	0	Spain (2)
Acrocephalus schoenobaenus	Sedge warbler	Long distant migrant	343	18	8	0	England (1) Spain (2)
Acrocephalus scirpaceus	Reed warbler	Long distant migrant	3478	1516	549	296	Belgium (7) France (16) England (22) Holand (2) Spain (5)
Aegitalus caudatus	Long-tailed Bushtit	Resident	131	84	369	197	-
Alcedo atthis	Kingfisher	Resident	520	358	85	8	-
Anthus pratensis	Meadow Pipit	Partial Migrant	0	0	40	3	-
Anthus spinoletta	Water Pipit	Partial Migrant	2	0	229	3	Spain (1)
Anthus trivialis	Tree Pipit	Partial Migrant	0	0	1	0	-
Caprimulgus europaeus	Nightjar	Long distant migrant	2	0	4	0	-
Carduelis cannabina	Linnet	Resident	11	0	12	0	-
Carduelis carduelis	Goldfinch	Resident	3	0	47	0	-
Carduelis spinus	Siskin	Partial Migrant	0	0	1	0	-
Certhia brachydactyla	Short-toed Treecreeper	Resident	6	0	78	26	-
Cettia cetti	Cetti warbler	Resident	1187	1458	320	607	-
Chloris chloris	Greenfinch	Resident	284	5	546	36	-
Cisticola juncidis	Zitting *cisticola*	Resident	33	12	19	1	-
Dendrocopos major	Great-spotted woodpecker	Resident	0	0	10	3	-
Emberiza cirlus	Cirl bunting	Resident	0	0	1	0	-
Emberiza pusilla	Little bunting	Partial Migrant	0	1	0	0	Holand (1)
Emberiza schoeniclus	Reed bunting	Partial Migrant	2001	284	104	1	France (3) Germany (4) Poland (2) Sweden (1)

Erithacus rubecula	Robin	Resident/ Partial Migrant	257	224	1000	683	-
Estrilda astrild	Waxbill	Resident	816	41	479	13	-
Ficedula hypoleuca	Pied flycatcher	Long distant migrant	58	5	301	47	Russia (1)
Fringilla coelebs	Chaffinch	Resident	2	0	294	28	-
Garrulus glandarius	Jay	Resident	1	0	16	0	-
Hippolais polyglotta	Melodious warbler	Long distant migrant	42	0	170	5	-
Hirundo daurica	Red-rumped Swallow	Long distant migrant	3	0	0	0	-
Hirundo rustica	Barn swallow	Long distant migrant	2376	8	713	1	-
Ixobrychus minutus	Little bittern	Long distant migrant	63	13	2	0	-
Jynx torquilla	Wryneck	Long distant migrant	4	0	3	0	-
Locustella luscinioides	Savi's warbler	Long distant migrant	364	186	51	16	-
Locustella naevia	Grasshopper-warbler	Long distant migrant	34	1	19	0	-
Luscinia megarhynchos	Nightingale	Long distant migrant	10	4	48	5	-
Luscinia svecica	Bluethroat	Partial Migrant	351	95	5	0	Belgic (2) France (8) Spain (1)
Lymnocryptes minimus	Jack snipe	Long distant migrant	0	0	1	0	-
Motacilla alba	White wagtail	Resident/ Partial Migrant	1	0	5	0	-
Motacilla cinerea	Grey wagtail	Resident	4	1	1	0	-
Motacilla flava	Yellow wagtail	Long distant migrant	6	0	0	0	-
Muscicapa striata	Spotted flycatcher	Long distant migrant	10	0	8	0	-
Parus ater	Coal tit	Resident	1	0	43	14	-
Parus caeruleus	Blue tit	Resident	390	279	805	407	-
Parus cristatus	Crested tit	Resident	0	0	6	1	-
Parus major	Great tit	Resident	88	30	502	156	-
Passer domesticus	House sparrow	Resident	1247	11	128	0	-
Passer montanus	Tree sparrow	Resident	446	7	184	2	-
Petronia petronia	Rock sparrow		0	0	4	0	-
Phoenicurus ochruros	Black redstart	Resident	4	0	9	1	-
Phylloscopus collybita	*Chiffchaff*	Partial Migrant	2009	164	1196	69	France (1) England (1)
Phylloscopus collybita ibericus	Iberian chiffchaff	Partial Migrant	13	0	27	15	-
Phylloscopus inornatus	Yellow-browed warbler	Partial Migrant	2	0	0	0	-
Phylloscopus throchilus	Willow warbler	Long distant migrant	850	19	323	1	Belgic (1) Germany (3)

							Norway (1)
Prunella modularis	Dunnock	Partial Migrant	2	0	3	0	-
Pyrrhula pyrrhula	Bullfinch	Resident /Partial migrant	0	0	61	4	-
Rallus aquaticus	Water rail		7	1	6	2	-
Regulus ignicapillus	Firecrest	Partial Migrant	5	1	47	14	-
Remiz pendulinus	Penduline tit	Partial Migrant	130	63	0	0	France (4) Spain (1)
Riparia riparia	Sand martin	Long distant migrant	245	0	97	1	Spain (1)
Saxicola rubetra	Whinchat	Long distant migrant	3	0	0	0	-
Saxicola torquata	Stonechat	Resident	6	0	42	6	-
Scolopax rusticola	Woodcock	Partial migrant	0	0	2	0	-
Serinus serinus	Serin	Resident	4	0	487	16	-
Streptopelia turtur	Turtle dove	Long distant migrant	0	0	1	0	-
Sturnus unicolor	Spotless starling	Resident	2	0	17	0	-
Sturnus vulgaris	Starling	Partial migrant	13	0	12	0	-
Sylvia atricapilla	Blackcap	Resident / Partial migrant	267	59	1208	382	France (1)
Sylvia borin	Garden warbler	Long distant migrant	12	1	487	45	Belgic (3) France(1)
Sylvia communis	Whitethroat	Long distant migrant	15	0	109	17	-
Sylvia melanocephala	Sardinian warbler	Resident	26	1	368	142	-
Sylvia undata	Dartford warbler	Resident	3	0	2	0	-
Troglodytes troglodytes	Wren	Resident	66	38	251	162	-
Turdus iliacus	Redwing	Partial migrant	1	0	2	0	-
Turdus merula	Blackbird	Resident	106	37	253	168	-
Turdus phylomelos	Song thrush	Resident / Partial migrant	12	2	83	15	Belgic (1) England (1) Sweden (1)

Cetti warbler as resident specie on Portuguese Wetlands

Cetti’s warbler is associated with dense and tangled, shrubby vegetation growing near water, as described by le Sueur (1980) and Fergunson-Less (1964). They build their nest inside dry and dead vegetation, even in the latter half of the breeding season when new green shoots are available. Portuguese birds tend to be smaller than those from further north, which probably represents a cline in the expected direction, with birds from colder more northern areas being larger. The size dimorphism is believed to be due to the polygynous habits of the species, as the result of competition among males (Bibby 1982). Males are larger than females, and males usually pair with one to three females, which lay one or two clutches per year (Bibby 1982, Bibby and Thomas 1984). The weight of the females increases during breeding season, but declines afterwards (Villarán 2000). Since the female incubates and takes responsibility for most of the care of the chicks (Bibby 1982), they may have a greater need than males to accumulate fat, as these activities must increase their energetic costs (Alivizatos et al 2011). Cetti’s warblers males sing sporadically in the winter and may defend territories. Hence, the possibility is that females disperse in greater magnitude because successful males need to occupy and defend their potential breeding territories from autumn onwards and are therefore prevented from dispersing to such an extent. Their diet includes aquatic invertebrates, perhaps more often in winter, presumably taken from water’s edge or by reaching down to the surface from raised stems. Seeds have been found to be an important food source during the winter (Fergunson-Less 1970).

Outline

Reedbeds have high conservation value in Europe. In Portugal reedbeds are an important habitat for breeding bird species, both resident and migratory, and as a stopover for species migrating between Europe and Africa. This thesis took advantage of an existing long-term data set on these bird species and further work was carried out to address the ecology of resident and migrating passerines in reedbeds of Central Portugal. The overall objective of this proposal is to understand the factors behind/affecting these fluctuations in the populations of breeding, wintering and transient reedbed passerines in Central Portugal. Our proposed study links climate, food abundance, bird habitat selection, reproductive success and survival rates. **To achieve the proposed goal, the following specific objectives were pursued:**

Chapter I – The relation of climatic variables with minimum stopover duration and body mass variation during the stopover period of migrant reed warblers *Acrocephalus scirpaceus* in Central Portugal using long term data set. Reed warblers are adequate to examine this aspect because their migration is characterized by short bouts of flight alternating with long stopovers to refuel.

Chapter II – The differences in migration ecology of two warblers in the wild using physiological measurements: the sedge warbler *Acrocephalus schoenobaenus* and the reed warbler *Acrocephalus scirpaceus*. These two species are very similar in size and morphology but exhibit marked differences in migratory strategies, with sedge warblers making longer flights with fewer stopovers than reed warblers.

Chapter III – The importance of the riparian and reedbed habitats on the annual life cycle of Cetti´s warbler. We describe the importance of each wetland type for the Cetti’s warbler, examining the seasonal variation in population structure during a long term study (10 years data). This dataset was complemented with a short term study (1 year) performed to evaluate health condition for each habitat type, and the role of food

resources and shelter conditions as potential drivers of the seasonal variations observed in each habitat.

Chapter IV – Evaluate annual changes in survival probability using Cetti warblers *Cettia cetti* as a model resident species for both riparian and reedbed habitats. Long term bird-ringing datasets, using constant effort ringing, provide information on annual changes in survival probability and recapture rates. Multi-state models with body mass (categorized into discrete mass classes), sex and age were used as the individual state variable.

Chapter V – The population patterns of European long-distance migrants, partial migrants and residents in two sites differing in habitat in Central Portugal using long-term data set from constant effort ringing sites. Understanding population patterns of these bird groups in Portugal is particularly relevant due to its strategic geographical position, before crossing to North Africa.

Chapter I

Reed warblers migrating through Portugal: a climatic influence on stopover ecology over the last decade.

Abstract

The arrival and breeding dates of small migrant birds have advanced throughout Europe. This study evaluates the hypothesis of a faster migration along the migratory route, which should lead to a decrease in stopover duration in staging areas over the last decades. Several climatic predictors were analysed as proxies to understand the stopover ecology of reed warbler *Acrocephalus scirpaceus* migrating through central Portugal. The minimum stopover duration of migratory reed warblers decreased significantly over the last decade during both the autumn and the spring migrations. During spring such reduction paralleled a higher gain in body mass in spring as compared to autumn. Such change in stopover patterns is likely to be related with the general improvement of environmental conditions along the migratory route, particularly a general increase in temperature. Warmer conditions en route during spring and in Northern Europe in summer should increase food availability, increasing the body condition of departing birds and the quality of stopover sites en route to Portugal, such that migrants will reduce the stopover duration at Portuguese reedbeds.

Keywords: Spring migration; Autumnal migration; environmental parameters.

Introduction

Climate change has advanced the seasons and thus the phenology of many organisms in the last decades (Crick et al 1997, Parmesan et al 2003). It is clear that the arrival and breeding dates of small migrant birds have advanced throughout Europe (Cotton 2003, Hüppop and Hüppop 2003, Both et al 2005), which can be explained by earlier departures due to increasing winter temperatures in sub-Saharan Africa (Cotton 2003) and/or a faster migration due to improved environmental conditions en route

(Both et al 2005). Both et al (2005) showed that male pied flycatcher *Ficedula hypoleuca* breeding in Holland arrive and breed earlier in years with higher temperatures in both stop-over sites in North Africa and at the breeding site. Birds may migrate faster during periods of warm weather (Richardson 1990, Schaub and Jenni 2001, Jenni and Schaub 2003), and this could partly explain their earlier arrival and breeding dates (Both et al 2005). If environmental conditions are improving en route we should detected a decrease in stopover duration in staging areas over the last decades, but this has not been examined. Departure dates from European breeding grounds have also advanced, and are related with elevated summer temperatures (Cotton 2003). Stopover duration is the most important factor in determining overall migration speed in long-distance migrant passerine birds (Schaub and Jenni, 2001).The amount of time a migrant will stay at a stopover site depends largely on the quality of such stopover, as was shown by Ktitorov et al (2010) with radio-tracking of migrant reed warblers (*Acrocephalus scirpaceus*), but the influence of climatic variation in stopover duration has received little attention.

The relationship between variation in atmospheric parameters and passerine migration parameters, such as stopover duration, is difficult to assess because many factors must be taken into consideration. During spring birds migrate at a consistently higher speed than in autumn (Fransson 1995, Bächler et al 2010). Nevertheless birds appear to select favourable wind conditions to migrate, and tail-winds for long-distance migrants are crucial to cover large ecological barriers (Liechti 2006). In addition, several recent studies found a significant relationship between large-scale weather phenomena such as the North Atlantic Oscillation index (NAO) during winter and timing of spring migration (Forchhammer et al 2002; Hüppop and Hüppop 2003; Vähätalo et al 2004). The NAO is characterized by an oscillation in atmospheric mass between the subtropical high-pressure zone of the Azores and the low-pressure zone of Iceland (Ottersen et al, 2001), Positive NAO index values during winter and early spring indicate warm air masses from the Atlantic moving eastwards, leading to higher temperature and precipitation (i.e. rainfall) in northwestern Europe. Negative winter NAO index values indicate weaker westerly winds, leading to a stronger influence of the continental winter high, which brings lower temperatures and less precipitation in northern Europe (Hurrell, 1995, Ottersen et al, 2001; Stenseth et al, 2003). The use of large-scale climatic phenomena reduce temporal and spatial variability into simpler measures such as the NAO (Stenseth et al, 2003), but we must born in mind that environmental factors sensed by the birds are variations in, amongst other factors, temperature and/or wind conditions (Hüppop and Hüppop 2003) that are correlated with large-scale climatic indices.

There is very little information about the effects of climate change and global warming on a complete migration cycle (Walther et al, 2002), and evidence for a prolonged breeding season and delayed or advanced autumn migration is not clear (Gatter 1992; Jenni and Kéry 2003, Cotton 2003, Both et al 2005, Saino et al 2009). The influence of environmental parameters on stopover duration, both before and after individuals cross important ecological barriers (but see (Calvert et al 2009; Deutschlander and Muheim 2009) are particular important to evaluate the hypothesis of a higher speed migration. Stopover sites in Portugal are important in these context given their geographical location before (autumn migration) and after (spring migration) the Atlantic Ocean and the Sahara desert.

In this study we decided to use large (winter NAO index) and medium scale environmental variables (temperature, precipitation and windspeed in North Africa, Central Portugal and Central Europe) because they are likely to reflect better the migratory journey of passerines from northern Europe, through Southern Europe and into Africa. In this study we used 10 years of data from migrant reed warblers in Central Portugal to assess whether minimum stopover duration has decreased both during autumn and spring migrations, and to evaluate the relation of climatic variables with minimum stopover duration and body mass variation during autumn and spring stopover periods. Reed warblers are adequate to examine this aspect because their migration is characterized by short bouts of flight alternating with long stopovers to refuel (Mills et al 2011). We predicted that years with good en route winter conditions, from North Africa to Central Europe, characterized by a higher NAO index, leading to higher temperature, precipitation and windspeed from North Africa to Central Europe) should decrease the stopover duration during the spring migration. Years with higher temperature and humidity in North Africa and Europe during winter/early spring should be related with higher food abundance, (Both el al 2010) meaning that a migratory bird would need to spend less time refuelling at a stopover. Those years are usually associated with south-westerly winds, which may also confer an advantage in terms of favourable tail winds. During autumn migration, the stopover duration should be less influenced by the NAO, given that this index has a stronger influence during winter (Stenseth et al 2003). In relation to medium scale environmental variables an increase in temperature and precipitation in Central Europe and Portugal should be associated with a decrease in stopover duration, and an increase in windspeed should be related with an decrease in stopover duration, because the predominantly northern winds will diminish flying time towards Africa.

Methods

Study area – Data on minimum stopover duration and body mass variation during the stopover period (hereafter termed body mass variation) of reed warbler was collected in Paul do Taipal reedbed (40°11'N 008°41'W), Mondego river Valley, Central Portugal between 2002 and 2012. The dense vegetation is dominated by common reed (*Phragmites australis*) with hedges of grey willow (*Salix atrocinerea*).

Bird sampling – Mist netting sessions were operated every 10 days, following the procedures of constant effort ringing sites (Cave et al 2010; Thomson et al 2009) from March to October, 2003 – 2012, and from June to October in 2002 . For the autumn migration from 2010 to 2012 we operated the ringing sessions every 5 days. The mist nets were set before dawn and operated for five hours after sunrise. In each session 120 to 240 m of nets were operated. Captured birds were ringed, weighed, aged and sexed according to Svensson and Hedenstron (1999). Minimum stopover duration of each individual was determined by calculating the number of days that elapsed from the first capture until the last recapture (Chernetsov 2012). It was assumed that birds did not leave the site between the first and last capture. Body mass variation corresponded to the difference in mass between the initial capture of an individual and its final recapture during each migratory season: spring migration = 15 March - end of May, autumn migration = 15 July - end of October.

Minimum stopover duration differs from the real stopover duration and Capture-Mark-Recapture (CMR) methods using daily ringing sessions are needed to calculate stopover duration (Schaub and Jenni 2001). This is also influenced by age class (juveniles vs adults) and the moulting state of the individuals (Schaub and Jenni 2001). All our birds were in a non-moulting state and we did not detect significant differences in the minimum stopover duration between adults and juveniles (t-test, $P < 0.05$). Our purpose in this study was to compare minimum stopover duration among years and to evaluate its relation with climatic variables. This is well justified because: a) every year we used the same method, i.e. we sampled birds at 10- days interval, and b) Schaub and Jenni (2001) provide an overall mean of 9.5 days for stopover duration of non moulting reed warbler, using CMR modelling of daily mist-netting data from Northern Europe to North Africa, c) the mean stopover duration obtained by Schaub and Jenni (2001) was longer for the Iberian Peninsula (Ebro Delta = 10 ± 2.0 days; Doñana 13 ± 7.0 days), with similar values to those obtained in our study from 2002-2004 (see results), and d) birds sampled at 5-day and 10-day intervals at our study site, from 2010 to 2012, presented similar minimum stopover durations (see results). This suggests that sampling at 10-days interval provide adequate estimates of minimum stopover duration for reed warblers.

Environmental predictors – We selected several environmental parameters that were likely to influence the minimum stopover duration of Afro-Palaearctic migratory passerines. Apart from large scale climatic variables such as the NAO index, we defined climatic variables from Central Europe, Central Portugal and North Africa (Table 1, Fig. 1), that are likely to influence minimum stopover duration in Portugal, during both the autumn (Europe to Africa) and the spring (Africa to Europe) migrations. Data from Central Europe was used due to the relative high number of individuals with French, Belgium and Dutch rings captured in our study site. NAO may represent different climatic influences over bird migration and was extracted from https://climatedataguide.ucar.edu/climate-data/hurrell-north-atlantic-oscillation-nao index-station-based and http://www.cpc.ncep.noaa.gov/products/precip/CWlink/daily_ ao_index/ ao.shtml, respectively. We used the extended winter NAO (mean NAO values for the period Dexember to March) , and the monthly NAO (The NAO index for the corresponding month that a particular individual entered our reedbed site, i.e. if a bird was captured in March we used the NAO index for this month). Spatially explicit data of the local environmental variables were extracted as raster composites (http://modis.gsfc.nasa.gov) and processed within ArcGIS 10.1 using the Spatial Analyst toolbox (Table 1). We used 8 environmental parameters that were likely to influence the minimum stopover duration during the spring migration (monthly NAO, Extended Winter NAO, Temperature, Precipitation and Windspeed for North Africa and Portugal), and 8 environmental parameters that were likely to influence the minimum stopover duration during the autumn migration (monthly NAO, Extended winter NAO, Temperature, Precipitation and Windspeed for Portugal and Central Europe).

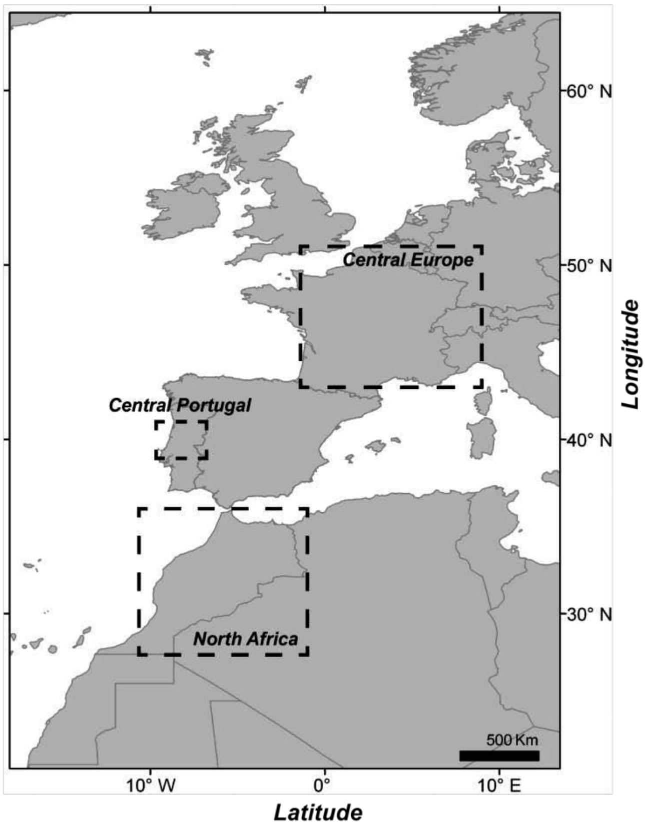

Figure 1: Geographical location of the study areas from where local environmental predictors (i.e. air temperature, precipitation and wind speed) were extracted.

Table 1: List of the environmental predictors used as explanatory variables on the regression models. NAO – North Atlantic Oscillation index.

Variable	Code	Derived Metric	Units	Influence proxy of?	Source
Monthly NAO	Mon_NAO	—	—	Monthly index of North Atlantic weather	http://gcmd.nasa.gov
Extended Winter NAO	DJFM_NAO	—	—	Index used as proxy of North Atlantic weather for December - March (i.e. spring migration)	http://gcmd.nasa.gov
Land Surface Temperature in Portugal during each migratory period	Temp_PT	Mean	°C	Local temperature conditions at the stopover site – central Portugal (39–42°N and 7–10°W)	Terra-MODIS http://modis.gsfc.nasa.gov
Land Surface Temperature in North Africa during each migratory period	Temp_NAfr	Mean	°C	Local temperature conditions in North Africa (i.e. spring migration path towards Portugal (28–37°N and 10°E–11°W)	Terra-MODIS http://modis.gsfc.nasa.gov
Land Surface Temperature in Central Europe during each migratory period	Temp_Euro	Mean	°C	Local temperature conditions in central Europe (42–51°N and 1°W–10°E)	Terra-MODIS
Precipitation in Portugal during each migratory period	Prec_PT	Mean	mm h^{-1}	Local precipitation conditions at the stopover site – central Portugal (39–42°N and 7–10°W)	http://modis.gsfc.nasa.gov
Precipitation in North Africa during each migratory period	Prec_NAfr	Mean	mm h^{-1}	Local precipitation conditions in North Africa (i.e. spring migration path towards Portugal (28–37°N and 10°E–11°W)	Terra-MODIS
Precipitation in Central Europe during each migratory period	Prec_Euro	Mean	mm h^{-1}	Local precipitation conditions in central Europe (42–51°N and 1°W–10°E)	Terra-MODIS
Wind speed in Portugal during each migratory period	WDSP_PT	Mean	m s^{-1}	Local wind speed conditions at the stopover site – central Portugal (39–42°N and 7–10°W)	http://modis.gsfc.nasa.gov
Wind speed in North Africa during each migratory period	WSPD_NAfr	Mean	m s^{-1}	Local wind speed conditions in North Africa (i.e. spring migration path towards Portugal (28–37°N and 10°E–11°W)	Terra-MODIS
Wind speed in Central Europe during each migratory period	WDSP_Euro	Mean	m s^{-1}	Local wind speed conditions in central Europe (42–51°N and 1°W–10°E)	Terra-MODIS http://modis.gsfc.nasa.gov

Data handling and statistical analyses – Most of our data on minimum stopover duration was collected from ringing sessions conducted every 10 days. This should have no influence on our annual comparison of stopover duration, because the same method was used every year, but comparisons with other studies using a different sampling interval and methods to estimate stopover duration must be treated carefully. To assess this point we used t-tests to compare the mean of the minimum stopover duration in autumn, calculated from ringing sessions at 10-days interval with: (1) the mean of the minimum stopover duration obtained from 5-days interval ringing sessions (2010-2012), and (2) with mean stopover duration given for reed warblers in the literature from daily ringing sessions and CMR modelling (Schaub and Jenni 2001). For this last comparison we used: a) our minimum stopover duration value estimated for 2002-2004, which is nearer the sampling year (1994-1996) given by Schaub and Jenni (2001), and (b) the overall minimum stopover duration for 2010-2012. We used a Spearman correlation to evaluate whether stopover duration, body mass variation and environmental predictors varied systematically along the study period.

The 8 selected environmental predictors were used to explain stopover duration of reed warblers for each migratory season. All environmental variables were standardized to have a mean of 0 and an SD of 1. Such standardization was executed by subtracting each absolute value by the respective average and dividing by the standard deviation value of the environmental variable. Thus, the coefficients of different habitat use models could be directly compared and interpreted, roughly determining the importance of each variable (Zuur et al 2007). We tested successively for environmental predictor trends in our data using generalized linear mixed-effects Models (GLMMs; *lme4* package (Bates et al 2013)). The minimum stopover duration was introduced as a fixed effect, and the year of capture was a random term, to control for possible differences between years. Environmental variables were firstly subjected to a cross-correlation analysis, in order to avoid problems with parameter estimations (Zuur et al 2007). One of each pairwise highly correlated variables (i.e. $r_s > 0.7$; Tabachnick and Fidell 1996) was excluded from the model. The variable retained from each pair was the variable that produced the smallest Akaike's information criteria (AIC; Burnham and

Anderson 1998) in the final model. Thus variables NAO_monthly, and Wdsp_NAfr were excluded from the spring migration models.

Upon model building, we began with a model with all the variables and the biological meaningful interactions, and deleted the non-significant variables step by step until obtaining the final models. We used the Akaike Information Criterion, corrected for small sample size (AICc), in order to identify the most parsimonious model for each migratory season from each candidate set of competing models (Burnham and Anderson 2002). We ranked all the models in accordance with their ΔAICc values, with models whose ΔAICc ≤ 2 being equally good (Burnham and Anderson 2002)), and selected the models with the lowest AICc. Finally, we performed model averaging with *MuMin* R package (Barton 2014) from models with ΔAICc ≤ 2, and thus extracted the averaged coefficients from predictors selected on such models. All model residuals were examined for potential inflated influence on the model output. All statistical procedures and data management steps were performed within the R environment (R Core Team 2013). Computations were carried out using several functions within different R packages, (psych, doBy, plyr, MASS, lme4, lmerTest).

Results

During the spring migration the stopover duration of reed warbler decreased significantly between 2003 and 2012 ($r_s = - 0.68$; $p < 0.05$; n=10 Fig. 2A), whereas body mass variation increased almost significantly over the same period ($r_s = 0.61$; $p= 0.061$; n=10; Fig. 2B). We found no significant differences on the mean of the minimum stopover duration from our 10-days interval ringing sessions (2010- 2012) and: 1) values calculated from our 5-days interval ringing sessions (2010 – 2012, $t_{205} = 0.97$; $p = 0.33$), 2) values given by Schaub and Jenni (2001) for non-moulting birds from daily ringing sessions ($t_{364} = 0.73$, $p = 0.47$). Moreover, we found no significant differences between our first study years (2002 – 2004: 16.11 ± 8.8) and the values obtained by Schaub and Jenni (2001) for Doñana in 1994-1996 (13 days of stopover duration; $t_{75} = 1.03$; $p = 0.31$).

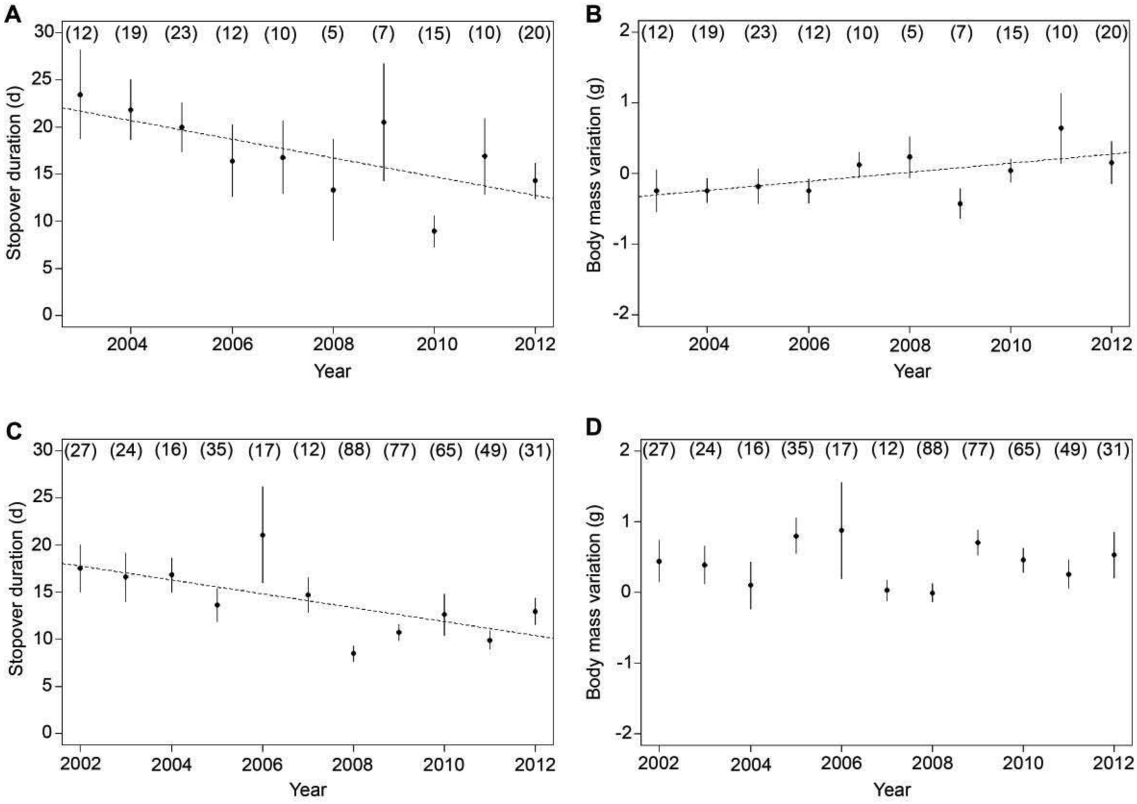

Figure 2: Mean (± SE) of minimum stopover duration (left column) and mean body mass (right column) during spring (top row: a and b) and autumn (bottom row: c and d) migrations (max. 11 years). Numbers in parenthesis indicate the sample size for each year (Spring migration n = 140; Autumn migration n = 441).

Temperature in Portugal and in Central Europe, and precipitation in Portugal showed a consistent trend from 2002 to 2012 ($r_s = 0.61$, 0.64 and -0.77 respectively, all $p < 0.05$), but the other environmental predictors did not change systematically (all $P > 0.06$). From all competing models, five models including Extended Winter NAO, Temperature in Portugal and North Africa and Precipitation in North Africa, and the most representative interactions influenced stopover duration during spring (Table 2 and 3). The minimum stopover duration increased with (1) a increase of Temperature in North Africa, and with a decrease of Extended winter NAO values (Figs. 3a and 3b).

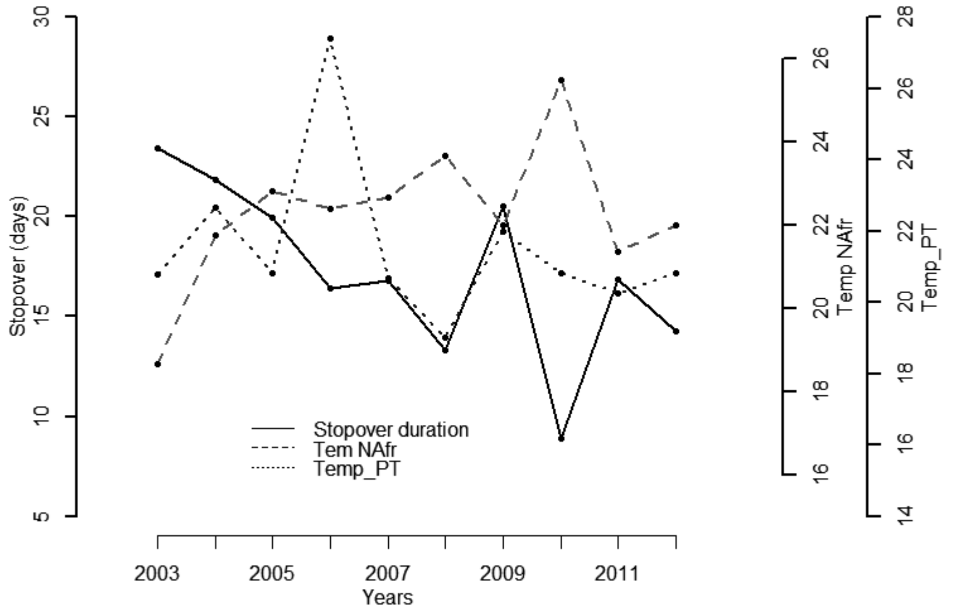

Figure 3a: Annual variation in minimum stopover duration, Temperature in North Africa (Temp_NAfr) and Temperature in Portugal (Temp_PT) during the spring migration.

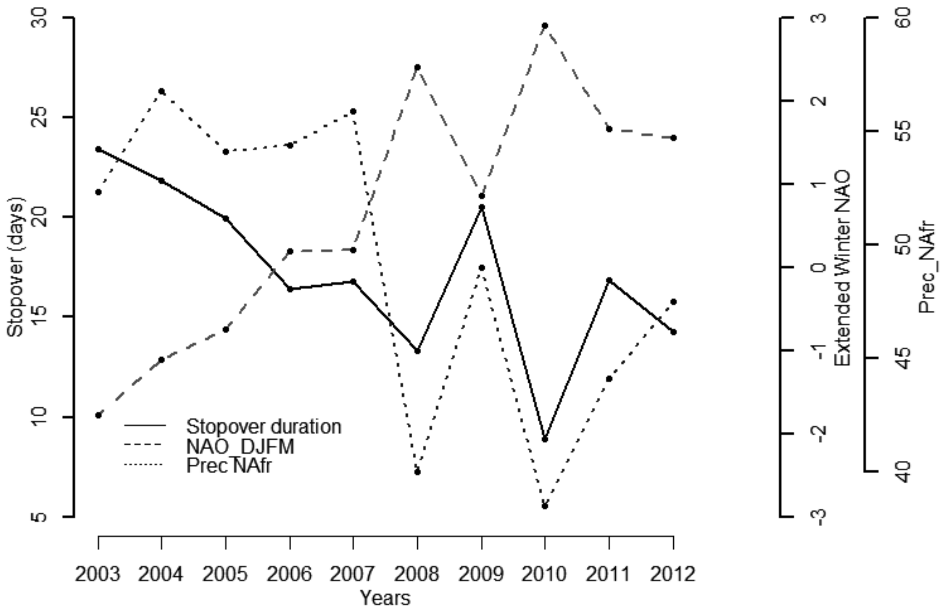

Figure 3b: Annual variation in minimum stopover duration, Precipitation in Portugal (Prec_PT) and monthly NAO (NAO_monthly) during the spring migration.

During autumn migration the minimum stopover duration decreased significantly between 2002 and 2012 (r_s = - 0.65; p = 0.03; n = 11, Fig. 2C), whereas the body mass variation remained constant over the same period (r_s = 0.00; p = 0.87; n = 11, Fig. 2D). From all competing models, one model including Precipitation in Portugal and North of Europe, Temperature and Wind speed in North of Europe, and the interaction between Temperature in North of Europe and Wind speed in North of Europe influenced the stopover duration during autumn migration (Table 2 and 3). The minimum stopover duration increased with (1) an increase of Precipitation in Portugal and Central Europe and with Temperature in Central Europe, and with a decrease in Wind speed in Central Europe (Fig.s 4a, 4b).

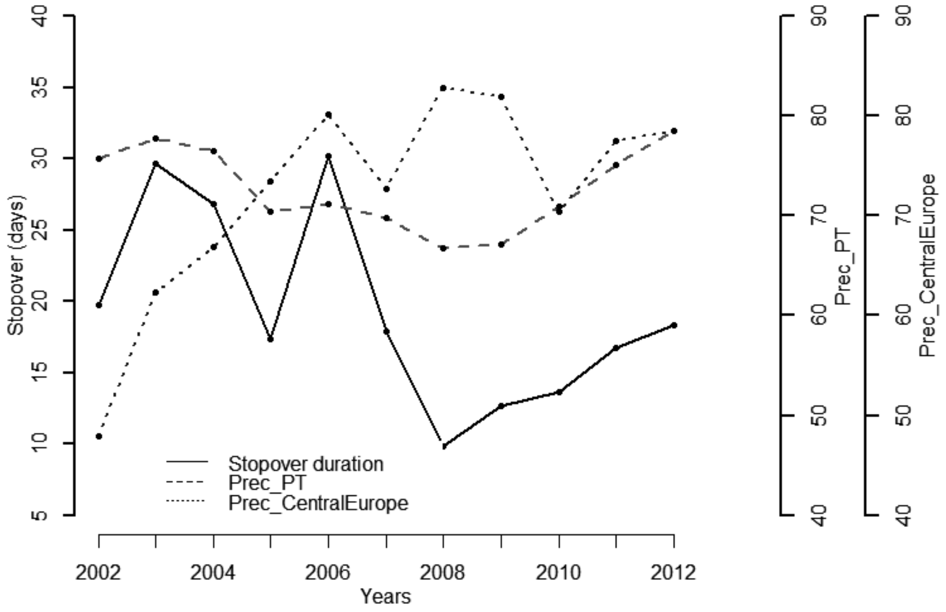

Figure 4a: Annual variation in minimum stopover duration in Precipitation in Portugal (Prec_PT) and in Central Europe (Prec_CentralEurope), during autumn migration.

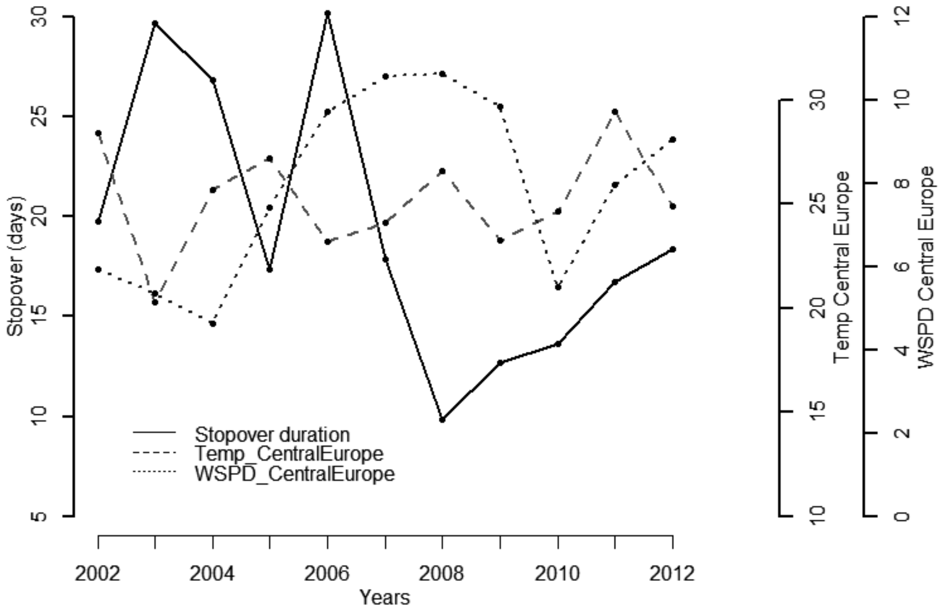

Figure 4b: Annual variation in minimum stopover duration in Wind speed in Central Europe (WSPD_CentralEurope, and Temperature in Central Europe (Temp_CentralEurope) during autumn migration.

Discussion

The reed warbler significantly reduced its stopover duration in Portugal over the last decade during both the autumn and the spring migrations. Minimum stopover duration may be underestimated because not all birds arrive or depart immediately after the first or last capture, and not all birds present were captured (Schaub et al 2004), Our results were similar to those obtained by Schaub and Jenni (2001) for other areas in the Iberian Peninsula (Ebro Delta = 10 ± 2.0 days; Doñana 13 ± 7.0 days), and by Rguibi-idrissi et al (2003) for Marroco (values ranged from 10 to 20 days), using CMR models from data collected daily. The similarity between our results and those of other studies using CMR models shows that our method is adequate to evaluate the relation of climatic variables with minimum stopover duration and body mass variation. Despite the fact that such similarity is no proof that a CMR model would not change our stopover duration results (Schaub et al 2001, Schaub et al 2004) the pattern detected in our study should present little bias because: 1) our results were based on a large sample size, 2) the same method was used every year, and 3) the quality of the Taipal reedbed, in terms of water level and density of reeds, was managed and remained relatively similar throughout the 10-year study period.

The Portuguese weather conditions over the last 10 years, characterized by a significant increase in temperature, a significant decrease in precipitation and strong oscillations in the NAO values, followed the global trend variation of these variables. The NAO index, used as a proxy of climate variability, has been associated with advances in the spring arrival dates for both short- and long-distance migratory birds in Europe (Both 2007, Hüppop and Hüppop 2003, Ottersen et al 2001). The decrease in minimum stopover duration detected in our study during both migratory periods may be explained by the fact that warmer conditions en route during spring and in Northern Europe in summer should increase food availability, increasing the body condition of departing birds and the quality of stopover sites en route to Portugal, such that when migrants stop at Portuguese reedbeds they do not need to feed extensively.

During autumn migration, temperature, precipitation and wind speed in Central Europe were all negatively correlated with minimum stopover duration. In relation to temperature, dehydration and hydric stress is amplified with its increase (Schmaljohann et al 2008). Birds flying under high temperatures will most likely have to spend more time resting and recovering from such stressful conditions than birds flying under more favourable weather conditions. Rainfall and adverse winds increase the energetic expenditure of flight, forcing the birds to delay departure from central Europe (Bayly 2007, Schaub et al 2004). Migrants facing such conditions may accumulate greater than usual energetic reserves in high-quality sites, possibly due to the influence of temperature in food abundance (e.g. (Chernetsov and Manukyan 1999, Chernetsov 2005)), in order to face the unpredictable weather conditions ahead and minimize migration time spent on stopover sites (McLaren et al 2013). In the absence of adverse weather conditions, Portugal seems a suitable location for refueling before crossing two great ecological barriers (i.e. Atlantic Ocean and Sahara desert). In addition, precipitation in Portugal was highly correlated with average of minimum stopover duration, further supporting the hypothesis that birds will wait for suitable flight conditions before departing. Because stopovers are energetically (Wikelski et al 2003) and temporally costly, birds fully refueled in stopovers prior to Portugal will most likely attempt to continue their migration towards Africa, without feeding in Portugal.

Conversely, being on the verge of the first great ecological barrier into North Africa, birds without sufficient energy to make the crossover must stop to feed and refuel. Since food abundance is directly connected to temperature, is it expected that migrants abundantly refuel in Portugal during periods of high temperature when they were unable to do so in their breeding sites or along their migratory route.

Table 2: Best model relating environmental parameters to explain stopover duration of reed warbler *Acrocephalus scirpaceus* during spring migration, and autumn migration between 2002 and 2012. The number of parameters in a model (k), the Akaike Information Criterion score (AICc), the difference between the given model and the most parsimonious model (Δ) are listed.

Spring migration			
Best Model selection	AICc	ΔAIC c	K
(1) NAO_DJFM + PREC_NAfr + Temp_PT + Temp_NAfr + NAO_DJFM * Temp_NAfr	1177.14	0	7
(2) NAO_DJFM + PREC_NAfr + Temp_PT + Temp_NAfr	1178.02	0.88	6
(3) NAO_DJFM + PREC_NAfr + Temp_PT + Temp_NAfr + NÃO_DJFM * Temp_PT	1178.08	0.94	7
(4) NAO_DJFM + PREC_NAfr + Temp_PT + Temp_NAfr + NÃO_DJFM * Prec_NAfr	1179.02	1.88	7
(5) NAO_DJFM + PREC_NAfr + Temp_PT + Temp_NAfr + Temp_PT * Temp_NAfr	1179.06	1.92	7
(6) NAO_DJFM + PREC_NAfr + Temp_PT + Temp_NAfr + Prec_NAfr * Temp_NAfr	1179.28	2.14	7
Autumn migration			
Best Model selection			
(1) Prec_PT + Prec_Euro + WSPD_Euro + Temp_Euro+ Temp_Euro * WSPD_Euro	1123.8	0	7
(2) Prec_PT + Prec_ Euro + WSPD_ Euro + Temp_ Euro + Temp_ Euro * Prec_ Euro	1130.4	6.6	7
(3) Prec_PT + Prec_Euro + WSPD_Euro + Temp_Euro	1136.5	12.6	6
(4) Prec_PT + Prec_Euro + WSPD_Euro + Temp_Euro + Prec_PT * Temp_ Euro	1136.8	13.0	7
(5) Prec_PT + Prec_Euro + WSPD_Euro + Temp_Euro + Prec_ PT + WSPD_ Euro	1138.1	14.3	7
(6) Prec_PT + Prec_Euro + WSPD_Euro + Temp_Euro + Prec_ PT + Prec_ Euro	1138.8	14.9	7

The significant decrease in minimum stopover by reed warblers in Portugal, duration both the spring and autumn migratory periods, agrees with the hypothesis of an increase in migratory speed (Both et al 2003), enabling birds to arrive and breed earlier in northern Europe (Cotton et al 2003). There is a strong evolutionary selection to minimize the time on migration (Marra et al 1998, Alerstam 2011), and Schaub and Jenni (2001) showed for sedge warblers *Acrocephalus schoenobaenus* that the best strategy is to begin migration as soon as possible. Therefore, our results may be an adaptive response to ongoing climate change. However, the complex relationships between climate change and the phenology of plants and insects along the migratory route may lead to mismatched stopovers and reproduction of long-distance migrants (Both and Visser 2001), with negative consequences for their populations.

Tabel 3: Model average coefficients parameters from the generalized linear mixed models (GLMMs) that best explained (lowest AICc, ΔAICc < 2) the variations of environmental parameters are provided. NAO_DJFM – North Atlantic Oscillation index of December-March. Temp_NAfr – air temperature in North Africa. Temp_PT – air temperature in central Portugal. Prec_PT – Precipitation in central Portugal. Prec_Euro – Precipitation in central Europe. Temp_Euro – temperature in central Europe. WSPD_Euro – wind speed in central Europe. Temp_PT – air temperature in central Portugal.

Spring migration – Model average coefficients					
Parameter	Estimate	SE	Adjust SE	Z Value	P
NÃO_DJFM	-0.68	0.17	0.17	4.07	<0.001
Prec_NAfr	-0.29	0.14	0.15	2.02	0.04
Temp_NAfr	0.33	0.11	0.11	3.07	0.002
Temp_PT	0.14	0.08	0.08	1.68	0.09
NÃO_DJFM * Temp_NAfr	-0.14	0.08	0.08	1.58	0.11
Temp_NAfr * Temp_PT	-0.09	0.05	0.05	1.67	0.09
NÃO_DJFM * Prec_NAfr	0.14	0.11	0.11	1.29	0.20
NÃO_DJFM * Temp_PT	-0.08	0.10	0.10	0.84	0.40
Prec_NAfr * Temp_NAfr	-0.07	0.12	0.12	0.62	0.54
Autumn migration – Model average coefficients					
Parameter	Estimate	SE	Adjust SE	Z Value	P
Prec_PT	0.46	0.24	0.24	1.88	0.06
Temp_Euro	0.49	0.07	0.07	6.69	<0.001
WDSP_Euro	-0-30	0.29	0.29	1.04	0.3
Pre_PT * Temp_Euro	-0.19	0.11	0.11	1.79	0.07
Temp_Euro * WDSP_Euro	-0.50	0.25	0.25	1.99	0.04
Prec_PT * WDSP_Euro	0.07	0.22	0.22	0.33	0.74
Prec_euro	0.54	0.44	0.45	1.22	0.22
Prec_Euro * Prec_PT	0.27	0.18	0.18	1.44	0.15
Prec_Euro * Temp_Euro	0.43	0.27	0.28	1.55	0.12

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Chapter II

Using triglycerides and glucose levels in blood to evaluate migratory strategies in passerines

Abstract

Migrant birds have evolved different strategies to deal with long distance movements with most using stop-over sites along their journeys. However, the frequency of these events limits the migration speed and therefore migrants face trade-offs between refueling events or carrying higher fuel stores. These require different physiological patterns which reflect different migratory strategies. Here we combined blood triglycerides and glucose levels to evaluate differences in migration ecology of two warbler species, the sedge warbler *Acrocephalus schoenobaenus* and the reed warbler *Acrocephalus scirpaceus*, at two stopover sites in Central Portugal. We also evaluated differences between these two migratory species and a resident species, the Cetti's warbler *Cettia cetti*. Sedge warblers presented significantly higher triglycerides values reflecting the increased anabolism of fat stored in adipose tissue attributed to the smaller number of stopovers during their migratory journey. Contrastingly, reed warblers presented lower triglycerides concentrations and higher glycaemia values attributed to longer stopover periods and presumably they do not make stopovers with extensive fattening at Central Portuguese reedbeds. We used also acoustic playbacks to attract birds directly from flight and measured flight metabolism rather than refueling. Migratory birds caught in active migration with acoustic playbacks presented higher triglycerides, presumably due to the mobilization of their fat stores when compared with birds that stopped naturally. The lower triglyceride values presented in Cetti's warblers point out that resident species do not use fat stores and do not fatten extensively. Our study suggests that triglyceride and glucose levels can be used in conjunction to evaluate differences on stopover strategies for migratory passerines.

Key words: Field analyses; Stopover; Migration; Fat mobilization; Physiological patterns; Acoustic playbacks.

Introduction

Migration often involves a long journey over deserts, oceans or mountains in fixed and oriented directions, and occurs primarily in association with seasonal changes in food availability. To be sustained in evolutionary terms, the associated costs and benefits of migration should be balanced (Gilg and Yoccoz 2010). Eurasian passerine migrants heading to wintering grounds in Africa may cross both the Mediterranean and the Sahara in an extensive non-stop flight of about 30-40 hours (Alerstam and Lindström 1990; Yosef and Chernetsov 2004), but a typical migratory journey is made up of flight periods, when fat load is used as fuel to cover distances, and stopover periods, when birds cease flying at a site and replenish their fat stores for the next flight period (Berthold 1996). For many species, most of their migration journey is spent in several stopover sites (Wikelski and Tarlow 2003). Fuelling at a stopover can be seen as the accumulation of potential flight distance, and is very important to describe and understand bird migration systems.

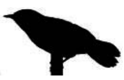
Fuelling in passerines can be evaluated by assessing their fattening rate and can be estimated from daily changes in individual body mass. However, for many species, measuring body mass which changes throughout the refueling phase may be problematic, because at least two, or more captures per individual within a short period are needed (Schaub and Jenni 2001). Measuring blood metabolites constitute an alternative to assess fuelling since they accurately reflect body condition (Guglielmo et al 2011) and allow inferring the fattening status. It is common practice to use plasma metabolites to measure the condition of passerines on migration, particularly triglycerides (TRIG) but also glucose (GLUC), glycerol, free fatty acids (FFA), β-hydroxybutyrate, and uric acid (Jenni-Eiermann and Jenni 1994; McWilliams et al. 2004; Landys et al 2005, Cerasale and Guglielmo 2010). TRIG is the storage form of lipids in adipose tissue (fat stores) and is primarily synthesized in the liver. High blood TRIG values can indicate either fattening (Smith and McWilliams 2010) or a later stage of the migratory flight (Gerson and Guglielmo 2013). Plasma TRIG levels increase during active migration of shorebirds (Landys et al 2005) and song birds, however the metabolites profiles can differ among stopover sites, suggesting differences in refueling rate (Schaub and Jenni 2001, Guglielmo et al 2002b), and stopover strategies.

Anteau and Afton (2008) found that an increase in body mass was positively correlated with plasma TRIG levels and negatively correlated with β-hydroxybutyrate levels in free-living lesser scaup (*Aythya affinis*), suggesting that plasma TRIG indicate fattening and β-hydroxybutyrate indicate metabolization of lipid stores. (Jenni-Eiermann and Jenni 1994) showed that TRIG had a stronger correlation with body-mass change than β-hydroxybutyrate, and increased during a mass gain period in Garden warblers (*Sylvia borin*). However the importance of TRIG during free flight remains unclear (Landys et al 2005) because most studies used only captive birds to conduct their experiments (Jenni-Eiermann and Jenni 1994). Lipids have several advantages over other classes of macromolecules as physiological fuels. For example, lipids can be stored within specialized adipose tissues that possesses little water content and high energy density which have relatively low mass-specific metabolic requirements (Blem 1976). Due to these characteristics, specialized lipid depots are found in some form in all nourished animals. High circulating TRIG levels might be caused by increased rates of lipid mobilization or reduced rates of lipid catabolism (McCue 2010). Total TRIG levels exhibit variable responses (i.e. increases or decreases) among starving animals (e.g. Dave et al 1975, Alonso-Alvarez et al 2003, Simpkins et al 2003), but other metabolites such as GLY and free fatty acids (FFAs) tend to follow more predictable patterns during starvation.

Blood GLUC levels should reflect the exceptional ability of migratory birds to rapidly mobilize, distribute, and oxidize endogenous lipids (Jenni and Jenni-Eiermann 1998, Gannes 2001, McCue 2010). Blood GLUC level is the most commonly measured physiological variable in starving animals (McCue 2010). However, not all animals undergoing induced starvation decrease the levels of GLUC in circulation, and birds are a good example of this (García-Rodríguez et al 1987, Lamsova et al 2004, McWhorter et al 2004). Glycerol (GLY) has been recognized as an indicator of fatty acid mobilization since it is released by adipocytes during lipolysis (Williams et al 1999, Guglielmo et al 2002a). Furthermore, Williams et al (1999) showed a strongly negative correlation between GLY levels and mass gain, being a plausible indicator of malnutrition. deGraw et al (1979) described that GLY and Cholesterol (CHOL) increase during periods of migration. CHOL can reflect an increased need for bile acids to improve the fat utilization (Bairlein 2002).

In the present study, we used TRIG, GLUC and GLY measurements to assess differences in migration ecology of two warblers in the wild: the sedge warbler *Acrocephalus schoenobaenus* and the reed warbler *Acrocephalus scirpaceus*. These two species are very similar in size and morphology but exhibit marked differences in migratory strategies, with sedge warblers making longer flights with fewer stopovers than reed warblers (Bibby and Green 1981). Therefore, if sedge warblers make fewer stopovers, and winter in the same areas as reed warblers, they should accumulate higher amounts of fat before their departure and during their fewer stopovers. Both species are nocturnal migrants, using acoustic markers for stop over site selection (Thomson et al 2003, Mettke-Hofmann and Gwinner 2004) thus allowing induced stops at specific sites by playing vocalizations at night (Mukhin et al 2008). The comparison of blood metabolites between birds that are "induced to stop" by playback and those that would naturally stop to satisfy their energetic needs is important in order to assess the body condition of migratory birds making a stopover.

We measured blood metabolites in these two migratory species and also on a resident species, the Cetti's warbler *Cettia cetti*. The fuel deposits on resident birds are considered residual when compared with those on migrants (Balança and Schaub 2005). reed warblers breed in our study sites in Central Portugal while sedge warblers only use it as stopover. Our study area is of particular interest for migratory birds because it could be their last stop before crossing to Africa. We purpose that: a) sedge and reed warblers will have higher TRIG values than Cetti´s warbler, except during the reproductive season, in which local breeding reed warblers and Cetti's warbler may show similar TRIG values; b) during migration sedge and reed warblers will differ in their TRIG values, because these two species exhibit markedly different stopover strategies; c) birds that are "induced" to stop during nocturnal migration should have higher TRIG values than birds captured without the use of acoustic vocalizations; d) both sedge and reed warblers that are "induced" to stop during nocturnal migration should present differences on TRIG, GLY and CHOL values. Because glucose levels readily increase after a feeding event, we expect blood glucose levels in Reed warblers to be higher than those in sedge warblers. Our work also assessed the reliability of blood metabolites to evaluate body condition in long-distance migratory passerines. In technical terms, our study used a portable



equipment to measure blood metabolites directly in the field, which should provide a faster, quicker and easier way to assess GLUC and TRIG levels in migratory passerines or other animals when compared to more conventional approaches that use stored blood samples from the field to measure metabolites in the laboratory. However, portable equipment may overestimate TRIG levels because it will measure total TRIG (TRIG plus GLY). In order to assess whether this is an important bias birds of the two species were tape lured at night to assess their physiological state before they engage in foraging activities and thus recover from the previous flight, and measured TRIG and CHOL (to provide an independent measure of circulating fat) in the field, and TRIG and GLY (the main degradation product of TRIG) in the laboratory.

Methods

Study area — We captured birds at two important stopovers sites for migratory bird species in central Portugal: the reedbeds of Paul do Taipal (40º 11’N 8º 41’W - 233 ha) and Paul da Madriz (40º 7’N 8º 38’W - 89 ha), in the Mondego Valley. These two sites are part of a network of wetlands stretching along the Mondego’s valley with dense vegetation dominated by bulrush (*Typha angustifolia*), common reed (*Phragmites australis*) and *Juncus sp*. (Paul do Taipal), and by common reed, *Juncus sp*., bulrush, common alder *Alnus glutinosa* and white willow *Salix alba* (Paul da Madriz).

Study species — Sedge and reed warblers are two nocturnal long-distance migratory Palaearctic passerines that winter in tropical Africa (Moreau 1972, Dowsett-Lemaire and Dowsett 1987). In Europe they are present in high numbers during spring and autumn migrations (Rguibi-Idrissi et al 2003). These species differ in their feeding ecology, diet and stopover behaviour (Green and Davies 1972, Catchpole 1973, Bibby and Green 1981), and some reed warblers breed in Portuguese reedbeds. The Cetti’s warbler is a largely sedentary, insectivorous passerine associated with dense and tangled shrubby vegetation growing near water (Cramp 1992). During the breeding season, which lasts from March to early July, males hold large territories and can breed simultaneously with up to 3 females (Bibby 2008).

Mist-netting and blood sampling — At each site, mist nets were erected before dawn and operated for five hours after sunrise to capture birds in two not consecutive days per week during both Spring and Autumn migration seasons of 2011 and 2012 (Table I) and autumn migration of 2014. Tape lure was used every other week in 2012 and during autumn migration of 2014. All birds were banded, and morphological measurements were taken (tarsus length, wing length, bill length, tail length and weight). During the breeding season there was one capture session per site for each 10-day period and no tape lure was used. The tape lure consisted of two Sony Creative Zen Mosaic EZ300 player loudspeakers, oriented to North. Songs of the two species, reed warbler and sedge warbler, were played simultaneously and continuously by two different acoustic systems, from 11pm until the end of the capture session.

Between 2011 and 2012 a blood sample (ca 75μl) from the first two captured individuals of each species was taken from the brachial vein into heparinized micro-haematocrit capillary tubes, and used immediately to measure TRIG and GLUC concentrations using portable TRIG and GLUC instruments (TRIG: Roche Accutrend GCT – precision 50 - 500 mg/dL (*+/- 6*mg/dL); GLUC: Roche *Accu-Check Advantage – precision 30 – 345mg/dL (+/- 5 mg/dL)*. Nets were visited every 30 minutes and blood was sampled immediately after the birds were removed from the net. Sedge warblers do not breed in Portuguese reedbeds, therefore their TRIG values during the breeding season were not evaluated. During the autumn migration (the most represented migratory season in terms of number of captures) of 2014 a blood sample (ca 75μl) was taken immediately from birds captured, from the brachial vein into heparinized micro-haematocrit capillary tubes and stored at 4 ºC. The samples were centrifuged in laboratory and we separated the plasma from red blood cells. Before laboratory analyses, plasma was diluted three-fold with 0.9% NaCl. Metabolites were measured via colorimetric enzymatic endpoint assays in 400 µL flat-bottomed microplates using a microplate spectrophotometer (BioTek Powerwave X340). We measured TRIG and GLY as described by Guglielmo et al (2002). In the field we analysed TRIG and CHOL using the portable machines.

Table 1: Sample size of birds captured during Spring, Breeding and Autumn Seasons, between March 2011 – October 2012, and birds captured with and without playbacks (Calls, no Calls), during Spring (15 March – 30 April) and Autumn (1 August – 15 October) migrations, between March 2012 – October 2012.

		Season		
Species	**Blood metabolites**	**Spring migration**	**Breeding season**	**Autumn migration**
Cetti's warbler	TRIG	50	31	208
	GLUC	50	31	208
reed warbler	TRIG	78	82	177
	Calls, No calls	35,68		25,61
	GLUC	81	80	128
	Calls, No calls	10,25		31,20
sedge warbler	TRIG	17	-	59
	Calls, No calls	5,12		19,40
	GLUC	15	-	75
	Calls, No calls	5;10		19,31

Data analyses — We used two-way ANOVA followed by post-hoc Tukey tests to evaluate: a) the effect of season (spring, breeding and autumn), species (resident Cetti's warblers and migratory reed warbler) and their interaction on TRIG and GLUC levels; b) the effect of playback on species (sedge vs. reed warblers), migrating season (spring vs autumn), and their interaction on TRIG and GLUC levels. Since there was no difference in TRIG and GLUC values between years ($p > 0.10$) we pooled data for both 2011 and 2012. To satisfy conditions of normality, all metabolite data were log-transformed. All statistical analyses were performed using the software R (version 2.15.2 – 2012/10/26 (R Core Team 2013). Computations were carried out using several functions within different R packages, (e.g. psych, doBy, plyr, MASS). The number of: i) Cetti's warblers, ii) reed warblers, and iii) sedge warblers with GLUC levels lower than 200 mg/dL, i.e. individuals in a state of hypoglycaemia (Motta 2011) were compared among seasons (breeding, spring and autumn migration) using a G test. Spearman correlations were used to evaluate the relationship between TRIG values and body mass of sedge and reed warblers, separately for each migratory season. Juvenile reed warblers were omitted from this correlation to exclude birds born at the study area, and thus to avoid birds that had not yet started migration. Results are given as means ± SD with a significance level at $p < 0.05$. Pearson correlation were used to evaluate the relationship in reed and Sedge warblers between a) TRIG values measured in the field and TRIG concentration obtained in the laboratory; b) TRIG values measured in the field and GLY concentration obtained in the laboratory; c) TRIG and Cholesterol values measured in the field; d) TRIG and GLY concentrations obtained in the laboratory.

Results

Seasonal variation between migratory and resident local breeders

TRIG values differed between reed warblers and Cetti's warblers ($F_{1,239} = 30.84$, $p < 0.001$) and between seasons ($F_{2,239} = 30.41$, $p < 0.001$), but there was no significant interaction between species and season ($F_{2,239} = 0.18$, $p = 0.84$; Figure 1.). GLUC values did not differ between species ($F_{1,218} = 0.79$, $p = 0.38$), but differed between seasons ($F_{2,218} = 30.93$, $p < 0.001$), and there was a significant interaction between species and season ($F_{2,218} = 5.68$, $p < 0.001$, Fig. 1.). Migratory and resident birds presented similar GLUC levels at all seasons (Fig. 1.). During the study period the number of birds with GLUC levels lower than 200 mg/dL did not differ significantly between seasons (16 % and 2.5 % for Spring, 11% and 2.5% for the Breeding season, and 46% and 22% for Autumn, respectively for the reed warbler and Cetti's warbler, n= 37, $G^2_2 = 1.01$, $p = 0.6$).

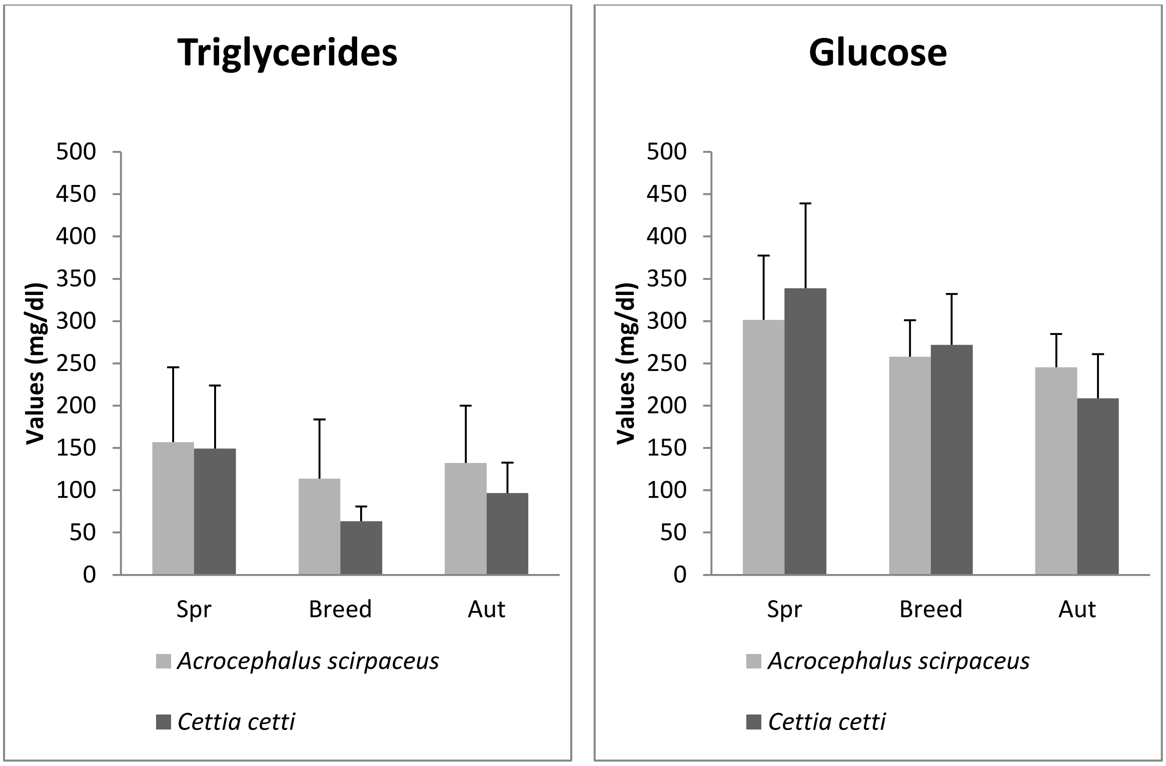


Figure 1: Triglycerides and Glucose values (mean + SD) of reed warblers (dark grey) and Cetti`s warblers (light grey) during Spring (Spr), Autumn (Aut) migration, and reproduction season (Breed).

Seasonal variation for species with different migratory strategies

Sedge and reed warblers differed in their TRIG values ($F_{1,190}$ = 98.84, $p < 0.001$), though there was no effect of season ($F_{1,190}$ = 0.02, p = 0.88), and no significant interaction between species and season $F_{1,190}$ = 1.75, p = 0.187, Fig. 2.). During spring migration reed warblers showed a significant positive correlation between TRIG values and body mass (Fig 3.). The correlations between TRIG and body mass for sedge and reed warblers were not significant for all other comparisons (all $P > 0.29$). GLUC values differed between sedge and reed warblers ($F_{1,290}$ = 5.23, p= 0,023), between season($F_{1,290}$ = 50.6, $p <$ 0,001), but there was not a significant interaction between species and season ($F_{1,290}$ = 2.53, p= 0.11, Fig. 2.). During the study period the number of birds with GLUC levels lower than 200 mg/dL did not differ significantly among seasons (3 % and 19 % for Spring migration, 25% and 53% for Autumn migration, respectively for the sedge and reed warblers, n= 32, G test $_2$ = 0.20, p = 0.66).

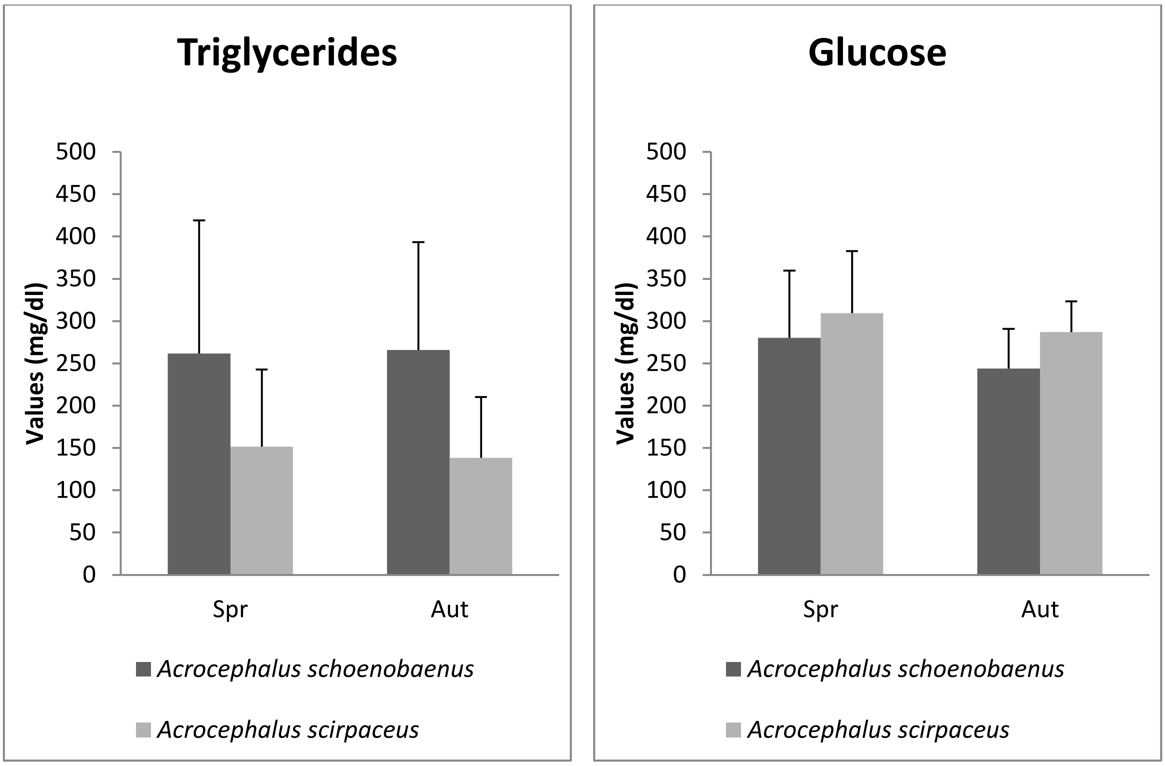


Figure 2: Triglycerides and Glucose values (mean + SD) of Sedge warblers and reed warblers during Spring (Spr) and Autumn (Aut) migration.

TRIG values differed between sedge and reed warblers ($F_{1,126}$ = 16.73, p < 0.001), between playback effect ($F_{1,126}$ = 8.61, p = 0,004), but there was no significant interaction between species and playback ($F_{1,126}$ = 3.44, p= 0.066; Fig.4). Reed warblers captured with playback showed significantly higher TRIG values than those caught without playback, though there was no playback effect on sedge warblers (post-hoc Tukey test).

For GLUC analyses there was no effect of species $F_{1,100}$ = 0.25, p =0.58) and playback ($F_{1,100}$ = 1.77, p = 0.19), but there was a significant interaction playback * species ($F_{1,100}$ = 6.35, p = 0.013; Fig.4). Reed warblers caught without playback presented higher GLUC values than reed warblers caught with playback (post-hoc Tukey test). Tape lured sedge warblers presented lower inter individual variation on TRIG and GLUC analyses than reed warblers.

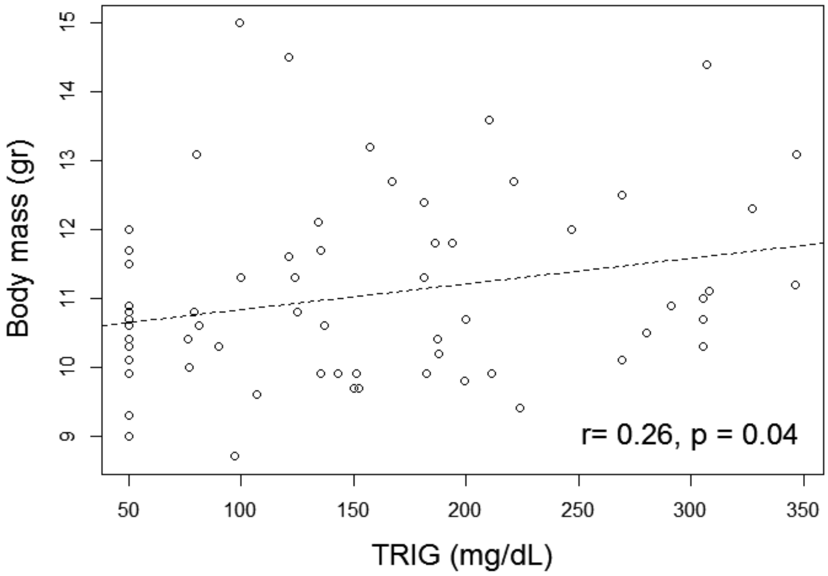


Figure 3: Relationship between Triglycerides values and body mass of reed warblers during Spring migration.

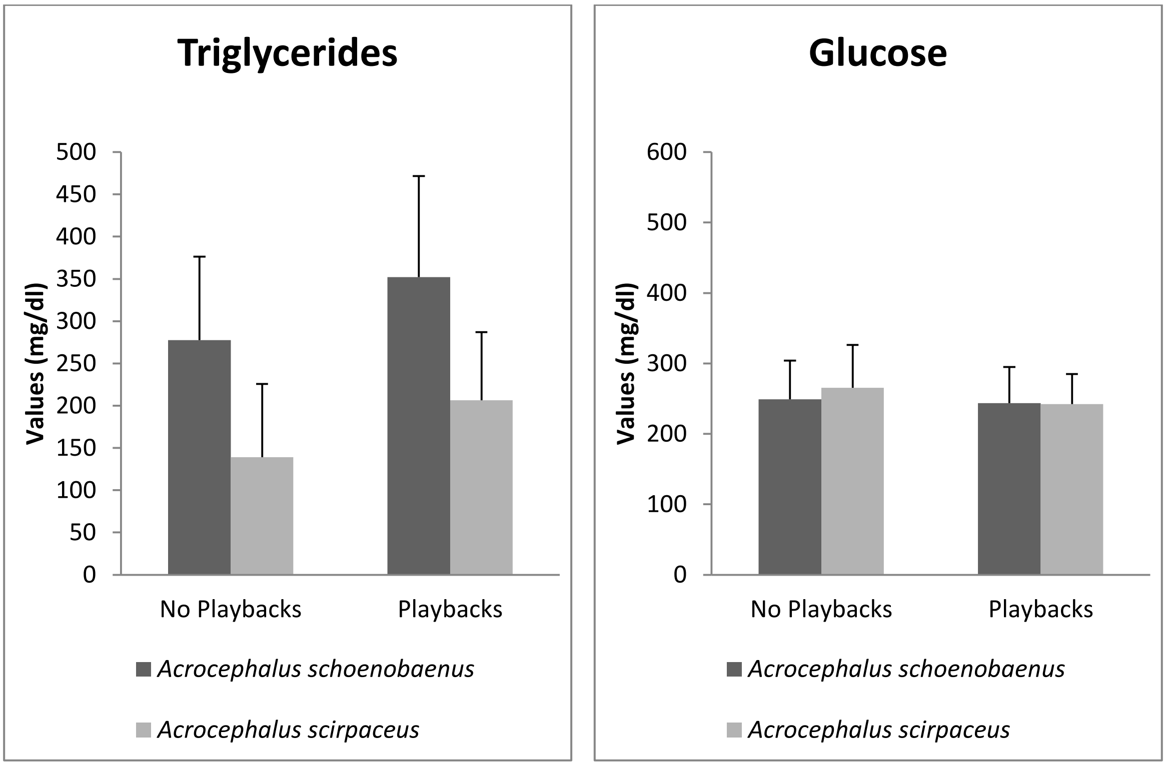


Figure 4: Triglycerides and Glucose values (mean +SD) of Sedge warblers and reed caught with or without playbacks.

TRIG and GLY laboratorial composition analyses vs field analyses

During 2014 autumn migration we captured 11 sedge warblers and 19 reed warblers. The birds were captured in the peak of the autumn migration. In laboratorial analyses sedge and reed warblers showed significant differences on plasma GLY and TRIG concentrations ($F_{1,28}$ = 5,48; p= 0.027; $F_{1,24}$ = 4.60; p = 0.042;Fig.5). Sedge warblers presented high plasma GLY and TRIG concentrations. These results agreed with our field results, as Sedge warblers presented high TRIG levels than reed warblers ($F_{1,36}$ = 11.02; p = 0.002). Moreover, sedge warblers showed significantly high CHOL levels than reed warblers ($F_{1,35}$ = 36.18; $p < 0.001$;Fig.5). During autumn migration (2014) we found a significant positive correlation (Fig.6) between: a) field and laboratory TRIG values (r = 0.32; $p < 0.001$); b) field TRIG values and laboratory GLY concentration (r = 0.46; $p < 0.001$); c) field TRIG and CHOL values (r = 0.62; $p < 0.001$); d) laboratory TRIG and GLY values (r = 0.39; $p < 0.001$).

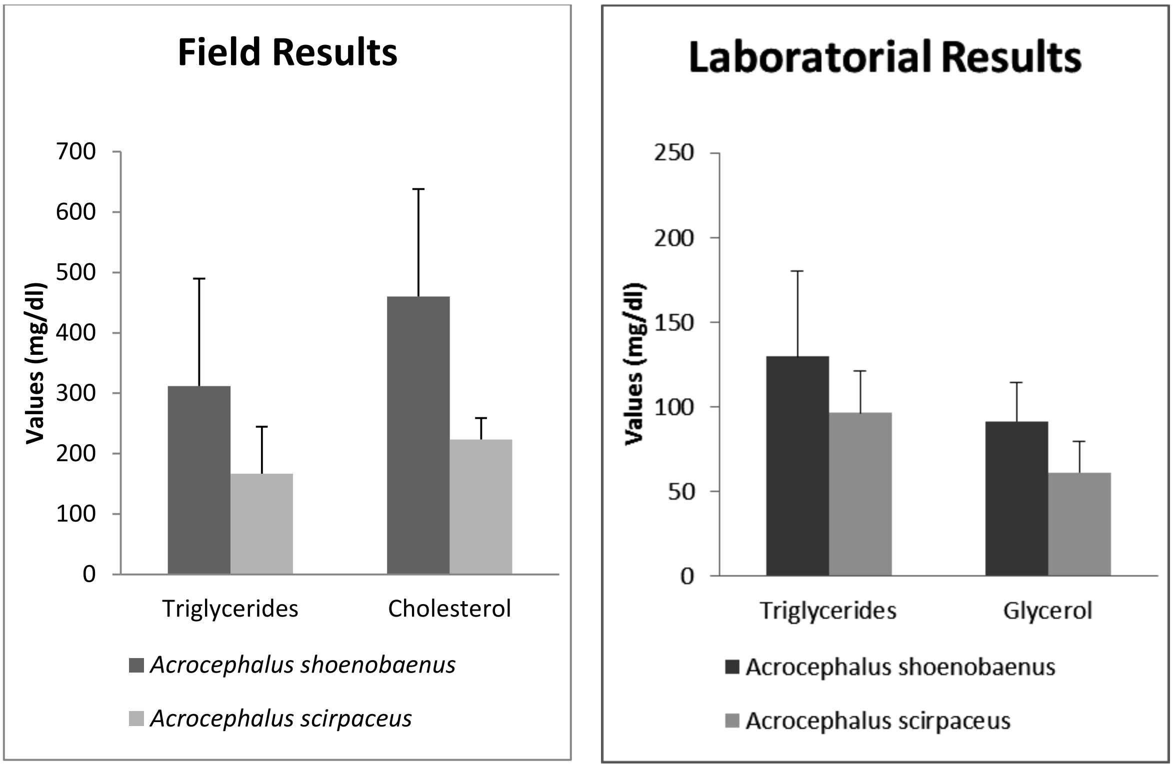


Figure 5: Triglycerides and Glycerol concentration (mean +SD) for laboratorial analyses of Sedge warblers and reed caught with playbacks. Triglycerides and Cholesterol values (mean +SD) on field analyses of Sedge warblers and reed caught with playbacks. Laboratorial results were transformed (1 mM = 88.825 mg/dL of TRIG; 1 mM = 88.652 mg/dL).

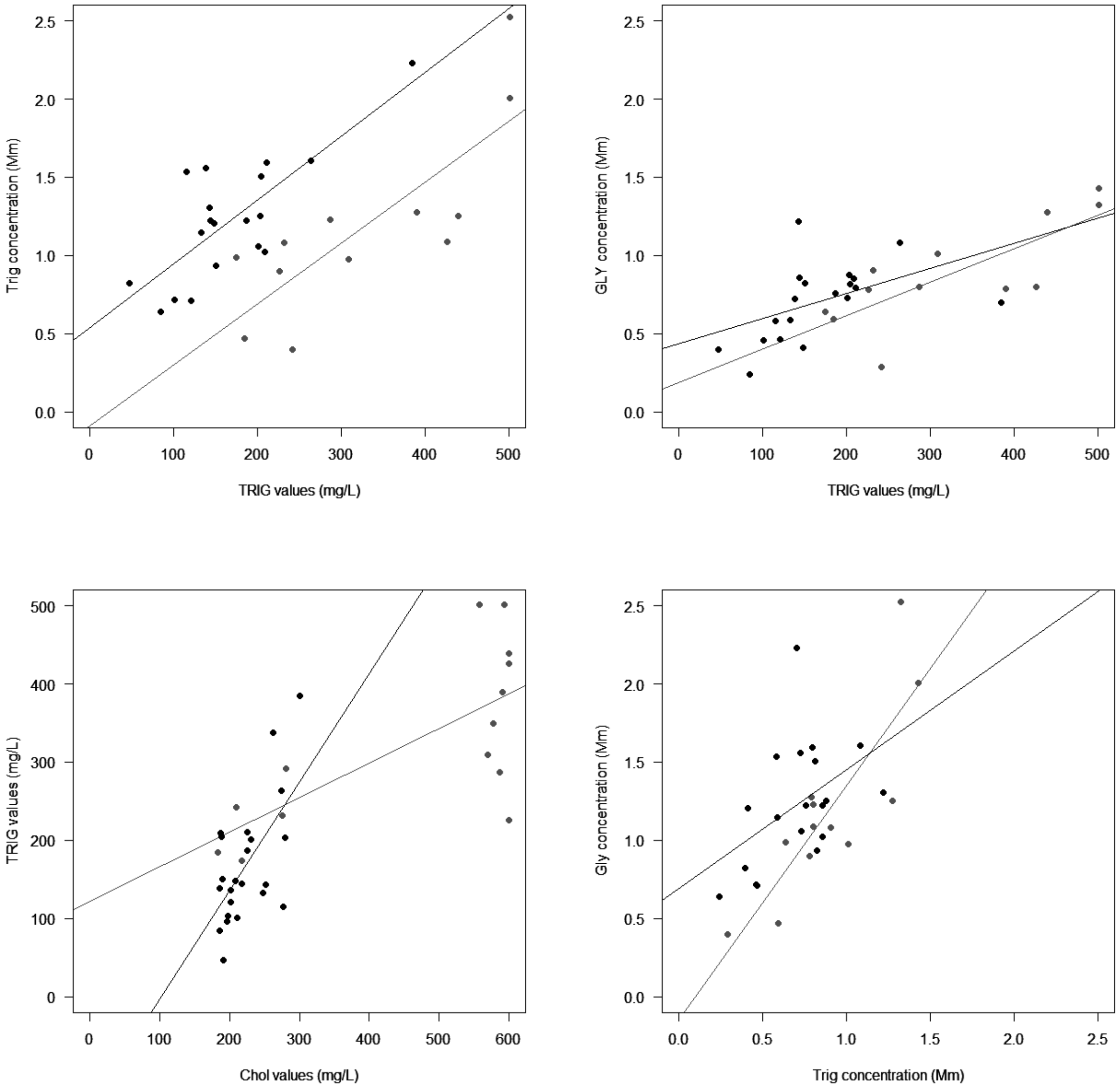


Figure 6: Relationship between: a) Triglyceride values measured in the field and in the laboratory; b) Triglyceride values measured in the field and Glycerol values measured in the laboratory; c) Triglyceride and Cholesterol values measured in the field; d) Triglyceride and Glycerol values measured in the laboratory. Reed warblers *Acrocephalus scirpaceus* (black circles) and Sedge warblers *Acrocephalus schoenobaenus* (Grey circles).

Discussion

Comparison of Triglyceride and Glucose levels between resident and migratory birds

Our study showed that resident Cetti's warbler exhibited lower TRIG values than the migratory reed warbler. As a sedentary species, Cetti's warbler does not need to accumulate as many fat stores as migratory species (Bibby and Green 1983, Balança and Schaub 2005). The higher TRIG values of the two migratory species are easily explained by the fact that they need to use their lipidic stores for energetic purposes in order to reach their destination. However, reed warblers breeding at Portuguese reedbeds showed similar TRIG values to those of Cetti's warbler during the breeding season, when they face the same constraints as the resident species. During spring, Cetti's warblers presented higher TRIG values than during both the autumn and Breeding seasons. Explanation may arise from the fact that spring (i.e. before breeding) is a period of high energetic demands and has costs, such as territory defence by males and egg formation by females, which will increase circulating TRIG.

Nevertheless the TRIG values of Cetti's warbler were lower than those of the reed warblers during both migratory seasons. To our knowledge, this is the first study clearly demonstrating that migratory birds show higher TRIG values than resident birds as previously predicted by several authors (e.g. (Mandim and Vezina 2012). We found no differences in GLUC levels between resident and migratory birds, and no differences among seasons in the number of birds with hypoglycaemia. The most likely cause for the five birds captured during the breeding season with hypoglycaemia was the increased activity and energetic demand caused by the reproductive season.

Comparison of Triglyceride and Glucose levels between migratory species with different strategies

We found that sedge warblers crossing Portugal, in both migratory seasons, had higher levels of TRIG in blood circulation than reed warblers. This should reflect higher fat reserve mobilization by sedge warblers, attributed to the smaller number of stopovers during their migratory journeys than those of reed warblers (Bibby and Green

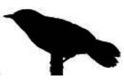
1981): it also suggests that sedge warblers use more lipid stores in each migratory step, due to longer fasting periods causing lower glycaemia levels.

Our results suggest that reed warblers do not make extensive fattening at Portuguese reedbeds. Furthermore, the reed warbler blood GLUC levels were significantly higher than those in sedge warbler. Since reed warblers stop more and with longer stopover periods, their migratory energetic needs are presumably lower. By feeding more frequently, GLUC levels can be maintained at the expense of catabolically faster substrates, namely dietary carbohydrates and glycogen stores, reducing the consumption of fat stores and thereby lowering circulating TRIG levels. The levels of blood metabolites such as GLUC are dependent both on the composition of energy reserves (Jenni and Jenni-Eiermann 1998) and on the metabolic preferences adopted by muscles under migration. A more pronounced dependence on lipid sources is typical of species with long migrations and the higher levels of TRIG found in plasma of sedge warbler corroborate such strategy. This preferential usage of lipids for such long durations has to be finely matched with GLUC availability. Adequate oxidation of lipids can only occur if GLUC stores are not fully exhausted and for that reason blood GLUC have to be kept under strict concentrations regimens to avoid misuse by other peripheral tissues. A low but steady level of blood GLUC warrants a longer duration of existing glycogen stores and that way ensures adequate use of most of the lipid stores. On the reverse, higher blood GLUC levels would deplete more rapidly the glycogen stores and limit the possibility to oxidizing lipids upon their exhaustion. This is because Portuguese reedbeds are likely to offer reed warblers many opportunities to replenish their fat and carbohydrate stores. Reed aphides, a major energy source for sedge warblers, are scarce in the Iberian Peninsula (Villarán 2000), making stopovers for this species less profitable and, as a consequence, they cannot replenish their glycogenic stores and are forced to spend longer fasting periods that ultimately consume their lipidic stores. These lipids will be mobilized from the stores accumulated in the liver and adipocytes (Scow et al 1970, Hurley et al 1986, Elia et al 1987), increasing TRIG, GLY and CHOL values in blood circulation. Their GLUC levels were lower than those of reed warblers', presumably because when we caught the birds they had already spent their dietary carbohydrates and glycogen energy sources. Sedge and reed warblers presented

higher GLUC levels in the spring migration than in the autumn, suggesting that in Spring, birds arriving from Africa had presumably depleted their lipid stores, and resort more extensively to carbohydrate sources to restore lipid stores by *de novo* lipogenesis.

Tape lured birds presented high TRIG levels in blood circulation, which can be explained by the fact that they were in active migration. Reed warblers captured without playbacks had significantly lower TRIG values, presumably because they were not as strongly dependent on fat stores at the time of capture. The fact that reed warblers caught without playback presented higher GLUC values, suggests that birds had access to food resources recently. Sedge warblers tape lured and not tape lured showed similar TRIG values probably because both fattened in Northern and Western Europe and are more dependent on fat stores throughout their migration. The lower inter individual variation observed in tape lured birds may indicate that these individuals were in a better condition and were able to continue their migration further South or North without making an anticipated stopover.

Our results suggest that TRIG and GLUC levels in blood circulation reflect differences between resident and migratory birds on mobilization and utilization of energy stores when in active migration. During the breeding season these differences were not found when comparing reed warblers with Cetti's warblers, showing that these species have a similar behavior in the use of stores in this period. By measuring blood metabolites such as TRIG and GLUC, immediately and directly from blood of free-living birds, we decreased the likelihood of underestimating their levels, as GLUC and TRIG are degraded in the blood over time (Saunders 2000). The evaluation of TRIG and GLUC levels in blood circulation showed clear differences between the two different migratory strategies of sedge and reed warblers. TRIG seem to be the most reliable and easiest to measure blood metabolite to assess differences in migratory strategy, whereas GLUC may be used to obtain supplementary information because it indicates whether birds had access to food recently. Our results, did not demonstrate any relation between body mass or fat stores and TRIG or GLUC levels, contrary to the previous suggestions of (Jenni-Eiermann and Jenni 1994).



Comparison of Triglycerides and Glycerol levels between migratory species with different strategies

For birds in active migration Sedge warblers presented high values of TRIG, CHOL and GLY than reed warblers. Plasma concentration of TRIG and in some cases phospholipids, increase when energy intake exceeds energy demand (e.g. fattening or refueling phases). Conversely, when energy demand exceeds intake (e.g. restricted feeding, fasting or active migration) stored lipids are mobilized from adipose tissue resulting in elevated concentration of plasma GLY. As anticipated, we observed an increase of GLY concentration due to TRIG degradation. Moreover TRIG showed significant differences between species confirming our initial idea that long distance migrants should present higher TRIG values on their route to Africa. Furthermore GLY results from lipolysis of TRIG in adipose tissue and muscle during periods of negative energy balance and extreme migration phase (Lindström et al 2000). We also observed that TRIG concentrations measured in the field with portable equipment can be overestimated, because total TRIG (TRIG plus GLY concentration) from laboratorial analyses was lower. These can be related with some degradation of samples during the period elapsing from blood collection to analyses of plasma in the laboratory. Moreover, our positive and linear correlation between TRIG concentration measured in the field with GLY and TRIG measured in the laboratory corroborates our initial idea that field analyses are a good method to evaluate migratory strategies.

Lyons et al (2008) reported that GLY increased at very high plasma TRIG concentrations, such as we measured for sedge warblers (mainly) during spring and autumn migration, probably because of very high rates of hydrolysis of plasma TRIG during the fatty acid uptake by muscles and other cells. The time period elapsing from blood collection to analyses of plasma in the laboratory (TRIG tend to decreased and GLY tend to increase) will influence the results obtained (Lyons et al 2008), which advocates the fact that field measurement should constitute the best method. In the field we reduce handling hazardous conditions and sample degradation in order to obtain accurate measurements.

Conclusion

Through the examination of blood TRIG and GLUC in two migratory species we showed that TRIG can indicate two different migratory status: active migration (high TRIG levels mobilized from adipose tissue) and refuelling at a stopover. Sedge warblers in active migration showed high TRIG values, because they need to mobilize TRIG from lipid stores. On the other hand, reed warblers did not show fattening activity, even when stopping at reedbeds in Central Portugal. We also showed that TRIG and GLUC blood levels varied in opposite directions. High TRIG values were associated with low GLUC values, and high GLUC values with low TRIG values. This agrees with the notion that TRIG is the best indicator of migratory strategy of long-distance migrants: short trips with many stopovers (reed warblers) and long trips with few or no stopovers (sedge warblers). Overall blood TRIG appears to be more closely related with fat mobilization in active migration. Metabolite profiles constitute a valid technique to assess stopover ecology but factors such as time between capture and blood sampling are known to affect the measurements of plasma metabolite and enzyme levels (Jenni-Eirmann and Jenni 1991; Guglielmo et al 2001; Guglielmo et al 2004). Therefore, bleed time needs to be reduced and blood samples processed and stored accurately until analyses (McWilliams et al 2004) in the laboratory. The use of portable machines provides an accurate alternative method without the need to store and process blood samples.

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Chapter III

The importance of reedbeds and riparian areas for Cetti's warbler *Cettia cetti* along its annual cycle

Abstract

In this study we describe the importance of reedbeds and riparian areas for the annual cycle of Cetti's warbler *Cettia cetti*, examining the seasonal variation in population structure and body condition using a long-term data set (10 years). Because seasonal variations in the differential use of each wetland type should be influenced by food resources and shelter conditions for roosting, a short term study (1 year) was performed to assess the diet, trophic niche and health condition of Cetti's warbler, and to examine differences in the abundance of food resources and temperature between the two wetlands. Our results reveled that reedbeds are very important for Cetti's warbler, supporting large numbers of (mainly) juvenile females during the post breeding period. Fecal analysis showed that predator insects (mostly coleoptera) were more important for Cetti's warblers on reedbeds, and diptera were more important for the riparian Cetti's warblers. These results were corroborated by stable isotope mixing models and reveal the opportunistic foraging behavior of Cetti´s warbler. The variation on the δ^{13}C values of juvenile secondary feathers was higher on reedbeds, indicating a wider provenance for birds that move into the reedbeds in late summer/early autumn. The δ^{1}3C values for the riparian area had a smaller variation suggesting that these birds are less dispersive. Overall, the higher abundance of higher trophic level food resources, and the more favorable minimum temperatures for roosting on the reedbed, makes this habitat particularly attractive for Cetti's warbler after the breeding season.

Keywords: reedbed passerines; trophic ecology; isotopes; dispersion.

Introduction

Reedbeds and riparian galleries are wetlands of considerable importance for wildlife and provide many ecosystems services to humans (Mönkkönen and Reunanen 1999, Bolger et al 2001). These two wetland types are very productive and provide habitats for many bird species during breeding, migration stopover, juvenile dispersion and wintering seasons (Machtans et al 1996). Many resident and migratory passerine species use both habitats during part or their entire life cycle, therefore riparian and reedbed habitats offer the opportunity for detailed studies of habitat requirements of individual bird species associated with them. Cetti´s warblers *Cettia cetti*, a small non-migratory brown bush warbler common in southern and central Europe, use extensively both habitats (Bonham and Robertson 1975, Bibby 1984), and is a good model species to evaluate the differential importance of both types of wetlands for insectivorous passerines throughout their annual cycle.

Cetti´s warbler present a clear sexual dimorphism in wing length and body mass (Bibby and Green 1983, Monticelli et al 2014), which is associated with the sexual selection involved in its polygynous breeding system (Bibby 1982), with up to three females breeding with one male. Males defend exclusive territories and females rear the chicks alone (Bibby 1982). As in most bird species, female Cetti’s Warblers disperse further than males, and juveniles disperse further than adult birds in search of a possible breeding territory (Paradis et al 1998). The moult of adult Cetti´s warblers lasts about 2 months (Bibby and Thomas 1984), beginning after the breeding period and completing by the end of August/early September. Therefore, to breed successfully, moult, disperse and winter Cetti’s warbler need highly productive wetlands. Bibby and Lunn (1982) defined Cetti´s warblers as a riparian bird. Favoured habitat described was marshy scrub or willow carr (Harvey 1977), and habitat requirements for nest building was an association with tangled vegetation such as brambles *Rubus* spp. (Ferguson-Lees 1964, Harvey 1977, Sueur 1980). The importance of reedbeds for Cetti´s warblers increases after the breeding season (e.g. Bibby and Lunn 1982, Balança and Schaub 2005), and females are described to move from the most suitable habitats (as riparian habitats, nearby scrub and water) into reedbeds (Bibby and Thomas 1984). However, a detailed analysis of the importance of each wetland throughout the entire annual cycle of the

Cetti's warbler in relation to population structure (age class and sex) is lacking, and long-term data is needed to evaluate the consistency in the use of both wetland types.

There is a large lack of detailed information regarding the diet of Cetti's Warbler, such as for many other small insectivorous birds. This is mainly due to the difficulty in recognizing their prey, usually tiny soft-bodied arthropods that decompose quickly during digestion (Molina et al 1998). Cetti´s warbler is a predominantly insectivorous bird species, but some seeds and vegetal contents can also be present on fecal samples (Molina et al 1998). A technique that has been under-used in ecological studies of terrestrial passerines is the comparison of the stable isotopic composition of animals with their diet. The use of isotope ratio of a consumer's tissues to assess diet relies on two points: (1) tissues reflect the isotopic composition of an animal's diet (Hobson and Clark 1992), and (2) primary producers at the base of food webs often differ in carbon and nitrogen isotopic composition (Farquhar et al 1989, Martínez Del Rio et al 2009, Robinson 2001). The stable isotope signatures of tissues integrate information over the period during which the tissue was synthesized (Bearhop et al 2002). In the case of metabolically inert tissues such as feathers, this signature remains unchanged over the time following synthesis, recording a discrete period in the past (Hobson and Clark 1992; Bearhop et al 2002). By contrast the isotopic signature of metabolically active tissues such as blood will change over time according to switches among isotopically distinct diets or movement between isotopically distinct habitats (Hobson and Clark 1992; Bearhop et al 2002). We measured stable-nitrogen isotope ratios (^{15}N:^{14}N, expressed as δ^{15}N) and stable-carbon isotope ratios (^{13}C:^{12}C, expressed as δ^{13}C) in blood, in the second secondary feather and in the most abundant prey Orders in the diet of Cetti's warbler. Nitrogen is enriched at each successive trophic level by 2 to 5‰ (DeNiro and Epstein 1978, Hobson and Clark 1992, Kelly 2000), whereas carbon is important to reflect the foraging location, which in terrestrial environments is enriched according to the proportions of C3, C4 and CAM plants within the foraging area (Hobson 1999), and it increases 0 to 1‰ per trophic level (Cherel et al 2007, Hobson et al 1994). Stable isotopes in Cetti's warbler blood and in their main prey groups were used in Bayesian mixing models to estimate proportions ingested of each main prey group. This is important to compare the importance of each prey group for birds in riparian galleries and reedbeds.

◆

In this study we assessed the importance of the riparian and reedbed habitats on the annual life cycle of Cetti´s warbler. We describe the importance of each wetland type for the Cetti’s warbler, examining the seasonal variation in population structure during a long term study (10 years data). This dataset was complemented with a short term study (1 year) performed to evaluate health condition for each habitat type, and the role of food resources and shelter conditions as potential drivers of the seasonal variations observed in each habitat. Because juveniles do not moult their secondary feathers (Cramp, 2006) their δ^{13}C give information about the origins of the birds that may move into the reedbeds in late summer/early autumn (Bibby and Thomas 1984). Because these birds should come from multiple surrounding riparian areas we expected them to show a larger amplitude in their δ^{13}C values, then the presumably more sedentary birds of the riparian habitat. The food resources and shelter conditions on each habitat should be important for juvenile dispersion. Therefore, we compared the diet and trophic niche (using stable isotopes of Carbon and Nitrogen) of birds in these two wetland types, and the temperature in both wetlands during the period when they are most used (July to December).

Methods

Study Site - Field data was collected from Cetti´s Warblers that were captured/recaptured using mist nets at two sites 8 km apart and differing in habitat characteristics: a reedbed (Paul do Taipal) and a riparian area (Paul da Madriz) both located in a flood plain of the river Mondego, near Coimbra. Paul do Taipal (N 40º10.70 W8º41.46) is permanently flooded and composed of common reed (*Phragmites australis*) and bulrush (*Scirpus lacustres)*, and is surrounded by a hedge of willow (*Salix* sp.) on the north margin, which is starting to encroach the area of common reed. Paul da Madriz (40º7’N, 8º38’W) is a mixed riparian forest/wetland with typical aquatic species such as the common reed, the bulrush, the European white water-lily (*Nymphaea alba*), and trees species as Common alder (*Ulnus glutinosa*) and Grey willow (*Salix atrocinerea*). The habitat was surrounded by mixed *Pinus spp., Quercus* spp. and agricultural fields. Both sites were designated Ramsar sites.

Population structure of Cetti´s warbler in reedbeds and riparian areas - To compared population structure of Cetti's warbler between the reedbed and the riparian area we used 10 years (March 2003 to March 2013) of captures/recaptures using mist-nets, hereafter named long-term study. Between 25 March and 22 July the mist-netting protocol followed the guidelines of the Portuguese constant effort sites scheme (PEEC), as part of a European project (Euro-CES: Constant effort ringing in Europe) promoted by EURING (one ringing session per 10 day period). In the remaining period there were 1-8 ringing sessions/month (median = 3). In each session 120-240 m (120 m between 2002 and 2007; 240 m after 2007) of nets were operated. Captured birds were ringed, weighted and aged (juveniles = birds from fledging until first breeding season, adults = birds after first breeding season) according to (Svensson 1999). Birds with wing length ≤ 58 mm and body mass < 12.0 g were sexed as females, and birds with wing length ≥ 59 mm and body mass ≥ 12.6 g were sexed as males (Monticelli et al 2014).

We evaluated monthly variations in the nº of adult males, adult females, juvenile males and juvenile females (corrected for the mist-netting effort), and assessed seasonal differences in population structure between the two habitat types, considering four seasons: breeding (March to May), moult (July to August), autumn (September to October) and winter (November to February). From July 2011 to February 2013 (hereafter termed short-term study) such data was complemented with the sampling of fecal samples to identify diet items, blood and feather samples to determine stable isotopes that inform about trophic interactions between Cetti's warbler and their prey, and the health condition of birds. From August to November 2012 we collected also arthropod samples and measured the air temperature to compare the abundance of food resources and shelter conditions between both habitats.

Diet Sampling and Trophic niche - During the short-term study, a total of 151 fecal samples were collected from Cetti's warblers during ringing sessions. Birds were kept in cleaned bags for up to 15 minutes to defecate. Samples were kept in 70% ethanol until processing in the laboratory. All hard remains in fecal samples (mostly fragmented arthropod parts) were separated using a binocular microscope. Due to the difficulty in identifying such arthropod parts, prey items were typically identified only to the taxonomic level of Order (Barrientos 2004). To identify arthropod fragments present in fecal samples, we used a reference collection, made with the body parts of whole arthropods captured in the two study areas, and several reference sources (Borror et al 1976, Burger et al 1999, Ralph et al 1985). We grouped the arthropods according to their trophic level and importance in the diet. Prey items were grouped in five categories: (a) Predatory insects (Coleoptera and Hymenoptera), (b) Generalist predators (Aranae), (c) Dipteran *predators* (Diptera), (d) Phytofagous insects (Hemiptera) and (e) Vegetable material (reedbed seeds). Items that were not identified were placed in a “Not Identify” category.

We measured $\delta^{15}N$ and $\delta^{13}C$ values in blood, in the second secondary feather and in the most abundant prey Orders in the diet. A blood sample (ca 75 µl) was taken into heparinized capillary tubes from the brachial vein of birds, from August 2012 to February 2013, that were using the sampling area for at least three weeks (i.e. birds that we recaptured), in order to reflect items ingested on the site from August 2012 to November 2012. The blood was transported to the laboratory and frozen. The blood was used to give information on the current trophic niche of the birds at each site, a few days to several weeks prior to sample collection (Bearhop 2008).

The second secondary feather was plucked only from juvenile birds and placed into individual labeled bags, thus reflecting the diet delivered by the female during the chick-rearing period. Adult Cetti`s warblers moult their secondary feathers after the breeding season, but juvenile birds retain these feathers until the end of their first breeding season, therefore the stable isotopes of juvenile secondary feathers will reflect their rearing location.

In the laboratory, feather samples were washed 3 x 5 minutes in a 2:1 chloroform-methanol solution. Then both samples (feathers and blood) were dried at 55 °C for a minimum of 24 hours. Feathers were cut into small pieces. Small amounts of cut feathers and pieces of dried blood were weighed (0.3 – 0.4 mg) and encapsulated. Prey samples (each category) were powdered and homogenized prior to SIA. Because high lipid concentrations in soft tissues of prey can result in depleted $\delta^{13}C$ values, lipids were removed using successive rinses in a 2:1 chloroform–methanol solution (Cherel and Hobson 2005). Finally an aliquot of 0.3 – 0.4 mg dry weight of each prey sample was weighed and encapsulated. The samples were analysed in a continuous-flow isotope-ratio spectrometry, using an Isotope Ratio Mass Spectrometer (Delta V Plus, Thermo Electron) (Inger and Bearhop 2008). The isotope ratio is calculated as $\delta X = [(R_{SAMPLE} / R_{STANDARD}) - 1] \times 1000$, where X (‰) is ^{13}C or ^{15}N and R is ^{13}C / ^{12}C or ^{15}N / ^{14}N (Kelly 2000, Inger and Bearhop 2008). For carbon, $R_{STANDARD}$ is the Vienna-Peedee Belemnite marine fossil limestone formation from South Carolina. For nitrogen, $R_{STANDARD}$ is the atmospheric nitrogen (Kelly 2000).

Food resources and shelter conditions – We used pitfall traps to infer arthropod availability on the soil, and sticky traps to estimate arthropods available on the vegetation, during the short term study. All arthropod sampling techniques have inherent biases. The two techniques that we used should be adequate to compare the abundance of arthropods between the two habitats (Cooper and Whitmore 1990). We used both techniques to sample 5 points in each habitat during 2012 (all separated by 100 meters), twice per month (hereafter termed Fortnights periods) during the period of higher abundance of Cetti's warbler: August to November (see results).

The Pitfalls traps were plastic containers with 0.2 L capacity, with the 2/3 of their volume with Ethanol 62%. The Sticky traps (40 cm x 10cm, model Viarma), were placed on vegetation 50 cm above the ground. The arthropods were identified to order level using a binocular microscope and placed in three size categories: a) less than 5 mm; b) between 5mm and 10 mm; and c) bigger than 10 mm. The total biomass of arthropods was estimated using the equation $W = 0.0305 L^{3.62}$ ($R^2 = 0.94$), where W is the Weight (mg); L is the Length (mm), that applies to all insect orders (Rogers et al, 1976).

To compare the shelter conditions between the two habitats we placed temperature loggers (USB Data Logger PCE- HT71) at each site, one for each habitat type, from 8 August - 17 October. The loggers registered temperatures every 30 min during these periods. We analyzed differences in the maximum temperature (the record at 14:00 h), and in the minimum temperature (the record at 06:00 h) between the two habitats.

Health condition – The body condition index was calculated as the residual from a linear regression of a body mass on wing length on first capture, during each season, for each habitat for the following groups separately: a) adult male, b) adult female, c) juvenile male and d) juvenile female. Triglycerides (TRIG), Glucose (GLU) and Hemoglobin (HEM) were measured in blood samples of individual captured during the short term study. To measure TRIG, GLUC and HEM concentrations we used portable instruments (TRIG: Roche Accutrend GCT – precision 50 - 500 mg/dL (*+/- 6* mg/dL); GLUC: Roche *Accu-Check Advantage – precision 30 – 345mg/dL (+/- 5 mg/dL); HEM:* Portable haemoglobin analyser URIT-12*;* Measuring range is 4.0 to 24.0 g/dL). Blood smears from each individual were also made, air-dried in the field, and later stained using May–Grundwalds–Giemsa in the laboratory, and scanned under 1,000 X magnification to count the number of white blood cells (WBC) per approximately 10,000 red blood cells (Ots et al1998), and the ratio of Heterophyls/Lymphocytes (H/L) on the basis of the examination of 50 white blood cells (repeatability of measurements on 50 and 100 white blood cells is very high (Norte et al 2009). The Triglycerides, Glucose and Hemoglobin values indicate the physiological condition at the moment of sampling, and the WBC an H/L values indicate infections or diseases, and these parameters reflect avian stress. These parameters allow us to compare the health condition of Cetti`s warblers between the two habitats.

Statistical analyses - All statistical procedures and data management steps were performed on the R environment (R Core Team 2013). Computations were carried out using several functions within different R packages, (psych, doBy, plyr, MASS, lme4, SIAR and SIBER). Response variables were tested for normality (Q-Q plots) and homogeneity (Cleveland dotplots) before each statistical test and were log-transformed when needed (Zuur et al 2009). Results are given as means ± SD with a significance level at $p < 0.05$.

Number of captures was standardized to reflect the number of individuals caught per hour per meter of per Month (log (Nº of birds / meters of net/ hour * 10000) + 1), and a Factorial ANOVA was used to evaluate: a) the effect of habitat (reedbed, riparian), Month (January-December), age (adults, juveniles), sex (Males, Females) and their 2nd order interactions on the number of captures (data were log transformed), using all data

from 2003 to 2013, b) the differences between seasons (breeding, moult, autumn and winter) in the number of captures.

We present the data as percentage of occurrence (% of fecal samples where a particular arthropod Order was present) per season (breeding, moult, autumn and winter), between July 2011 to February 2013. We used chi-square tests to evaluate differences in the % of occurrence of each Order (whenever expected frequencies were > 5) between habitats (within each season), and among seasons (within each habitat).

A Multivariate Analysis of Variance (MANOVA) (for $\delta^{13}C$ and $\delta^{15}N$) followed by one-way ANOVAs (separately for $\delta^{13}C$ and $\delta^{15}N$) was performed to compare stable isotope values of Cetti`s warblers in a) juvenile secondary feathers and b) blood between the reedbed and the riparian habitats. To estimate the main dietary sources to the diet of each habitat we adopted a Bayesian multi-source stable isotope mixing model (stable isotope analyses in R: SIAR; Parnell et al 2010) under R 2.15.2 (R Development Core Team 2011, www.R- project.org). All possible combinations of each source (Predator insects, Dipteran predator, General predators, Phytofagous insects and Vegetable material) contribution were examined using both isotope values ($\delta^{13}C$ and $\delta^{15}N$) from blood (during the post breading period) for both habitats (birds sampled in 2013), and from prey items collected in the two habitats. Although the isotopic value of animal tissues reflects that of diet, the transfer of the isotopic composition between prey and predator, the discrimination factor, should be estimated using controlled studies of predators fed with known isotopic diets (Caut et al 2009). In the model we used a trophic enrichment from arthropods to birds of 0.75 ±0.5 for $\delta^{13}C$ and 0.10 ± 0.5 for $\delta^{15}N$ (Caut et al 2009).

To analyse stable isotope data in the context of isotopic niche width between habitats and among seasons, we adopted the recent metrics based in a Bayesian framework (Stable Isotope Bayesian Ellipses in R: SIBER; (Jackson et al 2011), which allows for robust statistical comparisons. The area of the standard ellipse (SEAc) was adopted to compare niche width between habitats, and a Bayesian estimate of the standard ellipse and its area (SEAB) to test whether group 1 is smaller than group 2 (i.e. p, the proportion of ellipses in riparian habitat that were lower than reedbed habitat). We used the computational code to calculate the metrics from SIBER implemented in the package SIAR (Parnell et al 2010) under R 2.15.2.

We assessed differences in arthropod biomass (for each order we add soil and vegetation values) between habitats after converting number of individuals of each order to total biomass of individuals with < 10 mm. We categorized all sampling events into Fortnight periods of equal sampling effort. We used a Factorial Anova, followed by a post-hoc Tukey's HSD test, to assess differences in: a) total arthropod biomass between habitats, Fortnight period, and their interaction, and b) the arthropod biomass of each main order in the diet between Habitats, Season and their interaction. To account for deviations from assumptions of normality and homogeneity of variance, we log transformed all biomass data (Sokal and Rohlf 1995).

We used two-way ANOVA, followed by post-hoc Tukey's HSD test, to evaluate the effect of Habitat, Season and their interaction on body condition of: a) adult males, b) adult females, c) juvenile males and d) juvenile females, using data from the long-term study. To evaluate the effect of habitat on each physiological parameter for birds sampled during the short term study (triglycerides, glucose, hemoglobin, WBC index and H/L index) we used T-tests, separately for adults and juveniles (log transformed values to meet normality requirements). To evaluate the differences in maximum and minimum temperatures between the two habitats we used a t-test.

Results

Population structure of Cetti´s warbler in the reedbed and riparian area

The total number of Cetti's warblers captured (Fig. 1) differed between the reedbed and the riparian habitat ($F_{1,847}= 81.12$; $p < 0.001$), with a much higher number of captures on the reedbed. There were also two significant interactions: habitat*age ($F_{11,847}=17.93$; $p< 0.0001$), with juvenile females showing a great influx of individuals into the reedbed, and habitat*month ($F_{11,847} = 11.20$; $p < 0.001$) due to the strong increase of individuals in the reedbed from September to November. However the interaction habitat*sex was not significant ($F_{11,847} = 2.55$; $p= 0.11$). The riparian habitat presented much lower captures/densities of Cetti's warbler than the reedbed habitat, particularly from July to November. Both the riparian and the reedbed habitats had a significantly higher number of females than males (riparian: $F_{1,412} = 11.24$; $p< 0.001$; reedbed: $F_{1,412} = 39.27$; $p< 0.001$), and of juveniles than adults (riparian: $F_{1,412} = 73.20$; $p< 0.001$; reedbed:

$F_{1,412}$ = 11.42; p< 0.001). The months with higher number of birds were September to November (riparian: $F_{1,412}$ = 5.06; p< .001; reedbed: $F_{1,412}$ = 37.78; p< 0.001). For both habitats, there was a significant interaction Sex*Month (riparian: $F_{11,412}$ = 2.84; p< 0.001; reedbed: $F_{11,412}$ = 3.81; p< 0.001), and Age*Month (riparian: $F_{11.412}$ = 5.20; p< 0.001; reedbed: F $_{11,412}$ = 1.32; p< 0.001). However there was no significant interaction Sex*Age (riparian: F $_{11,412}$ = 2.52; p= 0.113; reedbed: F $_{11,412}$ = 1.47; p= 0.226).

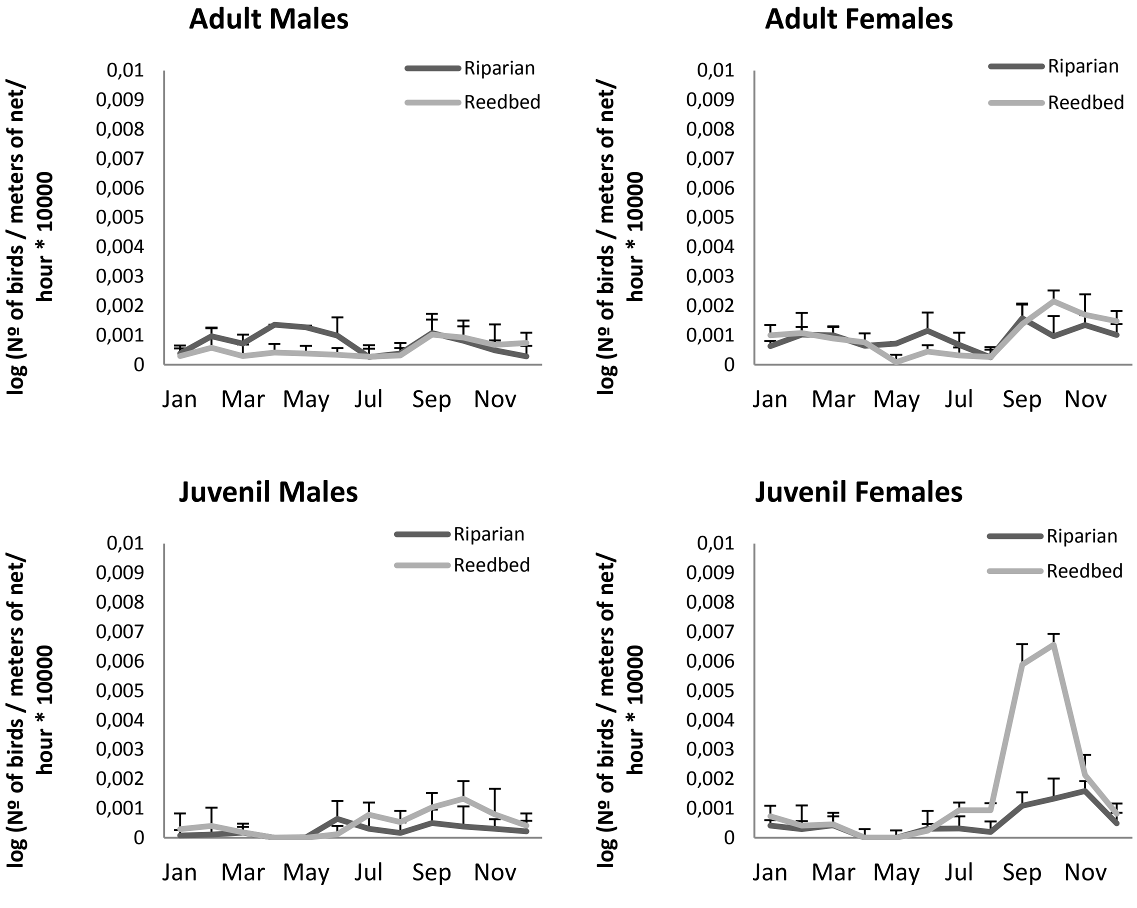

Figure 1: – Comparison of monthly variation in the abundance of Cetti´s warbler *Cettia cetti* between reedbed and riparian habitats. Values are mean ± SD of the number of birds captured per meter of net per hour * 1000 (log transformed values).

Diet and Trophic niche

Cetti’s warbler feed on a great variety of arthropods: Coleoptera, Diptera, Hymenoptera, Hemiptera e Araneae (Table 1). The most representative Order in the diet for both habitats at all seasons was Coleoptera, but during the winter and the breeding season Diptera and Araneae were also important (Table 1). A chi-square comparing the frequency of occurrence of each prey order between habitats (within each season) showed a significant difference for the occurrence of Coleoptera and Hymenoptera in autumn ($\chi^2_1 = 4.7$, $p= 0.031$ and $\chi^2_1 = 7.2$, $p = 0.007$).

Juvenile Cetti`s warblers from the two habitats were segregated by their overall isotopic values in secondary feathers (MANOVA, Wilk’s λ, $_{F2,22} = 4.04$, $p = 0.03$). The univariate analysis showed a significant difference between habitats for $\delta^{13}C$ (riparian birds: -23.4 ± 0.59, reedbed birds: -24.0 ± 0.67; $F_{1,23} = 4.80$, $p = 0.03$), but no significant differences for $\delta^{15}N$ (riparian birds: 9.07 ± 2.80, reedbed birds: 10.8 ±3.51; $F_{1,23} = 1.34$, $p = 0.26$). Birds from the reedbed showed a much larger amplitude of $\delta^{13}C$, likely indicating their wider provenance.

The results for blood isotopic values showed an overall difference (MANOVA, Wilk’s λ, $_{F2,25} = 0.33$, $p < 0.001$), and the univariate analysis showed a significant difference between habitats for $\delta^{15}N$ (riparian birds: 7.95 ± 1.90, reedbed birds: 11.8 ± 1.18; $F_{1,26} = 43.33$, $p < 0.001$) and no significant differences for $\delta^{13}C$ (riparian birds: 25.6 ± 0.45, reedbed birds: 26.0 ± 0.94; $F_{1,26} = 2.20$, $p = 0.15$). Birds from the reedbed showed a smaller range of $\delta^{15}N$ values, indicating the ingestion of a smaller spectrum of prey.

The SIAR mixing models outputs showed a clear segregation between the two habitats on the nitrogen values, and differences on isotopic signatures between prey items (Fig. 2). Predator insects were more important in the diet of reedbed birds, which agrees with the higher abundance of this insect group in the reedbed habitat. Cetti´s warblers from the riparian habitat were more generalists, ingesting dipteran insects, predator insects and generalist arthropod predators. The importance of seeds was marginal on reedbeds, which agrees with the diet data from fecal samples; however they may be important in riparian habitats (Fig.3).

Table 1: Seasonal variation in the diet (% of Occurrence) of Cetti`s warbler *Cettia cetti* in the riparian area and reedbed (moult (July – August), autumn (September – October), winter (November – January) and breeding (March to May). The 5 most important orders in the diet are indicated in bold. Sample size (no. of fecal samples for each period is shown in parenthesis). - = no data.

	2011						2012						2013	
	Moult		Autumn		Winter		Breeding		Moult		Autumn		Winter	
	Riparian	Reedbed	Riparian	Reedbed	Riparian	Reedbed	Riparian	Reedbed	Riparian	Reedbed	Riparian	Reedbed	Riparian	Reedbed
Predator insects														
Coleoptera	-	**50.0** (4)	-	**66.7** (6)	**75.0** (4)	**50.0** (18)	**50.0** (16)	**31.6** (19)	**33.3** (9)	**71.4** (14)	**63.6** (11)	**28.2** (39)	**71.4** (7)	**50.0** (4)
Hymenoptera	-	**25.0** (4)	-	**33.3** (6)	**25.0** (4	**22.2** (18	**25.0** (16)	**21.1** (19)	**11.1** (9)	**14.3** (14)	**45.4** (11)	**10.3** (39)	**28.6** (7)	**0.0** (4)
Dipterean predator														
Diptera	-	**25.0** (4)	-	**50.0** (6)	**25.0** (4)	**38.9** (18)	**25.0** (16)	**36.8** (19)	**22.2** (9)	**57.1** (14)	**18.2** (11)	**43.6** (39)	**71.4** (7)	**75.0** (4)
Phytofagous insects														
Hemiptera	-	**0.0** (4)	-	**16.7** (6)	**100.0** (4)	**0.0** (18)	**18.8** (16)	**0.0** (19)	**22.2** (9)	**14.3** (14)	**36.4** (11)	**12.8** (39)	**14.3** (7)	**0.0** (4)
General predators														
Araneae	-	**25.0** (4)	-	**16.7** (6)	**25.0** (4)	**16.7** (18)	**0.0** (16)	**36.8** (19)	**11.1** (9)	**42.9** (14)	**27.3** (11)	**35.9** (39)	**28.6** (7)	**0.0** (4)
Vegetable material														
Seeds	-	0.0 (4)	-	0.0(6)	0.0(4)	5.6 (18)	0.0 (16)	0.0 (19)	0.0 (9)	0.0 (14)	0.0 (11)	0.0 (39)	0.0 (7)	0.0 (4)
Other Groups														
Odonata	-	25.0 (4)	-	0.0 (6)	0.0 (4)	0.0 (18)	0.0 (16)	0.0 (19)	0.0 (9)	21.4 (14)	0.0 (11)	2.6 (39)	0.0 (7)	0.0 (4)
Chilopoda	-	0.0 (4)	-	0.0 (6)	0.0 (4)	0.0 (18)	0.0 (16)	0.0 (19)	0.0 (9)	0.0 (14)	0.0 (11)	0.0 (39)	14.3 (7)	0.0 (4)
Gastropoda	-	0.0 (4)	-	16.7 (6)	0.0 (4)	22.2 (18)	0.0 (16)	0.0 (19)	0.0 (9)	0.0 (14)	0.0 (11)	0.0 (39)	28.6 (7)	0.0 (4)
Not Identif.	-	100.0 (4)	-	100.0 (6)	100.0 (4)	100.0 (18)	100.0 (16)	100.0 (19)	88.9 (9)	100.0 (14)	100.0 (11)	94.9 (39)	100.0 (7)	100 (4)

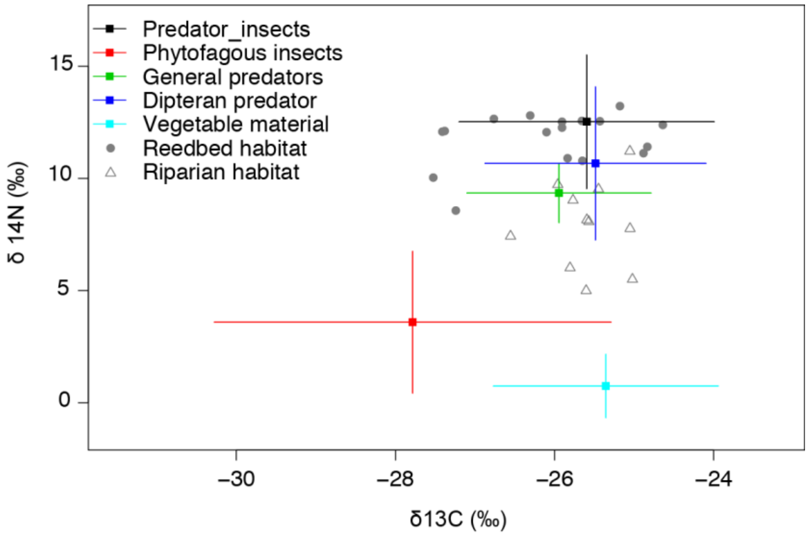

Figure 2: Stable isotope plot of nitrogen–carbon showing the isotopic signatures of blood from Cetti´s warblers *Cetttia cetti* and of its main prey (arthropod and vegetable material, mean ± SD) during autumn on the riparian and reedbed habitats.

The SIBER analysis indicate that the niche width was similar for the two habitats during the breeding period (SEA$_B$ (Blood): reedbeds birds = 3.28; riparian birds = 2.71, p = 0.53), but during the post breeding period reedbed birds showed a significantly larger niche width (SEA$_B$ (Secondary feather): reedbed birds = 7.36; riparian birds = 4.36, p = p = 0.038, Fig. 3).

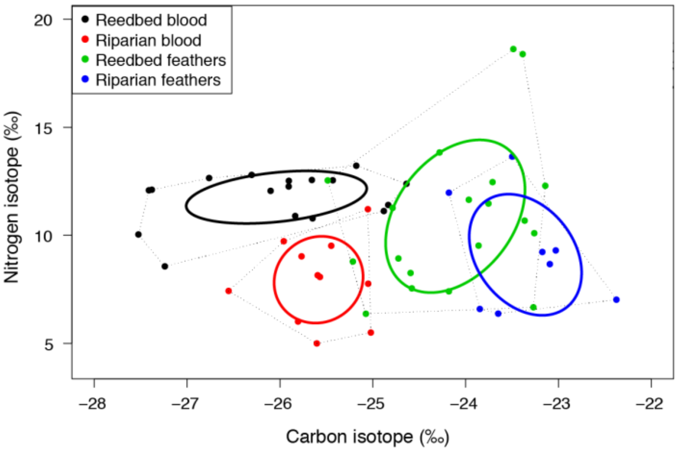

Figure 3: Stable isotope values (δ^{13}C and δ^{15}N) in blood and in secondary feathers on the reedbed and riparian habitats, during the sampled period. The area of the standard ellipses (SEAc) were represented by the solid bold lines (ellipses) and the layman metric of convex hull area (TA) by black dotted lines (see Jackson et al 2011, for more details on these metrics of isotopic niche width).

Food resources in reedbed and riparian areas

Both habitats presented similar overall abundance of food resources (Fig. 4), but when analyzing the biomass for each order separately we found significant differences between habitats and fortnights, on the abundance of Coleoptera, Diptera, Hymenoptera and Hemiptera (Fig. 4). For Coleoptera, there was an effect of habitat ($F_{1,48} = 6.99$; $p = 0.011$) and Fortnight period ($F_{6,48} = 8.44$; $p < 0.001$), but no interaction ($F_{6,48} = 1.21$; $p = 0.32$). Reedbeds had more Coleoptera during the second fortnight of August and October. For Diptera and Hymenoptera, there was an effect of Fortnight period ($F_{6,50} = 3.34$; $p < 0.001$, and $F_{6,48} = 17.68$; $p < 0.001$, respectively). For Hemiptera, there was an effect of Habitat ($F_{1,48} = 4.29$; $p = 0.044$), Fortnight period ($F_{6,48} = 4.33$; $p < 0.001$), and an interaction between these two factors ($F_{6,48} = 2.56$; $p = 0.031$). The riparian habitat presented more Hemiptera on the second fortnight of August than during November.

Health condition and shelter comparisons between riparian and reedbed habitats

The body condition, using data from the long term study, differed between the two habitats and seasons, for adult males, adult females and juvenile males (Table 2). For juvenile females there was only an effect of season, and a significant interaction habitat*season was obtained only for juveniles, both males and females (Table 2). Overall, the results indicate a higher body condition for birds of the reedbed, and the pos-hoc tests indicate also that during the breeding season adult males presented a higher body condition in the reedbed. Adult females presented a higher body condition during both the moult and breeding periods for both habitats than during autumn and winter. For Juveniles (both males and females) reedbed birds presented a higher body condition than riparian birds during the moult period, and, within reedbed juveniles the body condition during moult period was higher than during autumn and winter. During the short term study health condition parameters were similar between the two habitats, with the exception of a significantly higher glucose value for juveniles on the riparian area (Table 3). The mean of minimum temperatures registered was 12.7 ± 4.1 ºC and 14.2 ± 3.9 ºC, and the mean of maximum temperatures was 24.8 ± 4.1 ºC and 25.2 ± 4.1 ºC, respectively for the riparian and reedbed habitats. The minimum temperature differed significantly between the two habitats ($t_{140} = 2.22$; $p = 0.028$), but there was no significant difference for maximum temperature (t_{140}= 0.447; p = 0.656). This tentatively suggests that reedbeds present better conditions for roosting Cetti's warbler at night.

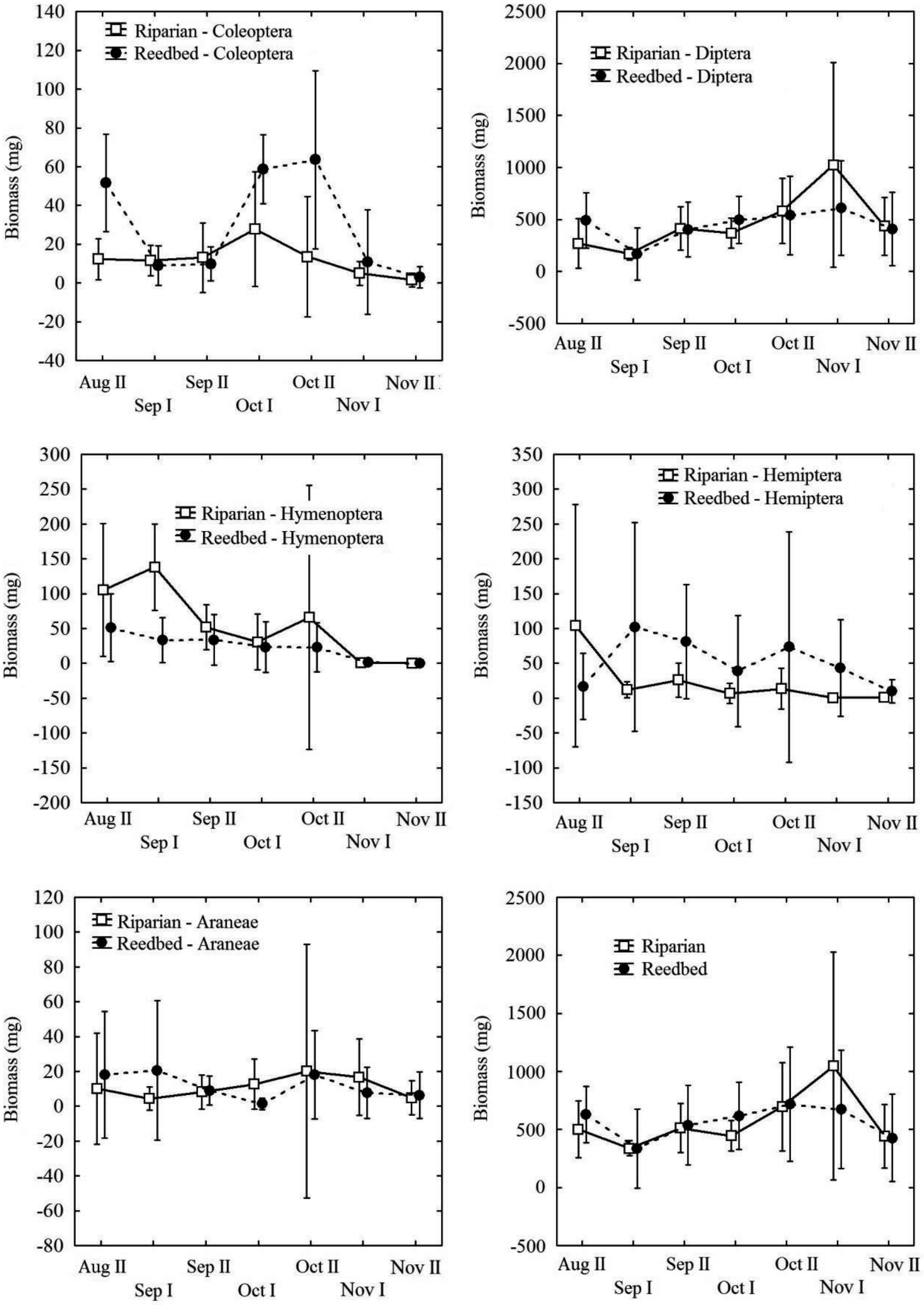

Figure 4: Mean ± SD of food available per Fortnight period (August to November 2012) on soil and vegetation (available biomass – dry weight (mg), using invertebrates with body size <10mm) in the riparian and reedbed habitats.

Table 2: Mean ± SD of Body Condition for each Age and Sex category (Adult Male, Juvenile Male, Adult Female, Juvenile Female), in riparian and reedbed habitats for all studied periods (breeding, moult, autumn and winter). The factorial ANOVA was performed to compare differences between Habitat, Season and their interaction. The significant results are in bold. Sample size (no. of samples for each period) is shown in parenthesis).

	Breeding	Moult	Autumn	Winter	Anova
Adult Male					Habitat: **$F_{1,647}$ = 6,39; p= 0.012** Season: **$F_{3,647}$ = 49,07; p < 0.001** Habitat * Season: $F_{3,647}$ =1,75; p = 0.16
Reedbed	1,06 ± 0,75 (103)	0,36 ± 0,90 (45)	-0,22 ± 0,82 (133)	0,01 ± 1,04 (91)	
Riparian	0,69 ± 1,00 (157)	0,50 ± 0,98 (18)	-0,38 ± 0,82 (59)	0,14 ± 0,90 (50)	
Juvenile Male					Habitat: **$F_{1,514}$ = 8,38; p= 0.004** Season: **$F_{3,514}$=6,88; p < 0.001** Habitat * Season: **$F_{3,514}$=3,38; p = 0.018**
Reedbed	0,68 ± 0,92 (24)	0,50 ± 1,02 (115)	0,04 ± 0,69 (190)	0,84 ± 1,08 (83)	
Riparian	0,08 ± 0,89 (30)	-0,26 ± 1,00 (18)	0,04 ± 0,73 (33)	0,30 ± 0,77 (24)	
Adult Female					Habitat: **$F_{1,927}$ = 18,31; p< 0.001** Season: **$F_{3,927}$ = 80,49; p < 0.001** Habitat * Season: $F_{3,927}$=0,16; p = 0.92
Reedbed	0,50 ± 0,88 (129)	0,55 ± 0,86 (46)	-0,39 ± 0,64 (233)	-0,40 ± 0,79 (257)	
Riparian	0,21 ± 0,88 (98)	0,29 ± 1,10 (25)	-0,59 ± 0,55 (59)	-0,63 ± 0,72 (88)	
Juvenile Female					Habitat: $F_{1,1251}$ = 0,28; p = 0.28 Season: **$F_{3,1251}$ = 30,59; p < 0.001** Habitat * Season: **$F_{3,1251}$=6,72; p < 0.001**
Reedbed	0,15 ± 0,85 (43)	0,34 ± 0,76 (163)	-0,22 ± 0,63 (618)	-0,17 ± 0,61 (208)	
Riparian	0,06 ± 0,57 (24)	-0,40 ± 0,70 (16)	-0,19 ± 0,58 (100)	-0,09 ± 0,61 (87)	

Table 3: Mean ± SD of Triglyceride (TRI) s, Glucose (GLU), Hemoglobin (HEM), WBC and H/L, for each Age category (Adult and Juvenile), in the riparian and reedbed habitats. A t-test was performed on log transformed data to compare means between Adults and Juveniles for each parameter. The significant results are in **bold.** Sample size (no. of samples for each age group) is shown in parenthesis.

	TRI (mg/dL)	GLU (mg/dL)	HEM (g/dL)	WBC	H/L
Adult					
Reedbed	107.70 ± 41.2 (17)	342.40 ± 77.2 (17)	15.58 ± 1.8 (15)	6.2 ± 2.6 (9)	0.12 ± 0.1 (9)
Riparian	115.53 ± 80.4 (13)	286.74 ± 84.1 (13)	16.48 ± 1.8 (10)	4.6 ± 2.1 (6)	0.41 ± 0.5 (6)
t-test	t_{28}= -0.20;	t_{28}= -0.21;	t_{23}= 0.99;	t_{13}= -1.32;	t_{13}= -2.14;
	p = 0.846	p = 0.834	p = 0.335	p = 0.208	p = 0.06
Juvenile					
Reedbed	91.68 ± 42.7 (12)	208.84 ± 45.0 (11)	14.70 ± 1.2 (5)	7.94 ± 4.5 (7)	0.57 ± 0.7 (7)
Riparian	98.0 ± 22.5 (10)	341.33 ± 107.1 (10)	14.65 ± 0.4 (2)	5.4 ± 4.4 (5)	0.16 ± 0.2 (5)
t-test	t_{20}= 0.30;	**t_{19}= 6.12;**	-	t_{10}= -0.83;	t_{10}= - 1.08;
	p = 0.773	**p < 0.001**		p = 0.477	p = 0.454

Discussion

Population structure of Cetti´s warbler in reedbeds and riparian wetlands

Our results show that reedbeds are very important for Cetti's warbler during the post breeding period, when large numbers of (mostly) juvenile females move to this habitat, which might be explained by a higher abundance of food resources and improved shelter conditions, when compared with riparian areas. Bibby and Thomas (1984)and Balança and Schaub (2005) found a similar pattern, with females dispersing into the reedbeds from about mid-August and reaching a peak in late September to early October.

The lower numbers of captures for both habitats during the breeding season can be attributed to sexual differences in behavior, which make males far more likely to be mist-netted than females (Bibby 1982, Monticelli et al 2014), which are incubating and, therefore, will not be captured so often. Cetti´s warblers need dense bushy vegetation to build their nests, and are reported to breed in dense bushy areas such as brambles along the riparian areas (Ferguson-Lees 1964, Sueur 1980), but apparently do not breed within common reeds (Balança and Schaub 2005). In our reedbed, Cetti's warblers might

breed along the hedges that surround the wetland (we captured/recaptured females with incubation patches in the reedbed) but feed on the reedbed during the breeding period, thus increasing their capture possibility. Similarly with other passerine bird species, female Cetti's Warblers should disperse farther than males, and juveniles should disperse farther than adults in search of a breeding territory (Paradis et al 1998). Recaptures of Cetti's Warbler ringed in France indicate that this species is largely sedentary, moving only small distances, usually no more than 10 km (Balança and Schaub 2005)

We found the same pattern observed by Bibby and Thomas (1984), i.e. a marked seasonal difference in the population structure of Cetti's Warbler between the riparian and the reedbed habitats, particularly during the post breeding period. The significant trend towards higher densities of Cetti's Warbler in reedbeds outside the breeding season may be associated with seasonal variation in territorial behavior. These can be explained because females disperse much more than males, which must occupy and defend their potential breeding territories from the post moulting period onwards, and therefore are prevented from dispersing. Monticelli et al (2014) suggests that females during that period may be forced to move towards suboptimal habitats and less profitable food patches by dominant males, and thus should demonstrate their high dependence on reedbeds during this dispersal period. In the autumn and winter, even in territorial species, there tends to be less territorial exclusion and thus a distribution according to resources (such as food) may be more apparent (Toth et al 2014).

Our results revealed differences in δ13C values on the juvenile secondary feathers, demonstrating that the two populations had distinct origins and confirming the initial idea that reedbeds were highly used by birds of distinct origins during the post moult and autumn periods. Moreover reedbeds showed a higher variation on the δ13C values of juvenile secondary feathers, and the SIBER feathers analyses confirmed the high amplitude for the δ13C values, confirming the wider provenance for birds that move into the reedbeds in late summer/early autumn. The δ13C values for the riparian area had a smaller variation (small data dispersion) indicating that individuals of this area disperse less and are more faithful to the riparian areas. (Wunder et al 2005) showed that δ13C in bird feathers from across a continental geographical area derives predominantly from local sources, rather than regional phenomena and confirming the importance of stable isotopes to evaluate habitat-specific differences.

Trophic niche and Diet

Predator insects (Coleoptera and Hymenoptera), Dipterean predators (Diptera), Phytofagus insects (Hemiptera) and Generalist predators (Araneae) were the most representative Orders in the diet of Cetti's warbler for both habitats, and were also reported in previous studies with this species in western France and Spain (Bibby et al 1983, Molina et al 1998). The seasonal changes in diet should be driven by seasonal changes in the arthropod community, which in turn should be associated with differences in the plant community between the two habitats, and with phenological changes in the plants species of each habitat (Root 1973, Pickett and Thompson 1978). In general, it appears that similar arthropod preys are important for Cetti´s warblers across much of its range.

The SIAR models strongly suggest that Cetti´s warblers fed on the most abundant arthropods of each area: a) predator insects were estimated to be very important for Cetti's warblers on reedbeds, which agreed with their high abundance in this habitat, and b) although diptera occurred more frequently in the fecal samples from reedbeds, the SIAR model results indicate a higher importance of diptera for the riparian Cetti's warblers, which agrees with the higher abundance of diptera in the riparian area during October/November (Fig. 5). This suggests the opportunistic foraging behavior of Cetti´s warbler, particularly during the post breeding period. Differences in food abundance between the riparian and the reedbed habitats may contribute to explain the much higher number of Cetti's warblers on the reedbed than on the riparian habitat during the post breeding period: we sampled arthropods extensively in both habitats and found that both the biomass of Predator insects (Coleoptera - the most important insect order on feaces) and Phytophagus insects (Hemiptera) were significantly higher in the reedbed.

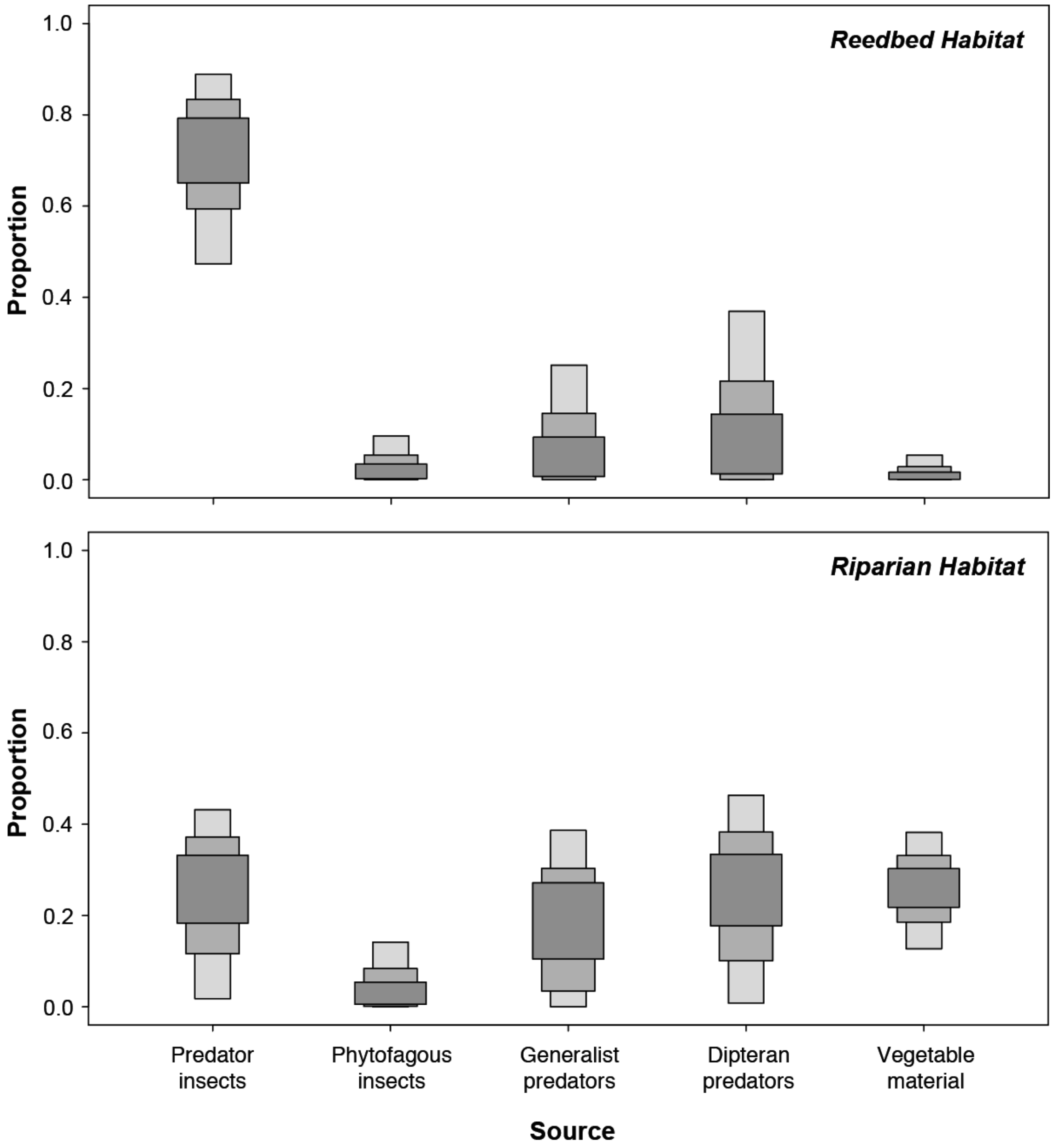

Figure 5: Estimated proportions, based on C and N isotopic signatures of blood, of the main food sources in the diet of Cetti´s warblers *Cettia cetti* from a riparian area and a reedbed during the post breeding period of 2012. Decreasing bar widths represent 50, 75 and 95% Bayesian credibility intervals computed by Stable Isotope Analysis in R (SIAR; Parnell et al 2010).

The significant difference in Cetti’s warbler δ^{15}N blood values between the two habitats is an evidence of the difference in isotopic niche between reedbed and riparian Cetti’s warblers: reedbed birds fed at a higher trophic level both during the breeding and the post breeding periods, whereas riparian birds were more generalists. Although δ^{15}N values of feathers showed no significant difference between the reedbed and the riparian habitats, the values were also higher for reedbeds. Because higher trophic level preys are likely to be more energetically profitable than lower trophic level prey (Post 2002), our results strengthens the value of reedbed areas for Cetti’s warblers.

Health Condition and Shelter

Our long term data series shows that Cetti´s warbler presented a better body condition on reedbeds, mostly during the post breeding period. This fact can be attributed to the high availability of food resources on reedbeds, which probably explains the strong movement of birds towards reedbeds during the post breeding period. Balança and Schaub (2005) show that Cetti´s warbler only moults body feathers during the post breeding dispersion, which does not support the statement that birds move into reedbeds to moult (Molina et al 1998). During the breeding season adult males showed a higher body condition than adult females. This should indicate differential costs and strategies for males and females during the breeding season, implying loss of body condition by females, which present a much higher parental investment than males (Bibby 1982). During the short term study the health condition of adults and juveniles was similar between the two habitats indicating that birds of the two areas had a similar physiological state.

Our results showed that the reedbed area should offer more favorable microclimates for roosting and protection during the night. The riparian habitat presented lower temperatures, meaning that birds should increase their metabolic rates to survive through the night, which should entail a higher risk of hypothermia in cold nights. This can be an important factor to explain the increase of individuals on the reedbed during the post breeding period. However, several studies have shown that birds are able to sense the decrease in body energy reserves (body mass) and to adjust the level of hypothermia accordingly Reinertsen and Haftorn (1986). Birds utilize hyperthermia together with other factors such as shelter and food available in order to save sufficient energy to survive through the night (Reinertsen 1983), mainly during the cold days.

Implications for Conservation

In summary, although Cetti’s warbler do not breed within extensive reedbeds our results show that this type of wetland is very important for this species during autumn and winter. We showed that reedbeds present a higher abundance of higher trophic level food resources and better shelter conditions, and both factors should be important to explain the large numbers of Cetti’s warbler dispersing into reedbeds in late summer. Other factor, such as a low predation rate in reedbeds, may also play a role but were not evaluated in this study. Our results show also that Cetti’s warbler moving into reedbeds in late summer should come from a wider range of surrounding local sources, which means that connectivity between riparian areas and reedbeds should be important for the conservation of this species. It is well known that reedbeds in Meditteranean areas are extensively used by large numbers of migrating passerines (Vilalta et al 2002) in late summer/early autumn, as well as by large numbers of wintering passerines (Brown and Atkinson 1996, Poulin et al 2002). Taken altogether these results highlight the need to protect reedbeds and riparian areas, and promote their connectivity.

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Chapter IV

Assessing the role of body mass and sex on apparent adult survival in polygynous passerines: a case study of Cetti's warblers in central Portugal

Abstract

Adult survival, an important fitness component, is usually (i) lower in lighter individuals due to their reduced ability to survive winter conditions compared to heavier ones, especially in resident species at northern temperate latitudes and (ii) lower in females compared with males due to higher reproductive costs incurred by females. In this paper, a capture-mark-recapture dataset of 649 Cetti's warblers (*Cettia cetti*) ringed seasonally at two wetlands in central Portugal over a 11-year period (2000-2010) was modelled in a multi-state framework to examine the influence of these individual covariates on apparent adult survival, while controlling for the presence of transient individuals in our study area. The probability of change in mass state ($\psi_{Light \rightarrow Heavy}$, $\psi_{Heavy \rightarrow Light}$) during the annual cycle was also estimated. Overall, birds survived better during spring-summer (breeding/moulting periods) compared with autumn-winter, but there was no effect of body mass on apparent adult survival probability. The modelling detected a significant interaction between sex and season, in which resident females survived better than resident males in spring-summer ($\phi_{RF} = 0.857 \pm 0.117$ and $\phi_{RM} = 0.698 \pm 0.181$) while the opposite pattern was found in autumn-winter ($\phi_{RM} = 0.440 \pm 0.086$ and $\phi_{RF} = 0.339 \pm 0.084$). In addition, Cetti's warblers had a tendency to lose mass in spring-summer ($\psi_{Heavy \rightarrow Light} = 0.560 \pm 0.063$) and to regain mass in autumn-winter ($\psi_{Light \rightarrow Heavy} = 0.701 \pm 0.069$). This pattern of body mass change during the annual cycle may reflect energetic costs to reproduction and moulting, and/or a response to increased starvation risk during winter. High body mass, however, did not increase adult survival in this population presumably due to the relatively mild winter weather prevailing in central Portugal. Survival estimates are more likely to be explained by important ecological and behavioural differences between the two sexes in polygynous passerines. Our results highlight that studies aiming to identify the main factors shaping survival and individual fitness in polygynous species should be conducted during different phases of their annual cycle.

Keywords: Fitness; reproductive costs; wintering; starvation risk; moulting.

Introduction

Survival is an important fitness component playing a central role in animal population dynamics, and understanding the factors that affect it is important in population demography and evolutionary ecology studies. While environmental conditions such as weather and food availability (Peach et al 1999, Robinson et al 2007a, Bocci et al 2010), and density-dependent processes (Tinbergen et al 1985, Lieske et al 2000, Saether et al 2002, Bocci et al 2010), are key factors shaping survival and hence population demography, individuals may be differently affected by these factors, depending on their particular attributes within a given population. For example, survival may be related to age (e.g. Clobert et al 1988), sex (e.g. Post and Götmark 2006a,b), or may vary between individuals according to their social status (e.g. Lundberg 1995, Schubert et al 2008).

In birds, particularly passerines, body mass is also an important determinant of fitness and survival of individuals (Houston and McNamara 1993, Covas et al 2002). For example, post-fledging survival and local recruitment rates are often positively correlated to body mass at fledging (Naef-Daenzer et al 2001 and references therein). There is also a positive relationship between adult survival and body mass, especially in resident populations at northern temperate latitudes, where the lightest individuals are those that carry low fat reserves and have thus reduced chances to survive during the cold winter months (Houston and McNamara 1993, Gosler 1996). However, high body mass has also survival costs presumably because the advantage conferred by carrying considerable amounts of fat reserves may be offset, at least for the heaviest individuals, by increased predation risk due to reduced flight efficiency (Covas et al 2002). As a result, birds might optimise their survival prospects by maintaining their body mass and fat reserves at a lower level than that of which they are capable (McNamara and Houston 1990, Witter and Cuthill 1993, Lima 1996).

Understanding the patterns of changes in body mass during the annual cycle of passerine species has also proved useful to unravel important aspects of their life history strategies (Moreno 1989). Empirical studies have shown that body mass usually increases during autumn and winter to reach a peak in mid-winter when the energetic requirements are highest (Cresswell 1998, Macleod et al 2005). Conversely, adults are often prone to lose body mass during nesting (Newton 1998, Boyle et al 2012), especially females (see e.g. Freed 1981) due to their higher parental investment (egg-laying, incubation, nestling feeding) compared with males (reviewed in Donald 2007).

Most studies examining the effect of body mass on the survival of songbirds are nevertheless based on monogamously breeding species. Extra-pair copulation and occasional cases of bigamy and polygyny are not rare in many species (Birkhead and Moller 1995, Pilastro et al 2002), and regular polygyny has been documented in 5% of species in both North American and European passerines. A peculiar trait of polygynous species is the size-dimorphism between the sexes, with males being larger than females (Searcy and Yasukawa 1981). In addition, polygynously-mated females incubate alone and male involvement in paternal care of offspring is dramatically reduced (Webster 1991, Bibby 1982). Studies examining the relationship between body mass, sex and adult survival have been previously conducted with several polygynous species of the new world family Icteridae (Johnson et al 1980, Searcy and Yasukawa 1981). However, the ‘survival’ estimates obtained and discussed in those studies actually referred to ad hoc return rates of ringed individuals and thus did not account for the potential bias introduced by unequal detection (recapture) probabilities frequently found between individuals or groups of individuals (e.g. males and females; Amrhein et al 2012). The risk of flawed inference from capture-mark-recapture (CMR) datasets can be reduced by relying on modern modelling techniques, which formally account for imperfect detection probabilities (Gimenez et al 2008). For instance, multi-state mark-recapture models can be tailored to investigate the relationships between individual state variables and survival probability, making them of great interest to address questions in evolutionary ecology (Nichols and Kendall 1995). Body mass, a proxy measure of individual fitness under selection pressure in bird populations (Covas et al 2002), is one example of such dynamic individual state variable that can be readily analysed in a multi-state framework (Nichols et al 1992). To our knowledge, however, very few studies have modelled mark-recapture data with body mass used as a state variable (but see Miller et al 2003, McGowan et al 2011), despite the fact that national ringing scheme procedures usually incorporate the collection of mass data and/or fat scores at each capture-recapture of individual birds (EURING 2010).

In the present study, we analysed a 11-year CMR dataset on the Cetti's warbler (*Cettia cetti*), a regulary polygynous species resident in most of south-western Europe. Multi-state models (Nichols et al 1992) with body mass (categorized into discrete mass classes) used as the individual state variable were developed to test the following hypotheses:

1. According to the starvation-predation risk trade-off, mass gain is adjusted during the annual cycle in order to maximise survival. Specifically, we expected individuals to (1.1.) gain mass during the winter (non-breeding) season in order to minimize the risk of starvation (McNamara and Houston 1990, Macleod et al 2005) and (1.2.) to lose mass, especially females, during the breeding season (Freed 1981, Boyle et al 2012);
2. Apparent adult survival might be lower during the winter season (i.e. compared to summer), with those individuals with low body mass (and therefore low fat reserves) exhibiting lower survival rates than heavier ones (Newton 1998);
3. A priori information on the peculiar ecology of this polygynous species suggests that females incubate eggs and raise their chicks alone, while males spend most of their time advertising and defending large territories during breeding. Previous studies on monogamous species with a strong parental investment of females compared with males found a strong reproductive cost for the former (i.e. due to high predation rate on females; Post and Götmark 2006a and reference therein). Alternatively, previous studies in a few polygynous (non-passerine) bird species (e. g. Angelstam 1984) reported significant mating costs lowering adult male survival. We thus expected that marked behavioural differences between male and female Cetti's warblers, particularly during the breeding season, may lead to sex-specific reproductive costs and adult survival rates.

Materials and methods

Study species and area - The Cetti’s warbler is a largely sedentary, insectivorous passerine associated with dense and tangled shrubby vegetation growing near water (Cramp and Brooks 1992). It is the sole European warbler species with a pronounced sexual size dimorphism and one of the few with a polygynous breeding system (Bibby and Thomas 1984). On average, males are bigger than females by c.26-32% in body mass and c.11-13% in wing-length with minimal overlap between the sexes (Bibby 1982, Bibby and Thomas 1984). During the breeding season, which lasts from March to early July, males hold large territories and can breed simultaneously with up to 3 females (Bibby 1982). Females take care of the incubation alone and provide most of the feeding to the nestlings (Bibby 1982). In late June – early July, adults start a post-breeding moult completed in c.60 days (Bibby and Thomas 1984).

The capture-mark-recapture (CMR) sessions were conducted at two wetland marshes, Paul do Taipal and Paul da Madriz, west of Coimbra, central Portugal. The two sites are classified as special protection areas (SPAs) and are part of a large network of wetlands stretching along the Mondego valley. Paul do Taipal (40º 11’N 8º 41’W) (233 ha), is a densely vegetated marsh resulting from the permanent flooding of long-abandoned rice fields. It supports a wide variety of breeding water birds and is of national importance for wintering ducks, as well as an important stop-over site for migrating passerines. Paul da Madriz (40º 7’N 8º 38’W) is located c.12km to the south of Taipal and is a smaller freshwater marsh (89 ha) surrounded by mixed pine-oak woodland and agricultural fields. At both sites, the dominant vegetation near water consists of reed (Phragmites australis) and bulrush (Scirpus lacustres).

Mist-netting protocol - From Feb. 2000 to Nov. 2010 (11 years), Cetti’s warblers were captured (mist-netted) and re-trapped at these two sites as part of a larger project including the study of reed warblers (*Acrocephalus scirpaceus*) and other migratory passerines. CMR sessions were conducted monthly (range 1-6 ringing sessions/month). During the breeding season (March - July), the mist-netting protocol followed the guidelines of the Portuguese constant effort sites scheme (PEEC), as part of a European project (Euro-CES: Constant effort ringing in Europe) promoted by EURING.

Ageing and sexing birds - At first capture, Cetti's warblers were ringed with a metal leg ring provided by the Portuguese Central Ringing Office and aged as either first-year (juvenile) or adult according to plumage characteristics (Svensson 1992). Maximum wing length was measured to the nearest 0.5mm and the birds were weighed to the nearest 0.1g with an electronic balance. Wing length and body mass were controlled at any subsequent recapture. Previous work in UK (Bibby 1982), Spain (Villaran 2000) and Portugal (Bibby and Thomas 1984) found a strong size dimorphism in the Cetti's warbler with females being shorter-winged and lighter than males. Based on the subset of birds caught with a brood patch, those individuals that had wing length ≤ 59 mm and were lighter than 12.0g were sexed as females while those individuals with body mass ≥ 12.0g and wing length ≥ 60 mm were sexed as males. A minority of individuals (ca. 3%), mainly juveniles, did not comply with both criteria and hence could not be confidently sexed. This might be due to the fact that juvenile males can have slightly shorter wings compared to adults (i.e. on average 1.6mm shorter; see Bibby and Thomas 1984) and/or because wing length measurement in the field is subject to error. In addition, it should be acknowledged that a degree of uncertainty, albeit small, existed when assigning sex for both adults and juveniles solely based on body mass and wing length measurements. Thus, intermediate birds with 12.0 to 12.4g in mass and/or wing length of 58 to 59mm were removed from the modelling (see below) in order to minimize the odds of incorporating misclassified Cetti's warblers with respect to sex.

Capture-recapture sampling periods - Despite a near-constant monthly mist-netting effort during the study period, one of the underlying assumptions of CMR models is that the recapture occasions are of negligible length relative to the time interval between them (Williams et al 2002). In order to select discrete capture–recapture sampling periods, the capture–recapture history dataset was plotted on a monthly basis (Fig. 1). For each year, a peak in capture -recaptures was apparent during the spring and autumn seasons. We thus opted for two annual recapture periods, 1 March to 30 April (spring) and 1 Sept. to 30 Oct. (autumn), for a total of 22 sampling periods from spring 2000 to autumn 2010. The recapture data obtained outside the sampling periods were not incorporated in the analysis and for birds that were caught multiple times during a recapture period, only one record/period was used during the modelling. Individuals were considered to survive between the mid-points of two successive recapture occasions, which corresponds to a 6-month survival rate estimated separately for the breeding/moulting season (1 April – 30 Sept) and the post-moulting/non-breeding season (1 Oct. – 30 March). Importantly, the terminology 'survival' used in this paper refer to an apparent survival ϕ, i.e. the probability of surviving between two successive sampling periods and remaining in the study area. As a result, the counterpart of 'survival' includes both mortality and permanent emigration from our study area (Williams et al 2002).

CMR data modelling - Multi-state capture-recapture models (Lebreton et al 2009 and references therein) were fitted using body mass class as a (stochastic) state variable (Nichols et al 1992), in which individuals were assigned to one of the following states: light female [9.0-10.4g], heavy female [10.5-11.9g], light male [12.5-13.9g] and heavy male [14.0-16.0g]. When individuals were capture-recaptured multiple times during a sampling period, the assignment to a mass class for that particular sampling period was made based on the mean mass measurement. Based on the assignment of the birds at each capture-recapture occasion as either light or heavy individuals, we modelled three kinds of parameters: apparent survival (ϕ), probability of change in, or movement between, mass class (ψ; state transition probability) and capture probability (p). During the modelling, transitions between mass states (ψLight → Heavy) and (ψHeavy → Light) were only permitted within each sex.

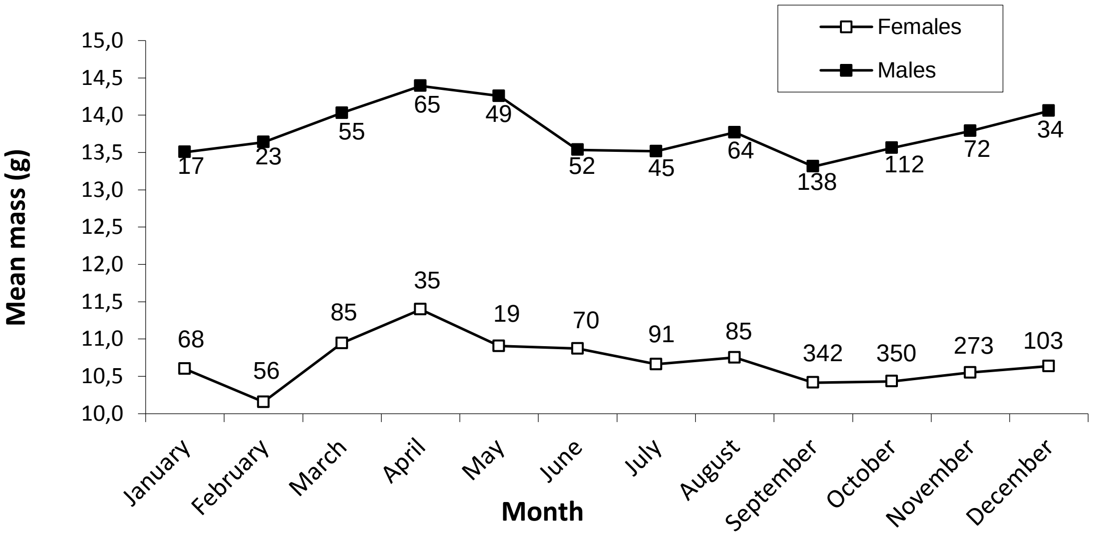

Figure 1: Annual cycle of variation in mean mass (± SE) of male and female Cetti´s warblers mist-netted in Central Portugal, 2000-2010. Sample sizes of monthly capture-recaptures are indicated below each respective plot labels. Data are shown separately for the subset females (9.0 – 11.9 g) and males (12.0 – 16.0 g).

European Cetti’s warbler populations are resident, although adults may disperse locally after breeding, especially females (Robinson et al 2007b). Most juveniles captured during the autumn period are also likely to disperse away from their natal sites (Robinson et al 2007b). Therefore, a large proportion of individuals captured in this study may be transients, i.e. individuals that are just “passing through” the area and thus unlikely ever to be recaptured, and failure to account for transient individuals may negatively bias the survival estimates (Pradel et al 1997). To overcome this bias, relative age models (Pollock 1975, Brownie and Robson 1983) were developed in a multi-state framework to estimate separately “survival” of newly-marked birds (of which a substantial proportion are transients) and survival of birds recaptured at least once following initial marking (= ‘resident’ birds; Prévot-Julliard et al 1998). In practice, this approach works by constructing models in which survival over the interval immediately following the first (initial) capture ($\phi 1$) differs from survival over subsequent intervals ($\phi 2$). In addition, the ratio $\phi 1/\phi 2$ can be used to estimate the resident probability of newly-marked birds ($\zeta = \phi 1/\phi 2$) and its counterpart, the transient probability ($1 - \zeta$) (Miller et al 2003). In the present study, the initial capture of first-year birds (juveniles) were only possible during the autumn capture-recapture sessions, i.e. the spring (March-April) capture sessions were too early for any recently-fledged birds to be mist-netted.

As a result, transient models allowed juveniles captured during their hatching year to become part of the pool of 'resident' birds only if they were recaptured during the following spring capture-recapture season or later. Cetti's warblers reach adult breeding propensity during their second calendar year, hence survival estimates of 'resident' birds in this paper refer to adult birds only.

The a priori set of competing models was developed based on biological reasoning and hypotheses to test (see "Introduction"). We had insufficient data to allow full time-specificity in survival which was permitted in the most general model instead to vary by year, sex and mass class, while both transition and capture probabilities were allowed to vary by season (spring-summer vs. autumn-winter), sex, mass class and site (notation: {ϕ(year × sex × mass class) , ψ(season × sex × mass class × site) , p(season × sex × mass class × site)} where '×' indicates a relationship that includes interaction terms). A set of candidate models (Table 1) was developed by modifying the structure of the general model. This included models with and without a transient effect on survival, models with additive effects (notation '+') between terms, and models with constant or seasonal variation in survival only. We also fitted models where season, sex, site and/or mass class effects were partially or entirely removed from the survival, transition or recapture components. Thus, as an example of notation, the model {ϕ(season + sex) , ψ(mass class × season) , p(site)} corresponds to the reduced-parameter model in which survival differs only between sexes and season (with additive effect), transition rates differ according to mass class (i.e., ψLight→Heavy ≠ ψHeavy → Light) and season (with interaction), and recapture probabilities vary by site (Taipal vs. Madriz). It can be also noted from this example that models that do not incorporate a mass class effect on transition probabilities would correspond to the case where the transition probabilities ψLight → Heavy = ψHeavy → Light.

Data analyses were performed using the package RMark (Laake and Rexstad 2008) as an interface to Mark (White and Burnham 1999). During model development, capture probability was considered as a nuisance parameter and was modelled first, followed by transition probabilities. Survival was the last variable modelled because the main interest in this study was on the factors (i.e., season, sex and mass effects) potentially affecting adult survival. During this stepwise progression, parameters that had not yet been a focus of modelling were allowed to be as general as possible (cf.

general model structure above). Akaike's information criterion corrected for small sample size (AICc) was applied to select the most parsimonious model(s) among the set of competing models (Burnham and Anderson 2002). AICc weights were also used to provide a relative measure of how well a model supports the data compared to other models (Burnham and Anderson 2002). A chi-square goodness-of-fit (GOF) test as available for the JollyMove (JMV) model (Pradel et al 2003) was performed in program U-CARE (Choquet et al 2005).

Results

Data set

Despite some slight differences between the two sexes, the annual cycle of variation in mean body mass reveals a common pattern for male and female Cetti's warblers mist-netted at two sites in central Portugal from 2000 to 2010 (Fig. 1): mean mass peaked in March-April (onset of the breeding season), then progressively decreased in May-June-July (chick-rearing period) to reach its lowest value in September-October (end of the moulting period). An upward trend in mean mass was subsequently apparent during the winter months, from November to January (Fig. 1).

Only those individuals weighed and measured (wing length) in March-April (high mean body mass) and September-October (low mean body mass) were incorporated in subsequent analyses (see "Methods"), which included a total of 649 individuals: 394 females and 109 males mist-netted at Taipal and 91 females and 55 males caught at Madriz. Before running our multi-state models, we checked whether or not there was a systematic variation in body mass with structural size (as expressed by wing length), by means of linear regression analyses. In both sexes, the fit of the regression lines was very poor ($R^2 = 0.05$ and 0.06 in females and males, respectively) despite a significant trend (both F-test with $P < 0.001$), which was mainly attributable to the high sample sizes involved in the analyses. Inter-individual differences in body mass in this dataset was thus likely to reflect inter-individual differences in fat/energy reserves, with no need to be adjusted according to structural size before inclusion in the modelling.

Sample size and goodness-of-fit test

The modelling was based on a total of 1122 capture-recaptures. The oldest Cetti’s warbler included in the models was a 7-year old female ringed as juvenile at Taipal in autumn 2003 and recaptured five times at the same site between spring 2004 and autumn 2010. The GOF test provided no evidence for lack of fit (χ217 = 11.65, P = 0.82), suggesting that the global model (see “Methods”) fitted the data adequately.

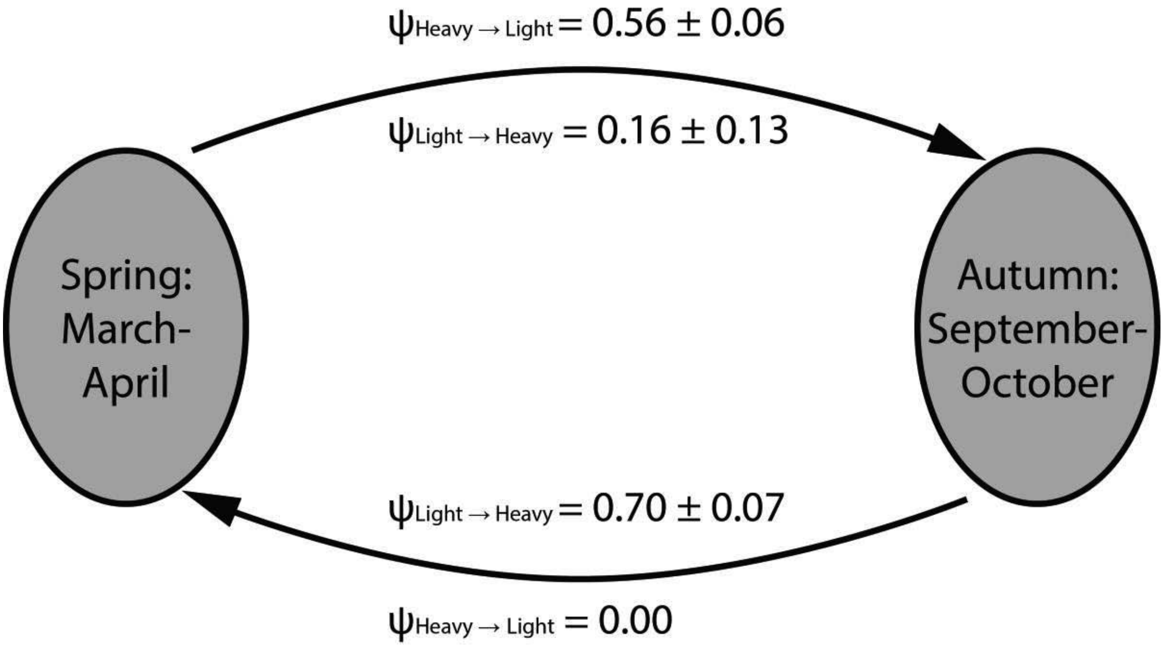

Figure 2: Seasonal transition probabilities (± SE) between mass-class states derived from models 1, 2, 3 in Table 1. Details of classes are light individuals (females {9.0-10.4g}, males {12.5-13.9g}) and heavy individuals (females {10.5-11.9g}, males {14.0-16.0g}). Capture-recapture seasons are spring (March-April) and autumn (September-October) each year (2000 – 2010). Note that the parameter estimate for the transition ψHeavy → Light between autumn and spring is on a boundary, so no SE is available.

Modelling recapture probability

Models with constant or seasonal variation in recapture probabilities (models 25, 23, Table 1) were much less supported by the data than models in which this parameter varied according to either sex (model 21, Table 1) or site (model 20, Table 1). Models with recapture probability modelled as a function of mass-state were also less supported compared to those with sex-specific or site-specific recapture probabilities (e.g. model 9 vs. model 8 and model 9 vs. model 5, Table 1). In fact, our top-ranked models (Table 2) suggested that the chances of recapture were nearly twice as high at the small wetland site (Madriz; $p = 0.371 \pm$ [SE] 0.045) than at the larger site (Taipal; $p = 0.208 \pm 0.035$). In addition, females were less frequently mist-netted than males, especially at Taipal (see model 3 in Table 2 for such estimates).

Modelling transition probability

Modelling transition probabilities as a function of mass-state variable alone was not a good fit when compared to those models incorporating additional effects (e.g. model 18 vs. models 13, 10 & 4, Table 1). The best competing models were obtained by modelling transition probabilities in relation to both mass-state and season with interaction, rather than additive, effects (e.g. model 4 vs. model 17, Table 1). Parameter estimates (± SE) of models with lowest AICc (models 1, 2, 3 in Table 1) suggested that during spring-summer Cetti's warblers switching mass-class had a 56% probability to transit from heavy to light mass-class, while in autumn-winter, birds switching mass-class had a 70% probability to transit from light to heavy mass-class; Fig. 2). It was noteworthy that none of the birds included in the present analysis showed a transition from heavy to light mass-states in autumn-winter (Fig. 2).

Table 1: Set of candidate models used to investigate apparent survival probability (ϕ), mass-state transition probability (ψ) and recapture rates (p) in the Cetti's warbler *Cettia setti*, central Portugal, 2000-2010. ϕ^* = transience effect on survival (i.e., first recapture interval following initial capture is modelled separately from all subsequent recapture periods). Subscripts in model notation are: 'Season' (spring-summer vs. autumn-winter), 'Sex' (male vs. female), 'Mass class' (light [female {9.0-10.4g}, male {12.5-13.9g}] vs. heavy [female {10.5-11.9g}, male {14.0-16.0g}] individuals), and 'Site' (Madriz vs. Taipal). Models with constant and year-specific parameters were also fitted to the data. (×) & (+) denote interaction and additive (parallel) effect between terms, respectively.

Model nº	Model	AIC_c	ΔAIC_c	AIC weight	K	Deviance
1	$\phi^*_{(\text{season})}\ \psi_{(\text{season} \times \text{mass class})}\ p_{(\text{site})}$	1290.00	0	0.659	10	751.52
2	$\phi^*_{(\text{season} \times \text{sex})}\ \psi_{(\text{season} \times \text{mass class})}\ p_{(\text{site})}$	1291.88	1.88	0.257	14	745.40
3	$\phi^*_{(\text{season} \times \text{sex})}\ \psi_{(\text{season} \times \text{mass class})}\ p_{(\text{sex} \times \text{site})}$	1294.15	4.15	0.082	16	743.68
4	$\phi_{(\text{season} \times \text{sex})}\ \psi_{(\text{season} \times \text{mass class})}\ p_{(\text{site})}$	1309.49	19.49	0.00	10	771.01
5	$\phi_{(\text{season})}\ \psi_{(\text{season} \times \text{mass class})}\ p_{(\text{site})}$	1310.86	20.86	0.00	8	776.38
6	$\phi_{(\text{season} \times \text{sex})}\ \psi_{(\text{season} \times \text{mass class})}\ p_{(\text{sex} \times \text{site})}$	1311.00	21.00	0.00	12	768.51
7	$\phi_{(\text{season} \times \text{mass class})}\ \psi_{(\text{season} \times \text{mass class})}\ p_{(\text{site})}$	1314.77	24.77	0.00	10	776.29
8	$\phi_{(\text{season})}\ \psi_{(\text{season} \times \text{mass class})}\ p_{(\text{sex} \times \text{mass class})}$	1317.16	27.16	0.00	10	778.69
9	$\phi_{(\text{season})}\ \psi_{(\text{season} \times \text{mass class})}\ p_{(\text{mass class})}$	1328.69	28.69	0.00	8	795.21
10	$\phi_{(\text{season} \times \text{sex})}\ \psi_{(\text{sex} \times \text{mass class})}\ p_{(\text{site})}$	1335.83	45.83	0.00	10	797.35
11	$\phi_{(\text{season})}\ \psi_{(\text{sex} \times \text{mass class})}\ p_{(\text{site})}$	1337.19	47.19	0.00	8	802.71
12	$\phi_{(\text{season} \times \text{sex} \times \text{mass class})}\ \psi_{(\text{season} \times \text{mass class})}\ p_{(\text{site})}$	1337.50	47.50	0.00	13	793.02
13	$\phi_{(\text{season} \times \text{sex})}\ \psi_{(\text{mass class} \times \text{site})}\ p_{(\text{site})}$	1339.59	49.59	0.00	10	801.11
14	$\phi_{(\text{season})}\ \psi_{(\text{site})}\ p_{(\text{site} \times \text{sex})}$	1340.25	50.25	0.00	8	805.77
15	$\phi_{(\text{season} \times \text{sex})}\ \psi_{(\text{site})}\ p_{(\text{site})}$	1340.78	50.78	0.00	8	806.31
16	$\phi_{(\text{year} \times \text{sex} \times \text{mass class})}\ \psi_{(\text{season} \times \text{sex} \times \text{mass class})}\ p_{(\text{sex} \times \text{mass class} \times \text{site})}$	1341.60	51.60	0.00	60	703.12
17	$\phi_{(\text{season} + \text{sex})}\ \psi_{(\text{season} + \text{mass class})}\ p_{(\text{site})}$	1342.75	52.75	0.00	8	808.26
18	$\phi_{(\text{season} \times \text{sex})}\ \psi_{(\text{mass class})}\ p_{(\text{site})}$	1344.61	54.61	0.00	8	810.13
19	$\phi_{(\text{season} \times \text{mass class})}\ \psi_{(\text{site})}\ p_{(\text{site})}$	1345.23	55.23	0.00	8	810.74
20	$\phi_{(\text{season})}\ \psi_{(\text{mass class})}\ p_{(\text{site})}$	1345.98	55.98	0.00	6	815.49
21	$\phi_{(\text{season})}\ \psi_{(\text{mass class})}\ p_{(\text{sex})}$	1355.76	65.76	0.00	6	825.28
22	$\phi_{(\text{sex})}\ \psi_{(\text{mass class})}\ p_{(\text{site})}$	1363.38	73.38	0.00	6	832.90
23	$\phi_{(\text{season})}\ \psi_{(\text{mass class})}\ p_{(\text{constant})}$	1364.03	74.03	0.00	5	835.547
24	$\phi_{(\text{constant})}\ \psi_{(\text{mass class})}\ p_{(\text{site})}$	1365.22	75.22	0.00	5	836.74
25	$\phi_{(\text{season})}\ \psi_{(\text{mass class})}\ p_{(\text{season})}$	1365.91	75.91	0.00	6	847.43
26	$\phi_{(\text{year})}\ \psi_{(\text{mass class})}\ p_{(\text{site})}$	1371.33	81.33	0.00	15	822.84

Modelling survival probability

Models with either year-specific or constant survival probabilities were not well-supported by the data compared to those with a 'seasonal' effect (e.g. models 26, 24 vs. model 20, Table 1). Models with survival influenced by 'sex' only were not a good fit (e.g. model 22 vs. model 20, Table 1) but those with an interaction between season and sex received substantial support from the data (e.g. model 10 vs. model 11 and model 5 vs. model 4, Table 1). Inclusion of mass-class as an additional effect did not improve model fit (e.g. models 7, 12 vs. model 5, Table 1). In fact, variation in survival was largely explained by factor 'season' in this dataset since removal of this effect from any of the competing models would result in a large increase in AICc values (e.g. model 4 vs. model 22, Table 1). Finally, models accounting for the presence of transient birds provided the best explanation of the data as judged by AICc-ranking (models 1, 2, 3 vs. models 4, 5, 6, Table 1). Average estimates of transient probability ($1 - \zeta$) from these 3 competing models (models 1,2,3, Table 1) suggested that most birds ringed in autumn (Sept. – Oct.) were transients in this population ($1 - \zeta = 0.809$ in males and 0.862 in females). A greater proportion of birds ringed in spring (March – Apr.) were residents ($1 - \zeta = 0.533$ in males and 0.663 in females) when compared to autumn.

Survival estimates of resident birds derived from the top-ranked model (model 1, Table 1) suggested that adult survival was twice as high in spring-summer than in autumn-winter (model 1, Table 2). This top model was nonetheless followed by two closely-competing models that included a ‘sex’ effect on survival (models 2, 3, Tables 1 & 2), so apparent adult survival estimates (± SE) were derived from models 1, 2, 3 using the averaging option in MARK (Fig. 3). These final estimates suggested that resident females in this population exhibited higher survival probability than resident males during the spring-summer season (ϕRF = 0.857 ± 0.117 and ϕRM = 0.698 ± 0.181) while during the autumn-winter season, resident males survive better than resident females (ϕRM = 0.440 ± 0.086 and ϕRF = 0.339 ± 0.084, Fig. 3).

Table 2: Survival and recapture estimates derived from the 3 best AICc-ranked models in Table 1. Recapture seasons are spring (March-April) and autumn (September-October) each year (2000 – 2010). The study was conducted at two distinct sites (Site 1: Madriz; Site 2: Taipal) in central Portugal.

Parameter	Estimate ± SE		
	Model 1 (AIC-weight: 0.659)	**Model 2** (AIC-weight: 0.257)	**Model 3** (AIC-weight: 0.082)
Adult male survival (autumn-winter)	0.371 ± 0.097	0.580 ± 0.022	0.566 ± 0.202
Adult female survival (spring-summer)	0.787 ± 0.177	0.999 ± 0.001	0.999 ± 0.001
Adult female survival (autumn-winter)	0.371 ± 0.097	0.278 ± 0.060	0.279 ± 0.060
Recapture probability of females (Site 1)	0.371 ± 0.045	0.371 ± 0.045	0.349 ± 0.062
Recapture probability of males (Site 1)	0.371 ± 0.045	0.371 ± 0.045	0.399 ± 0.065
Recapture probability of females (Site 2)	0.208 ± 0.035	0.208 ± 0.035	0.183 ± 0.038
Recapture probability of males (Site 2:)	0.208 ± 0.035	0.208 ± 0.035	0.286 ± 0.078

Discussion

In the present study, we analysed an 11-year ringing dataset of Cetti's warblers obtained at two national ringing stations in central Portugal. In particular, we sought to understand (1) the pattern of body mass change during the annual cycle, (2) the role of mass-class in explaining individual survival, and (3) the role of sex in explaining individual survival in this polygynous species with a high size-dimorphism between males and females. Several predictions based on life-history theory and other available studies were made (see "Introduction"), and these are discussed below at the light of our modelling results.

Recapture probabilities

In most mark-recapture studies, all survivors present in the study area cannot be detected at each sampling occasion. Consequently, the modelling of the recapture history dataset is needed to account for imperfect detection probabilities (i.e. detection probability < 1) thereby yielding unbiased survival estimates (Williams et al 2002). This situation is further highlighted by the low recapture rates achieved in the present study (all models with $p < 0.4$). Despite a similar mist-netting effort at both sites to meet the requirements of the Portuguese constant effort site scheme (i.e. equal no. of nets and re-trapping hours at both sites), our modelling detected a recapture rate at Taipal that was half that at Madriz, which seems consistent with the fact that Cetti's warblers can disperse over a much wider area at Taipal (233ha) compared with Madriz (89ha). In addition, our modelling detected a sex-specific effect on recapture rates (model 3), a pattern that should be connected to behavioural differences between the sexes. For example, during the nesting season, males are very active in defending and moving over large breeding territories (Cramp and Brooks 1992), while polygynously-mated females establish smaller territories within their male's territory and defend them against other females mated with the same partner (Bibby 1982). Cetti's warbler females are also particularly discreet during the incubation period and forage only over their small territories during the chick-rearing period (Bibby 1982). Thus, altogether, the limited spatial movements of females during the breeding season should make them less susceptible to capture than males (Amrhein et al 2012).

Mass-class transition probabilities

Our prediction that body mass varies seasonally was supported by the dataset. The actual pattern of body mass over the whole year suggested that mean body mass was usually high at the onset of the breeding season (March-April), but decreased thereafter and during the moulting period (Fig. 1). In fact, our models indicated that Cetti's warblers had a 56% probability to transit from heavy to light mass-class between spring (March-April) and autumn (Sept.-Oct.; Fig. 2). The opposite pattern was apparent between autumn and spring with a tendency to gain mass between both sampling periods (i.e. birds showed a 70% probability to transit from light to heavy mass-class; Fig. 2). Previous studies in monogamous species have reported mass losses in passerines during the breeding season (Freed 1981, Suarez et al 2005 and references therein), especially during the chick-rearing period (Boyle et al 2012). Our expectation was that mass loss during breeding might be mainly apparent in Cetti's warbler females that, in common with other polygynous species, have a much higher parental investment than males (Bibby 1982). Sex-specific differences in the pattern of mass loss were, however, not supported by our data (model 8 with ΔAICc > 45 units), indicating comparable energetic costs and/or strategies during reproduction in both males and females. Several reasons can be advanced to explain mass loss in both sex during breeding, including an energetic cost of reproduction (Bryant 1979, 1988, Nur 1988) or an adaptive response by which adults reduce their costs of flight during the rearing-period, thereby leading to an increase in the quantity of food available to their nestlings (Cichon 2001, Boyle et al 2012). Mass loss in Cetti's warbler males might also reflect energetic costs associated with defending their large territories during the breeding season. Previous experiments with small passerines have also reported decreasing fat levels and body mass during moult (Newton and Dawson 2011), although individual responses may differ according to factors such as sex and age (Gosler 1994). Our study suggests that Cetti's warbler adults may incur a significant mass reduction during their complete post-breeding moult (Cramp and Brooks 1992), presumably because moulting is an energetically-demanding phase during their annual cycle. Conversely, individuals in both sexes had a high propensity to regain mass in autumn-winter. Winter fat storage has been strongly correlated with body mass in resident passerine species (e.g. Gosler 1996), so this seasonal change in body mass seems consistent with a winter fattening pattern (Macleod et al 2005). In resident bird species, body mass is maintained at a higher level in winter compared with spring-summer, mainly as a response to increased starvation risk due to higher unpredictability of food resources and higher energetic requirements to survive longer/cooler nights (MacNamara and Houston 1990, Newton 1998, Macleod et al 2005). Therefore, we speculate that resident Cetti's warblers have a slight tendency to regain mass in autumn-winter in order to anticipate the reduced feeding opportunities they might encounter during midwinter (Lima 1986). We have no direct evidence of food reduction in winter at the study sites, but it can be noted that in central Portugal, most insectivorous passerines are summer visitors migrating to Africa in September-October, presumably as a response to the reduction in food availability at their breeding grounds.

Survival probabilities

Our top model (model 1) suggested that mortality is greatest during the non-breeding (autumn-winter) season, presumably because Cetti's warblers depend for their survival on a constant access to food resources, which are most unpredictable during winter when adult bird mortality is thus expected to peak (e. g. Peach et al 1999, Robinson et al 2004, 2010). Contrary to our expectations, however, we did not find that heavier birds had a greater survival probability than lighter ones during winter (model 7 with ΔAICc > 24 units). The absence of a relationship between mass-class and adult survival has also been reported for other species such as the European blackbird Turdus merula in central Italy (Miller et al 2003). In this study, Miller et al (2003) suggested that the relatively mild climatic conditions prevailing in their study area could explain the lack of a significant effect of body mass on survival. In fact, if carrying large amounts of fat, and hence being heavy, is advantageous to maximize fitness, we would expect this pattern to be more easily detected in populations living at northernmost latitudes (Lima 1986), where winter conditions can be much harsher

than in Southern Europe, such as in central Portugal (this study: minimum temperature recorded at closest weather station to our study area ranged 5-8°C; see http://www.windguru.cz/fr/index.php?sc=827) or central Italy (Miller et al 2003). As further evidence for this, Gosler (1996) found a negative relationship between survival and body mass/fat reserves in great tits Parus major from U.K. during winters when food availability was low, but failed to detect any such pattern during milder years when winter food was apparently more abundant. Considering additional factors such as a bird’s social status (i.e. dominant vs. subdominant bird) is also important to fully understand the effect of body mass on survival (Gosler 1996), but it should be acknowledged that our study protocol did not allow for such a separation between marked individuals.

A peculiar life-history trait of regular polygynous passerines, including the Cetti’s warbler, is the strong sexual size-dimorphism evolved as a response to sexual selection, with the largest males being those that mate with the highest number of females (Bibby 1982). In such mating systems, ‘stabilizing selection’ might occur as a mechanism to prevent further increase in male size (Searcy and Yasukawa 1981). Therefore, Searcy and Yasukawa (1981) predicted that male size might be limited by a survival selection where the largest and heaviest males have lower survival rates, a pattern that was nonetheless not supported by our results. Note, however, that we had insufficient data to assign Cetti’s warbler males to more than two mass-classes, perhaps making the detection of a stabilizing selection in males difficult. Further studies should seek to examine the role of body mass in explaining survival rates when individuals are grouped in more than 2 mass-classes (e.g., 3 or more mass-classes including light, intermediate and heavy males).

Our initial prediction that males and females may incur different fitness costs (particularly during the breeding season) was supported by the data. In fact, our modelling results pointed towards an interaction between factors ‘sex’ and ‘season’ (Fig. 3): during the breeding/moulting seasons, resident females had a tendency to survive better than resident males (0.86 vs. 0.70, respectively) while during the non-breeding season (autumn-winter), the latter survived better than the former (0.44 vs. 0.34, respectively). Combining these seasonal estimates yields a similar annual survival rate of c.0.30 in both sexes (0.86 x 0.34 and 0.70 x 0.44). This ad hoc annual survival estimate is slightly lower than that reported for many European species of similar size (see e.g. Siriwardena et al 1998 for survival rates of 31 different passerines) but is nonetheless in line with estimates reported for European warblers breeding in wetlands such as the sedge Acrocephalus schoenobaenus (Siriwardena et al 1998) and reed A. scirpaceus (Thaxter et al 2006) warblers. The modelling approach developed here only permitted the estimation of 'apparent' survival rates (Williams et al 2002), so the above estimates may also reflect a fair amount of permanent emigration from our study area. This also suggests that our sex-specific survival estimates may not represent a true pattern if, for instance, one sex has a much higher dispersal propensity than the other. In common with many other species, females are the more dispersive sex in the Cetti's warbler (Robinson et al 2007b), which could explain their lower apparent survival during the non-breeding season compared with males. However, Cetti's warbler females have a higher apparent survival rate than males during the breeding season, hence permanent emigration and dispersal may not be the sole factors accounting for the sex-specific variations reported here.

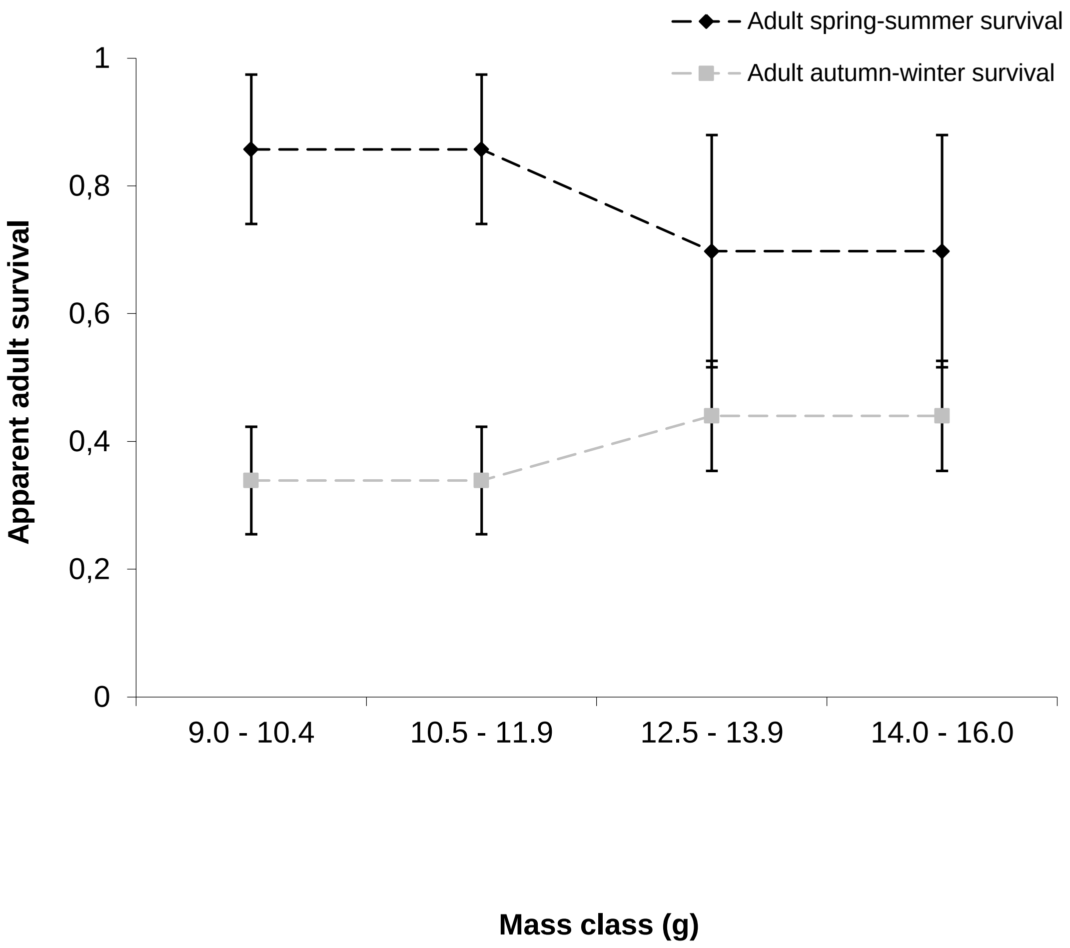

Figure 3: Apparent adult survival estimates (± SE) derived from models 1, 2, 3 in Table 1 using the averaging option in MARK. Details of classes are light individuals (females {9.0-10.4g}, males {12.5-13.9g}) and heavy individuals (females {10.5-11.9g}, males {14.0-16.0g}).

In many bird species, mortality rates are higher for females which suffer from high predation rates during both incubation (e.g. Post and Götmark 2006a in pied flycatchers *Ficedula hypoleuca*) and feeding of offspring (e.g. Post and Götmark 2006b in Eurasian blackbirds Turdus merula). However, this situation was not apparent here despite the reduced parental investment of Cetti's warbler males (Bibby 1982). Instead, our survival estimates suggest a higher survival cost in Cetti's warbler males during breeding. A possible explanation for this pattern may lie in higher predation levels on males by aerial predators, presumably because of their more conspicuous behaviour (i.e. during song display and territorial defence; Bibby 1982) compared to females. Behavioural studies have found that polygynous males spend more time per hour singing and advertising their territories than monogamous males (see e.g. Pilastro et al 2002 in the rock sparrow Petronia petronia), hence time exposure to aerial predators might increase substantially for Cetti's warbler males compared to other monogamous species, thereby explaining their low survival rates during the breeding season. Previous work with long-lived animals have also demonstrated a greater competition for mates in polygynous species compared to monogamous species, leading to skewed mortality rates towards males in the former situation (see Gaillard et al 2003 for several study cases with mammals). For instance, in the polygynous black grouse Tetrao tetrix, males have lower survival rates than females due to high predation rates during the lekking season (Angelstam 1984). We are, however, not aware of previous studies reporting any relationship between polygyny, male survival and predation risk in passerines.

The fitness advantage of Cetti's warbler females over males during the breeding/moulting seasons seems overridden by their higher mortality rates during the non-breeding season, although the failure of our modelling approach to account for possible sex-specific dispersal patterns suggests that interpretation of these results should be cautious (see above). Nevertheless, a higher dispersal propensity of females over males during the non-breeding season may expose the former to a greater mortality risk compared to the latter (Steifetten and Dale 2006). In the Cetti's warbler, males usually stay around their breeding territory year-round (author's personal data), which should promote high survival in winter. The lower survival of resident females during that period may be explained by intra-specific competition and dominance behaviour (Lundberg 1985), in which females may be excluded towards sub-optimal habitats and less profitable food patches by dominant males in winter when food is scarcer.

Conclusion

This study suggests that Cetti's warblers follow the predicted pattern of body mass change during the annual cycle of most passerines, in which adults have a tendency to lose mass during the course of the breeding season and to gain mass in autumn-winter. Body mass is not a good predictor of individual survival, consistent

with the hypothesis that minimum temperatures and winter conditions are probably too mild in central Portugal to be a major determinant of Cetti’s warbler population dynamics. Adult survival is more likely to be shaped by ecological and behavioural differences between males and females. Importantly, our modelling results highlight that further experimental and field studies aiming to identify the main factors shaping survival and individual fitness in polygynous species should be conducted during different phases of the annual cycle.

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Chapter V

Declining migrant populations at stopover sites in Portugal

Introduction

The Mediterranean basin has long been regarded an important ground for European partial migratory species, as well as an important stopover for European long-distance migrants, en route to and from Africa (Jonzén et al 2006). However, recent climate warming lead to a decrease in bird migration distances (Visser et al 2009) and shifted the wintering grounds of European bird northwards (Fiedler et al 2004, Telleria et al 2015). This should lead to strong seasonal changes of the avifauna community in the Mediterranean basin.

The increase of temperature in northern latitudes changed the migration cycle of a vast number of species, increasing earlier spring arrival at European and North American breeding grounds, particularly since the 1970s (Gordo 2007). Many studies showed shifts in the timing of bird migration during the last decades (Gordo 2007, Morrison et al 2013, Telleria et al 2015). Given the negative trend found between arrival dates and spring temperatures (Lehikoinen et al 2004, Root et al 2005, Gordo 2005), the advancement of spring migratory phenology is related with global warming (Gordo 2005). Other environmental changes, such as the frequency of severe weather events (Frederiksen et al 2008), and the continuous increase in Sahel rainfall, for example, can change the demography and distribution of some long distance migrant species. Reports of a strong reduction on Afro-Palaearctic migrant species have been associated to changes in Sahel weather, mainly in species that migrate further south to the humid West African forest and savannah (Hewson and Noble 2009, Thaxter et al 2010). Ockendon et al (2012) suggest that changes of climate conditions and land disturbance in Africa can lead to the decline in long distance migrants. However, Morrison et al (2013) showed that long distant migrants have decreased in Britain but not in Scotland. Such differences observed between populations in Britain and Scotland showed that poor conditions in wintering grounds in Africa alone, are unlikely to explain these population trends and seasonal interactions.

Some authors related the advances in spring arrival and delayed autumn migrations observed in Europe to a direct consequence of changes in the wintering area of birds (Visser et al 2009); however partial migrants (birds that do not cross the Sahara during wintering periods) deal differently with this problem: if the wintering areas present good conditions, they can leave their breeding areas later and migrate only shorter distances, thus staying closer to their breeding grounds (Coppack and Both 2003). Partial migrants can control better the cost of arriving and breeding than long distant migrants, which are less flexible on their migratory strategy (Gwinner, 1996). Onrubia and Telleria (2012) compared bird counts in Falsterbo (Sweden) and Gibraltar between 1997 and 2008 and showed a decrease in the relative contribution of partial migratory species in relation to long-distant migrants to the flux of birds moving across the Strait of Gibraltar. The long distance migrants that breed in Mediterranean Basin have declined over the last decades, and this can be related to with the size of breeding population (Onrubia and Telleria, 2012) and/or a result from spatial variation in climatic conditions (Morrisson et al 2013) and changes in environmental conditions in the African wintering areas.

Both et al (2010) predicted that long distance migrants are more vulnerable to climate change than residents or partial migrants, and showed that long-distance migrants decreased more markedly in Western Europe than in Northern Europe, because spring warming is more evident in Western Europe (Visser et al 2003, Both et al 2004). The Breeding, Migration and wintering periods of passerines throughout Europe may suffer a strong impact of environmental (particularly climate) changes, which will affect population abundance and migration patterns. This study focused in understanding the population trends of European long-distance migrants, partial migrants and residents in two sites differing in habitat in Central Portugal using 10 years of constant effort ringing data. Understanding population patterns of these 3 birds groups in Portugal is particularly relevant due to its strategic geographical position, before/after crossing to North Africa. Using data from two sites differing in habitat is also important in order to provide some control for potential habitat influences on bird numbers. Based on previous studies we predicted: a) a decrease in partial migrants during autumn migration, and no trend in long distant migrants; b) a decrease in the number of partial migrants wintering in Portugal; c) a decrease in

numbers of summer breeders and no trend for resident species; d) a decrease in the numbers of long distant migrants during spring migration.

Methods

Study area - We captured birds at two stopover sites for migratory bird species in central Portugal: the reedbed of Paul do Taipal (40º 11'N 8º 41'W - 233 ha) and the riparian area of Paul da Madriz (40º 7'N 8º 38'W - 89 ha), in the Mondego Valley. The two sites are classified as Special Protection Areas (SPA) and are part of a network of wetlands stretching along the Mondego valley. To examine and compare population trends of migratory birds crossing and wintering in Central Portugal we used 10 years of mist-netting data, collected from January 2002 to December 2012. Between 25 March and 22 July the mist-netting protocol followed the guidelines of the Portuguese constant effort sites scheme (PEEC), as part of a European project (Euro-CES: Constant effort ringing in Europe - CEE) promoted by EURING (one ringing session per 10 day period). In the remaining months there were 1-3 ringing sessions/period. In each session 120-240 m (120 m between 2002 and 2007; 240 m after 2007) of nets were operated.

Bird counts per season - The data for both the reedbed and the riparian habitats was filtered to select the species of interest (Table 1), the year range 2002-2012 and ringing period. The species of interest was chosen due to the high numbers of captures. For brevity, we denote by T = (b; s; y) a ordered triple in which b denotes the species, s the site and y the year. We begin by finding the total number of birds per period for each T, counting each bird only once in which period (i.e. recaptures were not considered). To do this, we assign to each possible T a group of 33 sets, i.e., one set for each period:

$$T \longmapsto (s_1, s_2, s_3, \ldots, s_{33}).$$

Accounting for ringing effort - We noted the number of sessions per season per year and site. This number was multiplied by the length of the mist nets used.

We did not take in consideration the duration of the sessions because it was the same for all sessions (5 hours). The result was an array of 33 numbers for each site and year:

$$(\text{site, year}) \longmapsto (e_1, e_2, e_3, \ldots, e_{33}).$$

To account for the ringing effort, we scale the raw counts according to:

$$c'_i = \begin{cases} 1000\,\dfrac{c_i}{e_i}, & \text{if } e_i \neq 0, \\ \text{NA}, & \text{if } e_i = 0. \end{cases}$$

Here, NA is a special symbol meaning “unavailable data". Note that $c'_i = 0$ is meaningful (the ringing effort was nonzero, but no birds of the species, year and site under consideration were detected in the period i. This must not be confused with NA: in the absence of ringing effort, the count is unknown, rather than zero. The factor 1000 was introduced for convenience, to avoid small numbers. It merely changes the units in which the effort is measured and has no other impact. Moreover each ringing period was placed in one of 4 wider periods: Wintering period (2 October to 14 March); Spring migration (15 March to 2 June); Breeding period (3 June to 1 August); Autumn migration (2 August to 1 October).

Separating populations.- In the case of Robins *Erithacus rubecula* and blackcaps *Sylvia atricapilla*, we attempted to distinguish between resident and wintering birds. To do this we considered the entire history of each bird, and checked if the bird was present in any of the breeding periods. If so, the bird was considered resident (even if it was present during the winter). Birds for which all existing records were confined to the winter period were considered wintering birds. A similar method was used in the case of reed warblers, to separate breeding birds from migrants. Again, the entire history of each individual was considered, and if the bird was present during the breeding season it was considered a summer breeding bird (we have ringed almost the entire breeding population of reed warblers in our study area), otherwise, it was considered a long distance migrant.

Data analysis.- We used a Generalized Linear Model (GLM) with a logit link function, that analyzed the effect of YEAR on each site for: a) partial migrants during autumn migration; b) long distant migrants during autumn migration; c) partial migrants during winter; d) residents during winter; e) summer breeders during breeding season; f) residents during breeding season; g) long distance migrants during spring migration and h) residents throughout the year. For definitions of terms see table 1.

Moreover we used a GLM to analyze the effect of the YEAR for the species that were more abundant at the two study sites: e.g. reed warblers and Robins. An offset was introduced in the model to include the effect of missed visits, following the data-adjustment methodology proposed by Peach et al (1998). Using this adjustment the expected total captures of juvenile and adults for years with incomplete coverage (missed visits) were calculated using all the data at the site from years with complete coverage (Peach et al 1998). Missed visits do not exceed more than 8 (in total of 33) per year. The analysis was performed in the software R 3.0.2 (R Core Team, 2013).

Results

Table 1: Bird pecies included in this study classified according to their status: long distance migrant [a]; partial migrant [b]; resident bird [c]. We indicate also the European global trend classification according to the European Bird Census Council (2014).

Species	Common name	Status	Population Trend in our study area	Population Trend in Europe (1980 – 2012)	Population Trend % (2003-2012) in Europe
Acrocephalus scirpaceus	Reed warbler	Long distant migrant	Decrease	Stable	7%
Cettia cetti	Cetti warbler	Resident	Stable	Increase	-15%
Emberiza schoeniclus	Reed bunting	Partial migrant	Stable	Decline	-18%
Erithacus rubecula	Robin	Resident/ Partial migrant	Stable	Increase	-3%
Ficedula hypoleuca	Pied flycatcher	Long distant migrant	Decrease	Decline	0%
Hippolais polyglotta	Melodious warbler	Long distant migrant	Decrease	Stable	44%
Luscinia svecica	Bluethroat	Partial migrant	Decrease	Decline	-7%
Muscicapa striata	Spotted flycatcher	Long distant migrant	Stable	Decline	0%
Sylvia atricapilla	Blackcap	Resident/ Partial migrant	Stable	Increase	36%
Sylvia borin	Garden warbler	Long distant migrant	Increase	Decline	-9%
Phylloscopus collybita	Chiffchaff	Partial migrant	Decrease	Increase	-8%
Phylloscopus throchilus	Willow warbler	Long distant migrant	Decrease	Decline	-7%

[a] **Long distant migrants**: Afro-tropical migrants that winter in Africa and breed in Europe.

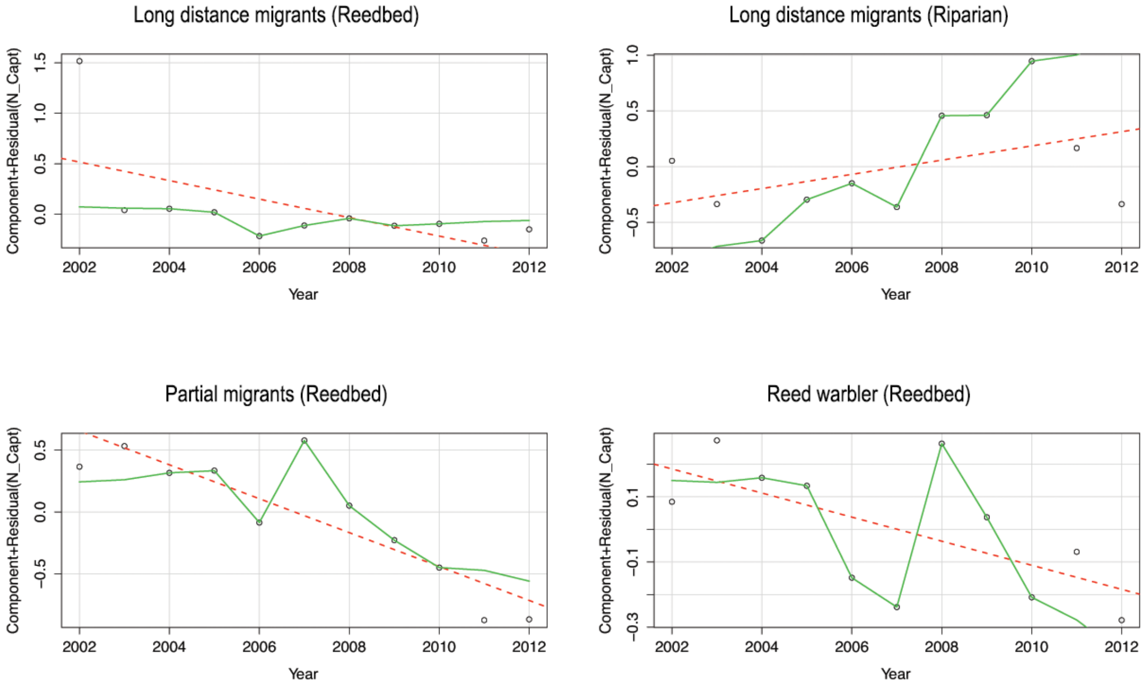
[b] **Partial migrants**: European migrants, that breed and winter in Europe.

[c] **Residents**: Species that spent all year in the study area.

The population Trend in Europe was calculated over the period 1980-2012 (EBCC 2014). The population Trend (%) in Europe (2002-2012) showed the % of variation in an index between the first (2002) and the last year of the period (2012).

Autumn migration

During the autumn migration (Fig.1) the number of long distance migrants showed no trend for both for the reedbed and the riparian area (Estimate = -0.071, SE = 0.04, t value = -1.94 p = 0.084; Estimate = 0.05, SE = 0.03; t value = 1.13, p = 0.287), and the number of partial migrants on the reedbed (no sufficient data was available for the riparian area) decreased significantly from 2002 to 2012 (Estimate = -0.117; SE = 0.03; t value = -4.37, p = 0.002). Reed warbler was the species most captured during this season on the reedbed, and showed no significant trend (Estimate = -0.04, SE = 0.02; t value = -2.26, p = 0.051). The bluethroat, a partial migrant captured on the reedbed, decreased significantly, thus showing the same trend as the overall partial migrants (Estimate = -0.113; SE = 0.02; t value = -5.70, p = 0.0003). On the riparian habitat the Garden warbler showed the opposite trend with a great increase during this period (Estimate = 0.129; SE = 0.03; t value = 4.33, p = 0.002).



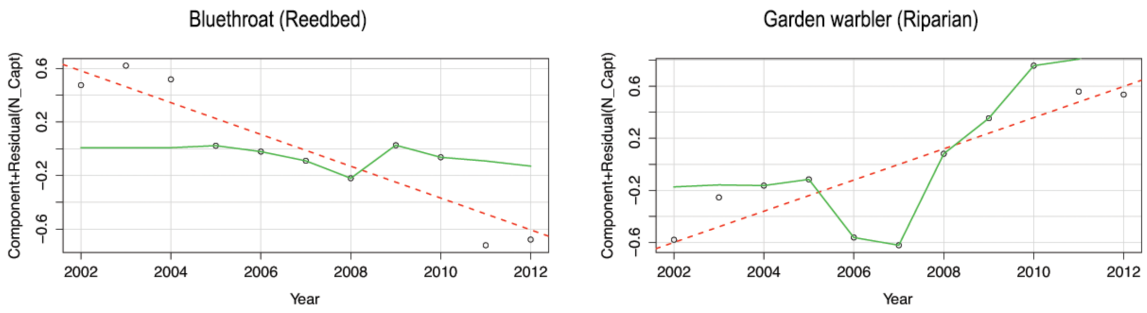
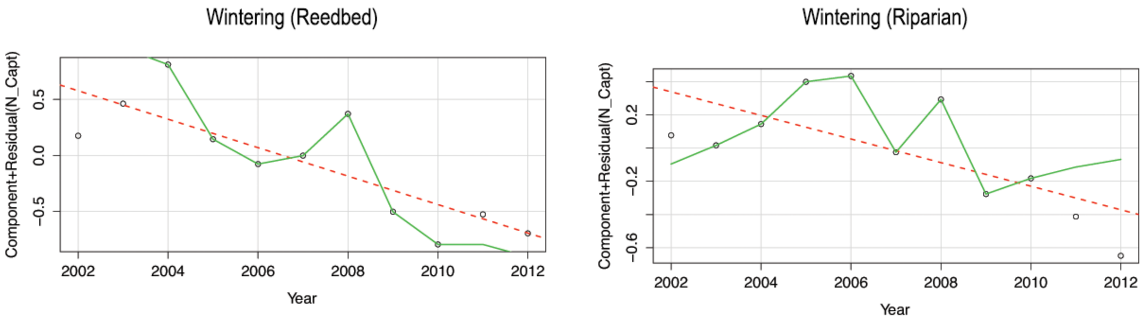


Figure 1: Bird trends during the autumn migration for both study sites. The green line represents the tendency and the points the scores (Standard residuals of the model).

Wintering

The numbers of wintering birds (Fig. 2) in Central Portugal showed a significant decreasing tendency for both the reedbed and the riparian area over the last ten years (Estimate = -0.120, SE = 0.04, t value = -3.42, p = 0.008; Estimate = -0.064; SE = 0.03; t value = -2.44, p = 0.037, respectively). The reed bunting followed this general pattern, as their numbers decreased significantly on the reedbed during the past 10 years (Estimate = -0.099, SE = 0.03, t value = -3.10, p = 0.013). Robins (Reedbed: Estimate = 0.015, SE = 0.05, t value = 0.31, p = 0.77; Riparian area: Estimate = 0.010; SE = 0.03; t value = 0.30, p = 0.77) and chiffchaff (Reedbed: Estimate = -0.14, SE = 0.07, t value = -2.00, p = 0.08; Riparian area: Estimate = -0.038; SE = 0.04; t value = -1.01, p = 0.34) showed no significant trend for both habitats.



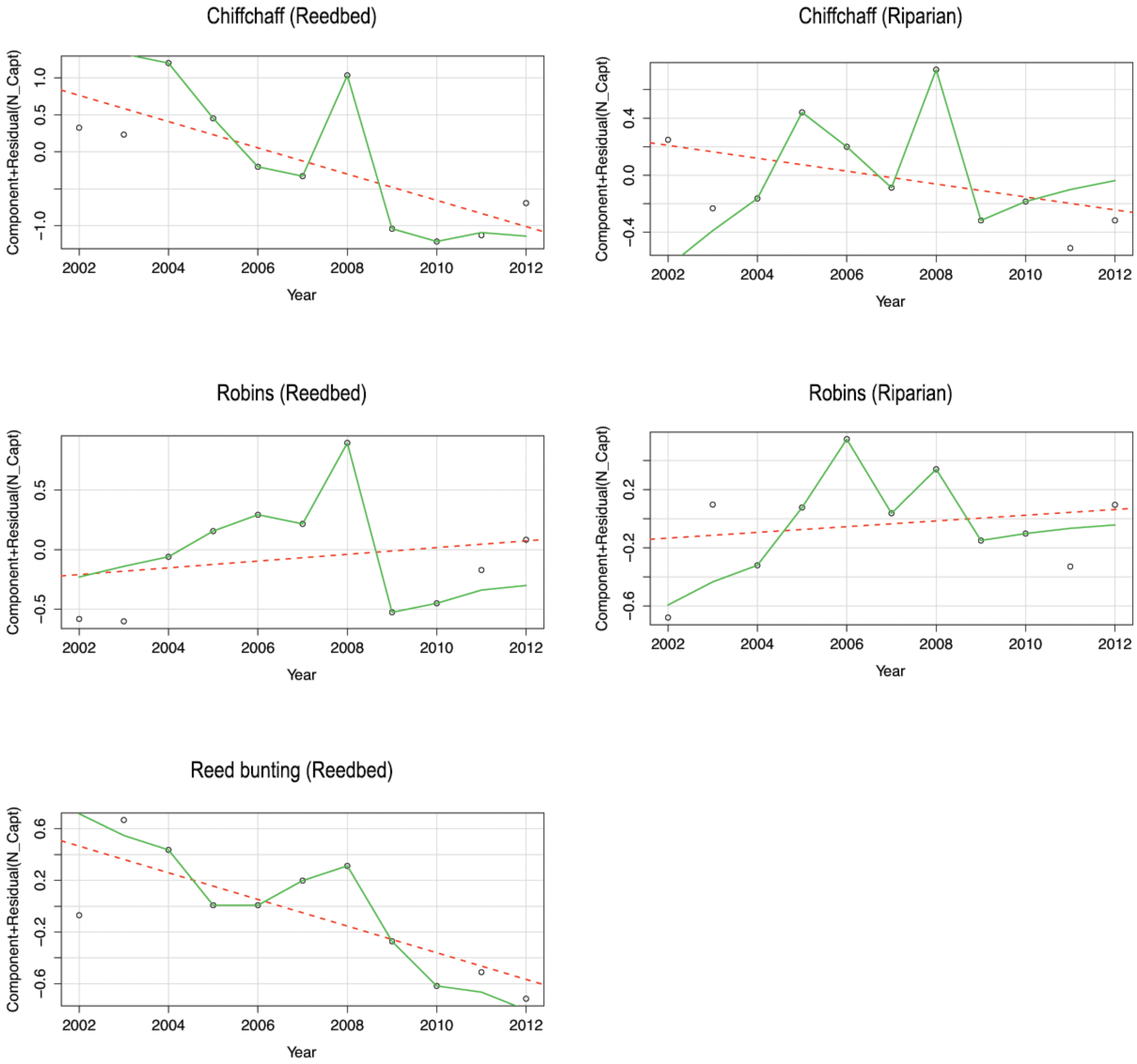


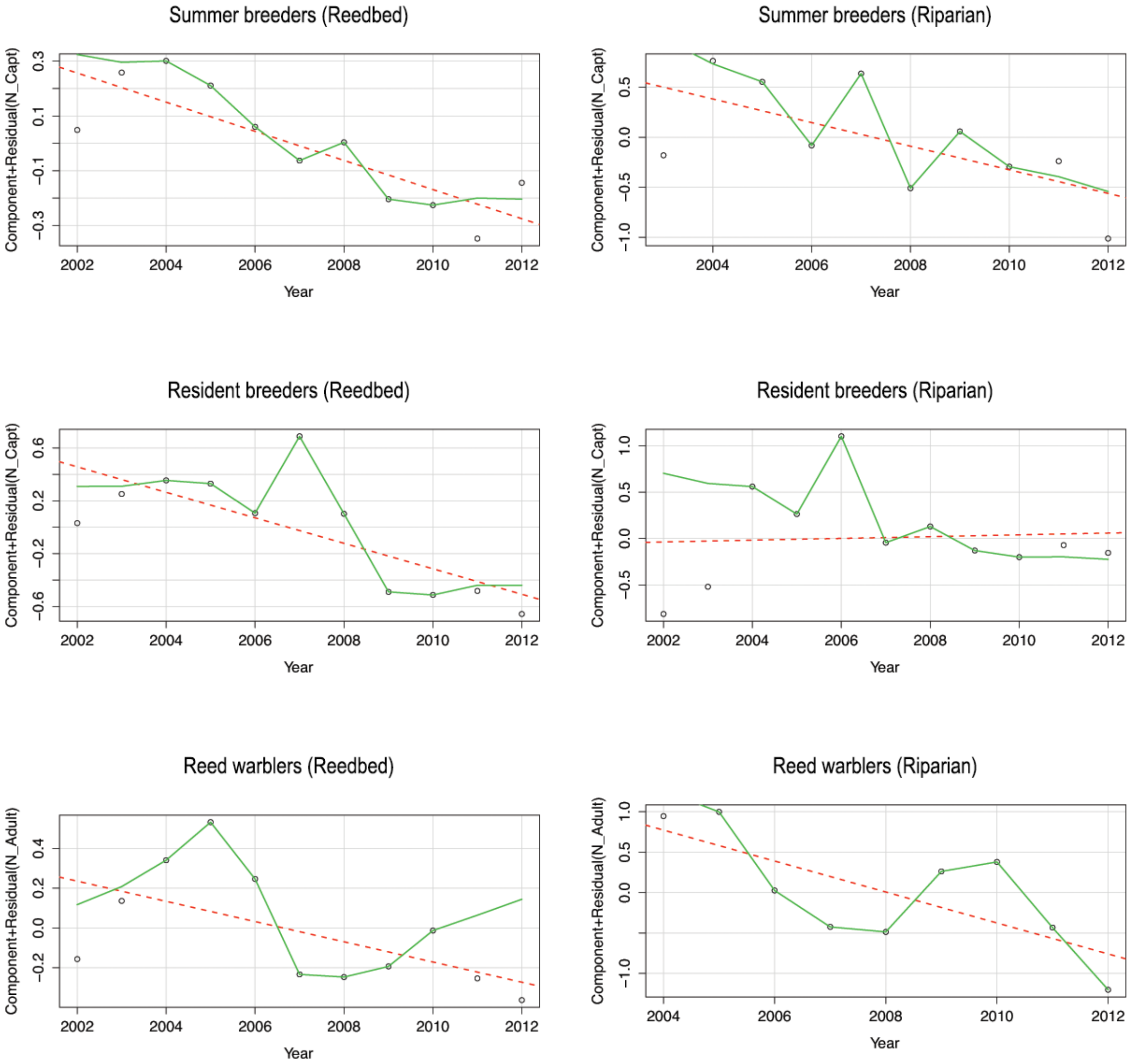
Figure 2: Bird trends during the Wintering for both study sites. The green line represents the tendency and the points the scores (Standard residuals of the model).

Breeding period

Both resident and summer breeders decreased significantly in the reedbed (Estimate = -0.056, SE = 0.01, t value = -4.79, p = 0.001; Estimate = -0.089, SE = 0.03, t value = -2.79, p = 0.021). For the riparian habitat, both residents and summer breeders showed no trend (Estimate = -0.09, SE = 0.06, t value = -1.63, p = 0.14; Estimate = 0.018, SE = 0.05, t value = 0.36, p = 0.73).

The reed warbler and the Savi´s warbler decreased significantly over the years on the reedbed (Estimate = -0.096, SE = 0.02, t value = -3.90, p = 0.006 and Estimate = -0.16, SE

= 0.05, t value = -3.09, p = 0.018). Reed warblers showed the same pattern on the riparian habitat with a significant negative trend (Estimate = -0.21, SE = 0.07, t value = -3.14, p = 0.016). However the resident breeders such as Robins (Estimate = 0.003, SE = 0.08, t value = 0.04, p = 0.97) and blackcaps (Estimate = -0.041, SE = 0.06, t value = -0.71, p = 0.5) showed no significant trend for the riparian habitat.



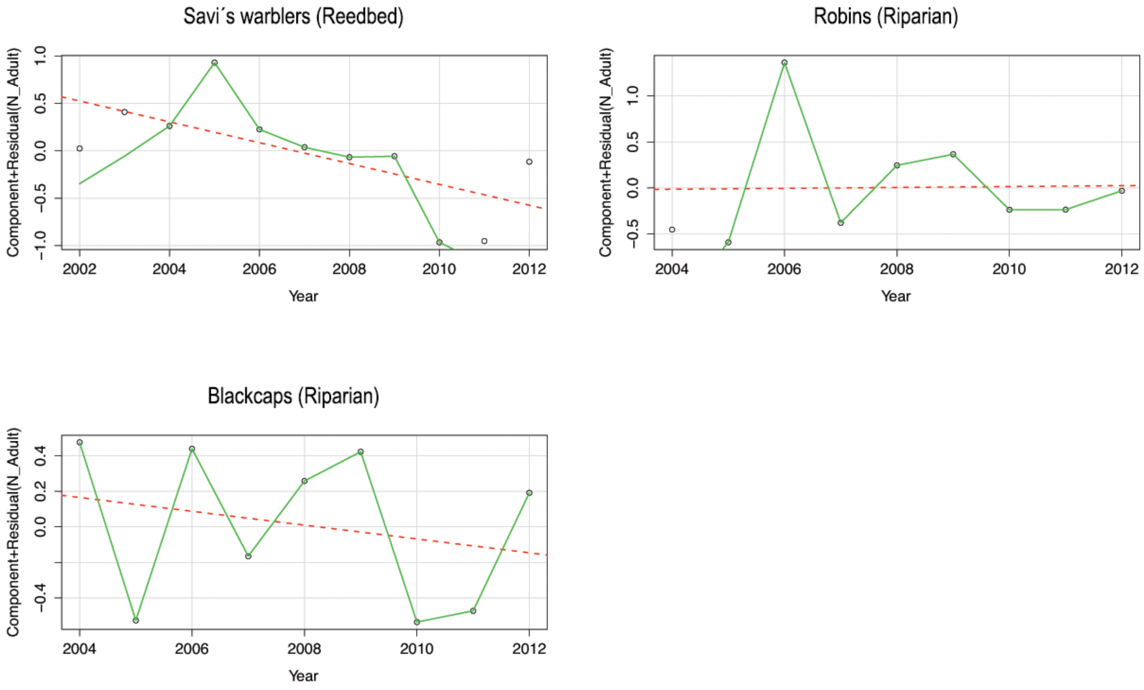


Figure 3: Bird trends during the breeding period for both study sites. The green line represents the tendency and the points the scores (Standard residuals of the model).

Resident birds

The abundance of resident birds decreased significantly on the reedbed over the past 10 years (Estimate = -0.08, SE = 0.03, t value = -2.59, p = 0.029), but showed no trend for the riparian habitat (Estimate = -0.026, SE = 0.03, t value = -0.96, p = 0.36). The main resident species present on the reedbed was the Cetti´s warblers, which decreased significantly from 2002 to 2012 (Estimate = -0.08, SE = 0.03, t value = -2.68, p = 0.025), but we found no trend for Cetti`s warblers on the riparian habitat (Estimate = -0.01, SE = 0.02, t value = -0.32, p = 0.75). Robins showed also no trend for both the reedbed and the riparian area (Estimate = 0.06, SE = 0.06, t value = 0.91, p = 0.39; Estimate = 0.05, SE = 0.04, t value = 1.39, p = 0.20, respectively).

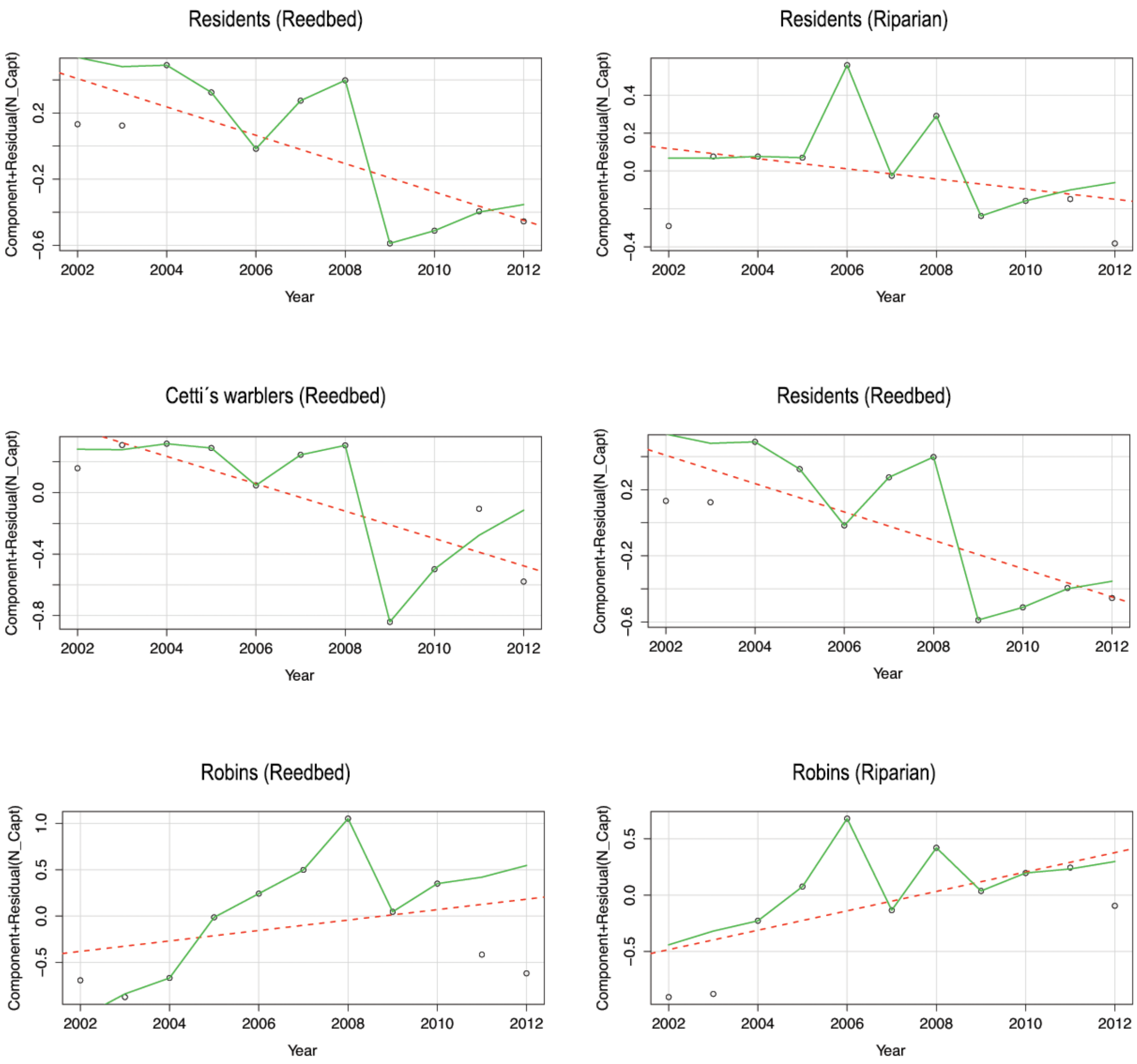


Figure 4: Resident Bird trends for both study sites. The green line represents the tendency and the points the scores (Standard residuals of the model).

Spring migration

During spring migration the captures on the reedbed decreased significantly (Estimate = -0.07, SE = 0.03, t value = -2.42, p = 0.037), but no significant trend was detected for the riparian habitat (Estimate = -0.08, SE = 0.09, t value = -0.88, p = 0.403). Reed warblers showed the same negative trend observed on the autumn migration, with a significant decrease on the reedbed (Estimate = -0.07, SE = 0.03, t value = -2.28, p =

0.048), and no significant negative trend on the riparian habitat (Estimate = -0.10, SE = 0.09, t value = -1.19, p = 0.267).

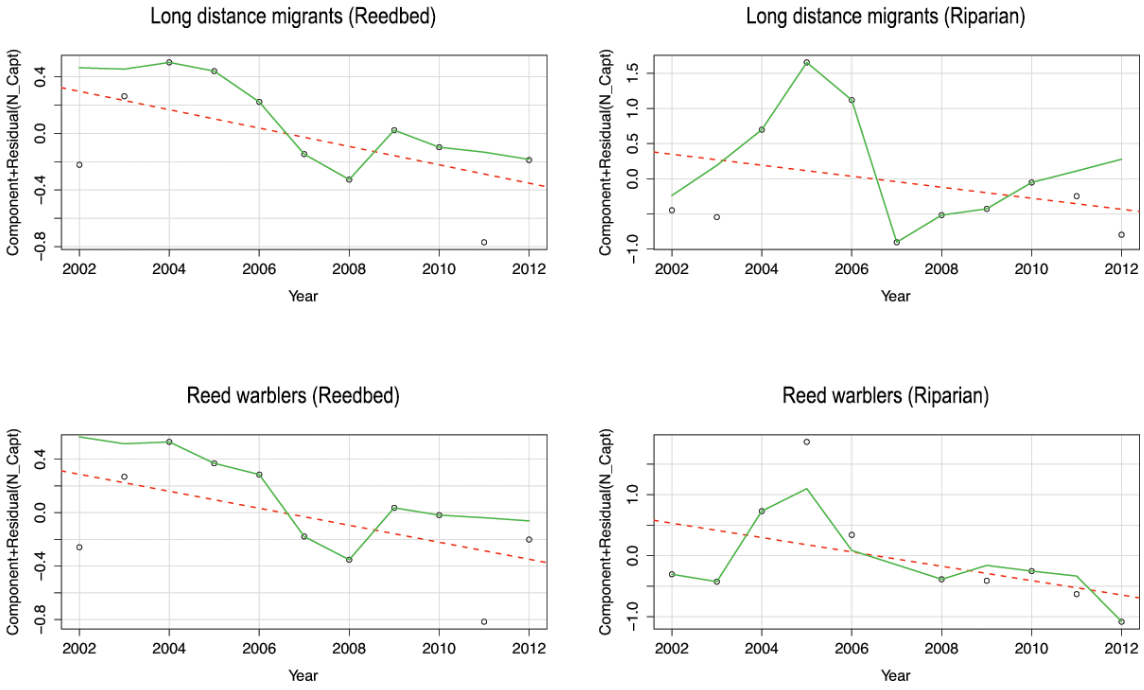


Figure 5: Bird trends during spring migration for both study sites. The green line represents the tendency and the points the scores (Standard residuals of the model).

Discussion

Our data on bird trends should be interpreted carefully because we analyzed data from only two ringing sites. However, we used two different wetland habitats to prevent the problems of differential habitat use for birds in migration. In general we obtained similar trends for most species in both habitats, and these trends were in line with those of common birds in Europe. Reports of a strong decline of both long distant and partial migrants in the Mediterranean (Birdlife international 2014, European Bird Census Council 2014) suggest that climate change is the likely reason for such decline. We found a great decrease of partial migrants wintering in both the reedbed and the riparian area of Central Portugal, and a few key species were responsible for such decrease. For example the reed bunting, chiffchaff and bluethroat showed a significant decline in our study area, and the same was observed throughout Europe (Birdlife international 2014, European Bird Census Council 2014). The decline of partial migrants wintering in Portugal can be associated with changes in the winter conditions and with the general trends in Europe. However, other factors may also play a role in explaining such decline as warming temperatures in northern Europe breeding areas or migration costs (Morrison et al 2013). The long distance migrants showed also a tendency to decline over the last decade (Both et al 2006). Contrary to the pattern described by Birdlife international (2014) and European Bird Census Council (2014) we observed an increase of Garden warblers during the autumn migration.

Trends for partial distance migrants during Autumn migration and Winter

In this study we observed a strong decrease of both long-distance migrants and partial migrants in reedbeds and riparian areas in Central Portugal. We expected that a continuous increasing of winter temperatures should lead to further declines of partial migrants and probably an increase of resident's birds, if their habitat condition does not change over time. Partial migrants have a high genetic variation in terms of migratory behaviour, and can change from being partially migratory to resident in a few generations (Pulido 2001). The global warming seems to be the strongest factor to explain the most significant reduction in partial migrants. However, to make predictions it is important to bear in mind the weather unpredictability and the climate change (Lemoine and Bohning-Gaese 2003). Climate change is expected to lead to increased trophic mismatches, resulting in declining population sizes (Moller et al 2008). Partial migrants may profit from increased temperatures in Northern Europe, and decrease their migratory movements to southern Europe. Anon (2003) showed that suitable winter habitats have increased markedly in Denmark and further south, and this fact can explain the strong decrease of wintering birds in southern Europe. Partial migrants can balance their relationship between the advantages/disadvantages to migrate (Rabenold 1979, 1993). They may profit from increased temperatures during migration in Europe, whereas birds breeding at more southern latitudes such as the Mediterranean migrate earlier and temperatures during migration for these populations have not increased (Both and Marvelde 2007). Adriaensen and Dhondt (1990) showed that residents Robin were clearly selected for by having both higher survival and higher reproductive success

than migrants. Our results showed similar patterns to those described by Telleria et al (2015) that compared the foreign birds captured in Spain with trends of the same species in northern European countries.

Trends long distance migrants during Breeding, Spring and Autumn migration

Long distance migrants showed a strong decrease in the breeding season and spring migration, which should be related with the vulnerability of long-distance migrants to climate change: (1) migrants face stronger competition from residents because resident populations increased owing to milder winters (Berthold et al 1998, Lemoine and Bohning-Gaese 2003, Ahola et al 2007); (2) climate change leads to a deterioration of wintering grounds (Peach et al 1991, Sillett et al 2000); and (3) increasing human populations and the associated increases in grazing pressure, fuel wood consumption and conversion of woodland to intensive agriculture in sub-Saharan Africa (Jones 1995, Norris et al 2010) may also be impacting migrants.

Our data does not support the first hypothesis because residents also decreased in reedbeds, and showed no trend for the riparian habitat. Buse et al (1999) showed that climate change can lead to resident population decline, because a further advance of the food peak may reduce reproduction. However the trend observed on riparian habitat during autumn migration can be related with the great input of Garden warblers. The abundance of garden warblers has varied alongside with that of other trans-Saharan migrant warblers (Siriwardena et al 1998), probably reflecting the influence of changes in their wintering environment. Despite large short-term fluctuations in abundance, the Common Bird Census/Breeding Birds Survey data suggest that the population may be in long-term decline. The increase in our riparian area may be related with the increase of fig trees in the surrounding area, providing superabundant food resources for this species during migration.

Our data supports the decreasing trend of long distance migrants during the breeding season in Europe (Visser et al 2009). This suggests that there may be a common cause determining the between-species correlation in population trends, which may well be habitat degradation and/or climate-related habitat change at the shared wintering

grounds (Gordo 2005, Sanderson et al 2006, Morrison et al 2013). Halupk et al (2008) showed that reed warblers can advance and extend their breeding season allowing more pairs to raise two successful broods during the season, which may be a direct consequence of climate change. The severe droughts occurred in Sahel (Africa) in the 1970s and 1980s (Baillie and Peach 1992, Foppen et al 1999, Zwarts et al 2009), can also be related with a strong decrease in breeding population numbers in Europe as observed for common whitethroat, sedge warbler and redstart (Zwarts et al 2009). However, other factors can influence the short-term population fluctuations and long-term population trends (Böhning-Gaese et al 1994, Böhning-Gaese 1995, Taper et al 1995). Presently Western Europe shows warmer springs, associated with more narrow peaks of food abundance, which can be the main reason for the decline of long distance migrants (Both et al 2010).

Conclusion

Our results concerning the factors that affect both long distance and partial migrants indicate that migrant passerines are very sensitive to climate changes, and warming on Northern latitudes may induce ecological pressures during the migration cycle. We need to keep monitoring the populations and climate change to investigate and understanding these complex ecological interactions. The migratory birds showed a great fluctuation over the last decade in Portugal, and we expected that changes continue affecting the migratory populations in the climate warming context. We also expected that migrants can adjust their migratory timing by either phenotypic plasticity and/or an evolutionary response to avoid the population’s risks. Moreover if partial migrants can indeed adapt better to warming in Northern Europe, long distant migrants will have to adjust their migratory cycle (Lemoine and Bohning-Gaese 2003).

Partial migrants -long migrants relationships provide an effective tool to predict the impact of climate change over the years on populations that breed and winter in Europe. Portugal has a good geographical position to link the wintering with the summer breeding populations. It requires also an understanding of the ecology of long distance migrants in Africa wintering grounds and in the European breeding grounds to understand the decline of summer breeders in Portugal. The numerical reduction in wintering and partial migrants agrees with the study of Tellería et al (2015) for Spain using ring recoveries. According to Tellería et al (2015) as global warming is predicted to continue there will be an increasing in the number of sedentary populations in Europe and a reduction in the number of partial migrants in the Mediterranean.

General Discussion

Migratory birds en route at Portuguese reedbeds

The present study focused on understanding the factors affecting the fluctuations in the populations of breeding, wintering and transient reedbed passerines in Central Portugal, an habitat particularly important for migratory birds. To understand passerine migration as a whole we link climate change, food abundance, bird habitat selection, and survival rates. To assess the broad and fine scale challenges that migrants face en route between breeding and wintering areas we used a long-term data set on these bird species addressing the ecology of resident and migrating passerines in reedbeds of Central Portugal. Environmental changes, including climate change, and a lack of direct conservation efforts, are reflected in the fact that a large numbers of migrant birds along their migratory route are endangered. Migration success depends on the availability of high-quality habitats (Stopovers) that provide adequate food resources, particularly in areas along migration corridors that support large numbers of birds during migration. In this context, reed warblers are a good model species to relate the stopover ecology and environmental parameters.

Here, I used several environmental parameters, to document the variation in stopover variation, both during spring and autumn migration, over the last decade. Overall, the stopover duration in Portugal tend to decrease, due to the warming in Europe and a significant decrease in precipitation. The warmer conditions in Northern Europe, have been associated to early arrivals and higher food availability in the North, and can explain the decrease in minimum stopover duration at Portuguese reedbeds. The higher food availability in Northern Europe should affect the fat stores of passerines that migrate through Portuguese reedbeds. This study also demonstrated that birds with a strategy of fewer stopovers along the migration route (e.g. sedge warblers) and wintering in Sub-Saharan Africa, accumulate higher amounts of fat before they begin their migratory journeys, increasing their fat loads when cross Portuguese reedbeds. Indeed, physiological parameters are a powerful tool to discriminate migratory strategies: passerines making fewer and shorter stopovers exhibit higher triglycerides values, than birds that stop more often en route (e.g. reed warblers). Evaluating physiological parameters allows us to estimate the dynamics of mass change, or fattening, in free-living migratory birds caught only once, an approach that is far better

to the static estimates of “condition” based on single body-mass measurements at the time of capture.

Portuguese Wetlands birds

Wetlands have high conservation value in Europe. As stated before Portuguese reedbeds offer an important habitat for breeding bird species, both resident and migratory passerines. Riparian galleries are wetlands of considerable importance for wildlife. These two wetland types are very productive and provide habitats for many bird species during breeding, migration stopover, juvenile dispersion and wintering seasons (Machtans et al 1996). Many resident and migratory passerine species use both habitats during part or their entire life cycle, therefore riparian and reedbed habitats offer the opportunity for detailed studies of habitat requirements of individual bird species associated with them. In this study I showed that reedbeds and riparian habitats presented different contributions for the annual cycle of a resident species Cetti´s warbler. The results demonstrate that Cetti´s warblers move in large numbers to reedbeds during the post breeding period, mainly juvenile females, which might be explained by a higher abundance of food resources and improved shelter conditions in this habitat, when compared with riparian areas. Other explanation is that females disperse much more than males which must occupy and defend their breeding territories, and therefore are prevented from dispersing. Apparently the females can be forced to disperse towards suboptimal habitats and less profitable food patches, indicating the high dependence on high quality reedbeds, mainly during the post breeding period. Our modelling detected a recapture rate for the reedbed habitat that was half of that for the riparian habitat, which seems consistent with the fact that Cetti’s warblers can disperse over a much wider area on reedbeds, when compared with riparian areas.

The post breeding period (autumn – winter) mean a high demand of energy, however Cetti´s warblers can gain mass. Moreover, during the breeding season the adults showed a tendency to lose mass. Contrary to our expectation and because Cetti’s warbler females have a much higher parental investment than males (Bibby 1982), females do not showed differences to males in terms of mass loss. Resident bird species

should maintain body mass at higher levels, reducing the starvation risk, and avoiding the unpredictability of food resources and higher energetic requirements (MacNamara and Houston 1990, Newton 1998, Macleod et al 2005).We have no direct evidence of food reduction in winter at the study sites, but it can be noted that in central Portugal, most insectivorous passerines are summer breeders migrating to Africa between August-October, or partial migrations wintering in the area. However the tendency over the last decade is a sharp decline, mainly of partial migrants and summer breeders. These results are corroborated by other studies showing that partial migrants from northern Europe have decreased in the Mediterranean area, which appears to be a direct consequence of climate change on avian migration.

According to our results many species can follow climatic changes at least to some extent, decreasing stopover duration or changing their wintering grounds in Europe. For long distance migrants, it is difficult to assess how closely are they able to follow phenological changes in other trophic levels in order to maintain synchronization with peaks of food availability. Furthermore, the migrants cannot anticipate the weather conditions on their breeding grounds. We showed that the tendency towards a decrease in stopover duration and the sharp decline of partial migrants in Central Portugal applies to a larger number of other species too. Climate may impact on the species' food sources (Both et al 2006, Holmes 2007, Sandvik et al 2008) or change habitat quality (Treinys et al 2008). Changes on the timing of migration and related changes in arrival time into the breeding/non-breeding grounds will also affect species (Both et al 2006). Furthermore, population responses to direct and indirect effects of climate change may not be linear due to demographic regulation and density -dependence (Ådahl et al 2006, Holmes 2007). More research is needed into how climate influences population abundances.

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