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COMPARATIVE ECOLOGY OF WILD COLUMBIFORMES NATIVE TO EUROPE

An analysis of movement behaviour, diet composition and haemosporidian infections

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Comparative ecology of wild Columbiformes native to Europe - An analysis of movement behaviour, diet composition and haemosporidian infections

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Giessen, May 2022

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CONTENTS

ABSTRACT

Behaviour encompasses all interactions of an animal with other organisms and with its environment. Studying animal behaviour can provide important information for species conservation and management. In this thesis, I aimed at closing fundamental knowledge gaps on different behavioural-ecological aspects of the three migratory species of Columbiformes (order of doves and pigeons) native to Europe. The species studied include one of the most rapidly declining breeding birds in Europe, the European Turtle Dove (*Streptopelia turtur*). As a Palearctic-African migratory species, it is the only long-distance migrant among the European native wild Columbiformes. On the contrary, Common Woodpigeons (*Columba palumbus*) and Stock Doves (*Columba oenas*) are both short-distance migrants. They belong to the most common European breeding birds or have a stable population trend, respectively. All three species are regularly hunted in several European countries.

In my thesis, I examined the wintering areas, migration flyways and phenology of European Turtle Doves by means of analysing feather-isotope values (*chapter 1*) and satellite tracking data (*chapter 2*). My findings highlight the use of several migration flyways and suggest that individuals breeding in different parts of Europe may occupy separate African wintering grounds. Tracking of Common Woodpigeons with GPS-GSM transmitters (*chapter 3*) showed that individuals breeding in Germany are facultative partial migrants, which can switch their migratory strategy (resident vs. migrant) between years. Tracking data evaluated along with land cover data indicated that both European Turtle Doves and Common Woodpigeons adapt their foraging areas and distances to the availability of food resources (*chapters 2* and *3*). A comparative assessment of diet components, analysed from faecal samples through DNA metabarcoding (*chapter 4*), revealed a variation in the presence and frequency of occurrence of diet items between the three species. Identified diet components were mainly seed-bearing plants (Spermatopsida). Furthermore, comparisons with previous studies suggest distinct regional intraspecific differences in diet composition. An evaluation of haemosporidian parasite infections (*Plasmodium* sp., *Haemoproteus* sp. and *Leucocytozoon* sp.) from avian blood samples (*chapter 5*) demonstrated interspecific differences in lineage diversity, overall and genus-specific prevalence. The observed infection pattern supported the assumption that long-distance migrants harbour a higher parasite prevalence and diversity compared to resident or short-distance migratory species.

Summarising, the findings of this thesis (i) enable a better interpretation of different behaviours observed in the Columbiformes, (ii) help to comprehend how behavioural habits are influenced by ecological causation and (iii) emphasize the distinct behavioural diversity and plasticity present among species as well as individuals. Furthermore, the results contribute to a greater understanding of the general and specific ecological requirements of the species of Columbiformes. This can help to optimise present or to plan effective future management and conservation strategies that reconcile the challenges of game bird management on the one hand and species conservation on the other hand.

ZUSAMMENFASSUNG

Verhalten umfasst alle Interaktionen eines Tieres mit anderen Organismen und mit seiner Umwelt. Die Erforschung des Verhaltens einer Tierart kann wichtige Informationen für das Management und den Schutz einer Art liefern. Ziel meiner Dissertation war es, grundlegende Wissenslücken hinsichtlich verschiedener verhaltensökologischer Aspekte von drei in Europa einheimischen und migrierenden Wildtaubenarten (Ordnung Columbiformes) zu schließen. Zu den untersuchten Arten zählte die Europäische Turteltaube (*Streptopelia turtur*), die eine der am stärksten rückläufigen Vogelarten in Europa ist. Europäische Turteltauben, welche in Subsahara-Afrika überwintern, sind die einzigen Langstreckenzieher unter den in Europa einheimischen Taubenarten. Dahingegen sind Ringeltauben (*Columba palumbus*)*,* die in Europa zu den häufigsten Brutvögeln gehören, und Hohltauben (*Columba oenas*), die einen stabilen Bestandstrend aufweisen, Kurzstreckenzieher. Alle drei Wildtaubenarten gehören in mehreren europäischen Ländern zu den legal jagdbaren Vogelarten.

Im Rahmen der Dissertation untersuchte ich die Überwinterungsgebiete, Zugrouten und Migrationsphänologie von Turteltauben anhand einer Analyse von Isotopensignaturen von Federn (*Kapitel 1*) und Satellitenortungsdaten (*Kapitel 2*). Die Ergebnisse zeigten auf, dass unterschiedliche Individuen verschiedene Zugwege nutzen und dass möglicherweise eine räumliche Trennung in den afrikanischen Überwinterungsgebieten zwischen Individuen aus unterschiedlichen Brutgebieten existiert. Das Tracking mit GPS-GSM-Sendern von in Deutschland brütenden Ringeltauben (*Kapitel 3*) ergab, dass einige Individuen ihre Zugstrategie (Standvogel oder Zugvogel) von Jahr zu Jahr ändern. Eine Auswertung der Trackingdaten gemeinsam mit Daten zur Landbedeckung deutete daraufhin, dass sowohl Turteltauben als auch Ringeltauben Habitatnutzung und Flugdistanzen während der Nahrungssuche an die Verfügbarkeit von Nahrungsressourcen anpassen (*Kapitel 2* und *3*). Eine vergleichende Betrachtung der Nahrungszusammensetzung der drei Wildtaubenarten ergab, dass das Vorhandensein sowie die Häufigkeit von Nahrungsbestandteilen zwischen den drei Arten variierten (*Kapitel 4*). Für die Ermittlung der Nahrungsbestandteile wurden Kotproben mittels DNA-Metabarcoding analysiert. Bei den identifizierten Nahrungsbestandteilen handelte es sich hauptsächlich um Samenpflanzen (Spermatopsida). Vergleiche mit früheren Studien deuteten auf regionale Unterschiede innerhalb einer Taubenart hinsichtlich der Zusammensetzung der Nahrung hin. Eine Auswertung von Infektionen mit Hämosporidien (Gattungen: *Plasmodium* sp., *Haemoproteus* sp. und *Leucocytozoon* sp.) in Blutproben verschiedener Wildtaubenarten zeigte interspezifische Unterschiede in der Diversität genetischer Linien sowie der allgemeinen und gattungsspezifischen Prävalenz der Blutparasiten auf (*Kapitel 5*). Das beobachtete Infektionsmuster stützte die Annahme, dass Langstreckenzieher verglichen mit Kurzstreckenziehern oder Standvögeln eine höhere Parasitenprävalenz und -vielfalt aufweisen.

Die Ergebnisse der vorliegenden Dissertation, im Allgemeinen und zusammenfassend betrachtet, (i) ermöglichen eine verbesserte Interpretation des bei den Taubenarten beobachteten Verhaltens, (ii) helfen zu verstehen, wie bestimmte Verhaltensweisen durch ökologische Ursachen beeinflusst werden, und (iii) verdeutlichen die ausgeprägte Vielfalt und Plastizität im Verhalten sowohl der jeweiligen Taubenarten als auch der Individuen. Darüber hinaus tragen die Ergebnisse zu einem besseren Verständnis der allgemeinen und spezifischen ökologischen Anforderungen der Wildtaubenarten bei. Dieses bessere Verständnis kann dazu beitragen, effektive Management- und Artenschutzstrategien zu planen oder bereits etablierte Maßnahmen anzupassen, welche sowohl die Anforderungen des Managements jagdbarer Arten als auch des Artenschutzes berücksichtigen.

1 | GENERAL INTRODUCTION

Why, how, where and when do organisms move? Studying the movements of animals is often motivated by its importance for many ecological patterns. Migratory and foraging movements can affect ecological processes including the spread of diseases, inter- and intraspecific competition and the ability of species to cope with global as well as local environmental changes (Nathan et al. 2008; Spiegel et al. 2016; Miller et al. 2019; Shaw 2020). Besides migration and foraging strategies themselves, these ecological processes can lead to differences in individual fitness and subsequently can influence population dynamics (Palacín et al. 2016; Dadam et al. 2019). Birds, constituting the most diverse lineage of living tetrapod vertebrates, belong to the most mobile organisms. These ubiquitously distributed organisms include around 10,900 extant species, which exhibit an extraordinary diversity in ecological, morphological and behavioural traits, thus occupying a variety of different ecological niches (Winkler & Leisler 1985, Gill 1995; Prum et al. 2015; Stiels & Schidelko 2018; Gill et al. 2021).

1.1 The order Columbiformes

Pigeons and doves (Columbiformes) are one of the oldest and most diverse extant lineages of birds (Soares et al. 2016). In many cultures, pigeons and doves can be found as a symbol of gentleness, love, sacrifice, grace and peace, especially in religions and belief systems. Nevertheless, they currently are one of the most threatened avian orders. Of the 354 extant species of Columbiformes worldwide, 72 species (20%) are threatened with extinction and 47 species (13%) are categorized as 'near threatened' (Wood & Cerbini 2021). Despite this, the order has not received a level of attention proportionate to the number of threatened species, in particular considering the vital ecological roles of many Columbiformes, e.g. as keystone species in tropical forests, seed dispersers, or important prey items (Gibbs et al. 2001; Kissling et al. 2009; Santos et al. 2019; Panter & Amar 2021; Wood & Cerbini 2021). Notwithstanding their relatively conserved anatomy and morphology, Columbiformes display a wide range of variations in their ecological adaptations (Soares et al. 2016). Except for the polar regions, pigeons and doves are widespread over all continents, albeit in varying numbers of species and individuals. The greatest species richness occurs in the tropical zones, particularly in the Southeast Asian and Oceanic regions (Rösler 1996; Gibbs et al. 2001).

1.2 Species of the order Columbiformes native to Europe

In contrast to the species-rich tropics, in Europe, besides the rock pigeon and domesticated feral pigeon (*Columba livia* and *C. livia* f. *domestica,* Gmelin 1789, respectively) as well as a few endemic species on the Canary Island, only four species of native wild Columbiformes occur (Gibbs et al. 2001; Gill et al. 2021). These are the Common Woodpigeon (*Columba palumbus*, Linnaeus 1758), the Eurasian Collared Dove (*Streptopelia decaocto*, Frivaldszky 1838), the European Turtle Dove (*Streptopelia turtur*, Linnaeus 1758) and the Stock Dove (*Columba oenas*, Linnaeus 1758).

All four species are listed in Annex II of the Council Directive 79/409/EEC and therefore can be legally hunted. However, the species-specific protection status, hunting effort and regulations differ between European countries.

This thesis has not considered the Eurasian Collared Dove as it only spread from its original distribution range in the Oriental region over the European continent throughout the 20th century (Gibbs et al. 2001; Rocha-Camarero & de Trucios 2002). In addition, it is a resident species in Europe, compared to the three other species, known to perform migratory movements (Gibbs et al. 2001; Baptista et al. 2015). Therefore, my thesis focuses on the remaining three species of wild Columbiformes native to Europe, namely the Common Woodpigeon, European Turtle Dove and Stock Dove. These species are briefly introduced in the following sections.

1.2.1 The Common Woodpigeon - From forest dweller to city resident

The Common Woodpigeon (henceforth Woodpigeon) is widely distributed throughout Europe (Fig. 1). With a population size of around 41 to 57 million mature individuals, they are one of the most common breeding bird species in Europe (BirdLife International 2021). Notwithstanding its great abundance and despite being considered by some as an agricultural pest, which causes damage to different crops, and the most important European game bird species (Murton 1960; Slater et al. 2001; Höfle et al. 2004; Butkauskas et al. 2013), the Woodpigeon receives relatively little attention in general as well as in scientific research (König et al. 2015). It was once a typical woodland species, which inhabited deciduous, mixed and coniferous forests, but an increase in population size since the mid-20th century has resulted in an expansion in its breeding habitats to suburban and urban areas (Tomiałojć 1976; Slater et al. 2001; Bea et al. 2011). Thus, the Woodpigeon is classified as synanthropic species nowadays (Kurucz et al. 2021). Possibly due to their high adaptability Woodpigeons now occur in almost all natural and man-altered habitats and their European population continues to increase further (Gibbs et al. 2001; Bea et al. 2011; Sakhvon & Kövér 2020; PECBMS 2022).

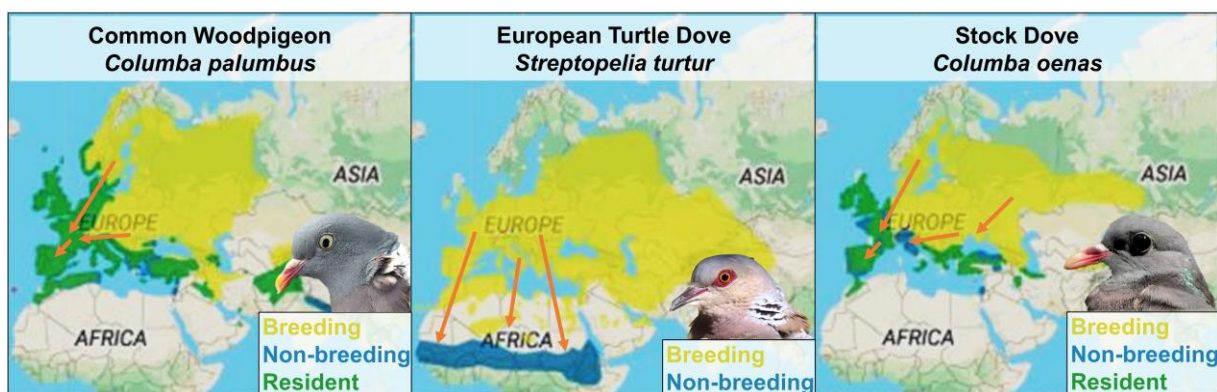*Figure 1* Maps of breeding and non-breeding areas, i.e. wintering areas, of Common Woodpigeons, European Turtle Doves and Stock Doves. Maps adapted from BirdLife International (2022). Orange arrows depict a simplified course of migration directions from breeding to wintering areas (adapted from Möckel 1988 for Stock Doves; Cohou et al. 2007 for Common Woodpigeons, Marx et al. 2016 for European Turtle Doves).

1.2.2 The European Turtle Dove - One of Europe's most rapidly declining species

Given its rather vast breeding range (Fig. 1), it can be assumed that the European Turtle Dove (henceforth Turtle Dove) occurs in multiple habitats and landscapes. Often the Turtle Dove is associated predominantly with farmland. However, research indicates that it occupies a more diverse range of habitats, often combining open ground areas, such as arable land or grassland, with hedgerows, trees, or small woodlands, i.e. an ecotone between agricultural areas and forest (reviewed in Carboneras et al. 2022).

The Turtle Dove, a trans-Saharan migrant, is one of many migratory landbirds within the Afro-Palaearctic system that is declining (Carboneras et al. 2022). The European population has declined by almost 80% since 1980. Consequently, the species has been classified as globally threatened ('vulnerable'; BirdLife International 2021). Nevertheless, the severity of the population decline varies between countries (Fig. 2). Despite the ubiquity of decline, the detailed causes remain uncertain, in particular, because certain pressures, such as food availability or predation pressure, affect different stages of the Turtle Dove life cycle, making it difficult to estimate their relative contribution to the overall decline (de Vries et al. 2022). The main causes suggested to contribute to the ongoing population decline are habitat loss and deterioration at breeding and wintering grounds associated with changes in agricultural practices, illegal killing and unsustainable levels of legal hunting (Fig. 2; Fisher et al. 2018; Lormée et al. 2020; Moreno-Zarate et al. 2021; Carboneras et al. 2022).
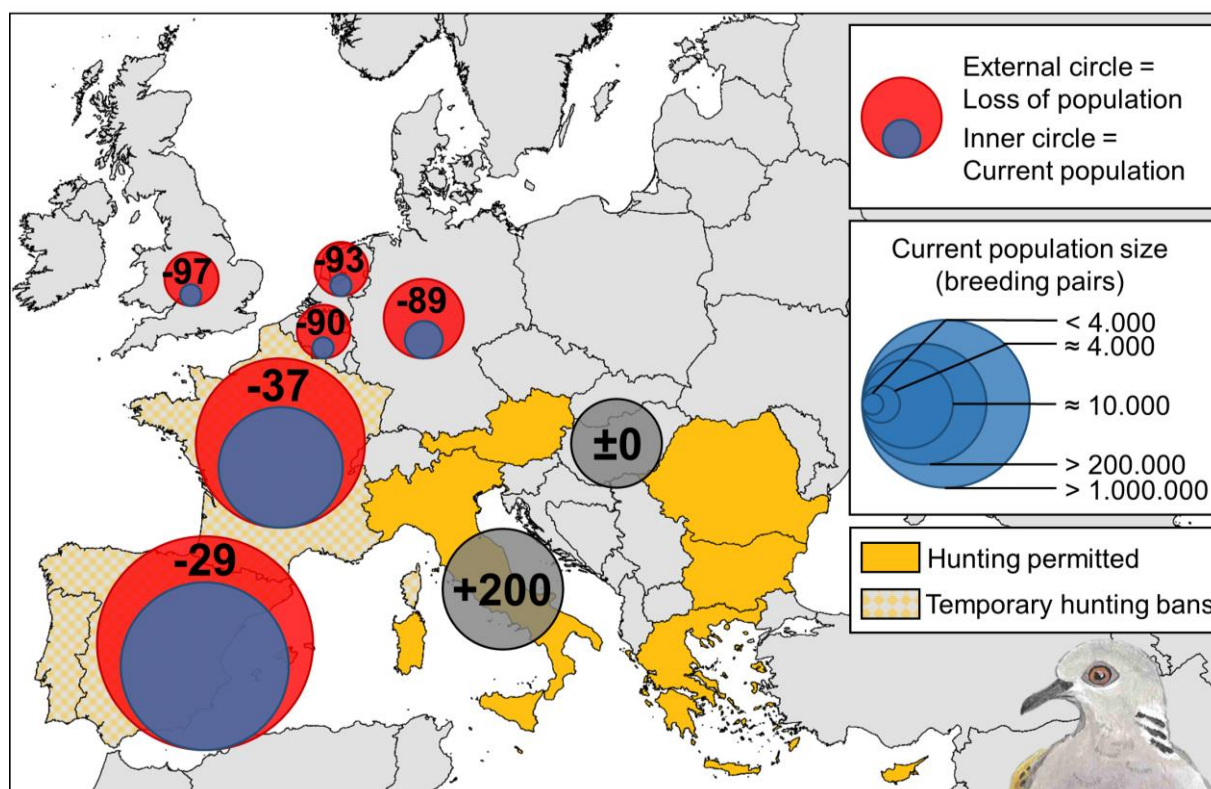
Figure 2 Long-term breeding population trends of Turtle Doves per European country for which sufficient data is available. The population trend is reflected by the numbers given in the respective circle ('-' corresponds to decline [%] and '+' to growth [%]). Yellow colour marks EU countries in which Turtle Doves can be legally hunted or in which hunting is generally permitted, but a temporary hunting ban exists (for hunting seasons 2020/21 and/or 2021/22). Modified figure according to FACE (2020).

1.2.3 The Stock Dove – The cavity-nesting columbiform species

The Stock Dove occurs almost all over Europe (Fig. 1) and inhabits mainly agricultural land with plenty of trees, woodland edges and broad-leaved forests, especially old forest stands (Möckel 1988; Gibbs et al. 2001; Kosiński et al. 2011). In contrast to most other wild doves and pigeons, which usually construct an open nest by accumulating some twigs to a platform nest, Stock Doves are cavity-breeders (Möckel 1988; Gibbs et al. 2001). Therefore, the occurrence and density of Stock Doves depend on the number of available trees with suitable nest holes, such as holes excavated by Black Woodpeckers (*Drycopus martius*, Linnaeus 1758; Kosiński et al. 2011). Also, artificial nest boxes are accepted as nesting opportunities by Stock Doves (Möckel 1988; Møller et al. 2016).

The European Stock Dove population declined at the beginning of the 20th century. This decline was attributed mainly to a lack of adequate breeding sites due to changes and modernisation in forestry management, such as the increase of spruces or loss of old broad-leaved forest stands (Hillerich 1984; Möckel 1988). Since around 1970, after increased protection efforts, the population has been stable and has even increased locally (Möckel 1988; Møller et al. 2016; BirdLife International 2022). Nowadays, Stock Doves are classified as 'least concern' in Europe and Germany (Ryslavy et al. 2020; BirdLife International 2021).

1.3 The relevance of behavioural studies in species conservation and management

With animal species increasingly facing threats from changing climate, diseases, habitat loss and other pressures from human activities, accelerated rates of species decline and extinction risk are observed (Swift et al. 2018; Soroye et al. 2020; Sattar et al. 2021). Thus, for effective conservation and management, it is important to have accurate information about the status and dynamics of natural populations (Swift et al. 2018). The study of animal behaviour can provide a valuable contribution to solving conservation and management problems (Sutherland 1998; Buchholz 2007). Attempts to relate the behaviour of animals to conservation are increasing in the academic literature (Caro & Sherman 2013). To be more precise, the behaviour of an animal is relevant to conservation biology as behaviour can affect the persistence of species, e.g. by dispersal and settlement decisions or learned foraging techniques (Reed 2002). Moreover, it is stated that the study of animal behaviour is crucial for solving issues of coexistence between wild animals and humans (Blackwell et al. 2018), especially in the case of legally huntable species.

Although the Turtle Dove is a rapidly declining species and the Woodpigeon is one of the most common species in Europe, large gaps in our knowledge regarding different behavioural aspects of these and related columbiform species exist. For instance, little is known about the migration patterns of the Turtle Dove (Marx et al. 2016), the dietary habits of the Stock Dove and Woodpigeon (Dunn et al. 2018), or the effects of diseases on mortality and fitness of columbiform host species (Fisher et al. 2018). In the presented thesis, I aimed to obtain a comparative and integrated picture of three behavioural-ecological aspects of the three aforementioned species of Columbiformes: movement, dietary and disease ecology.

1.3.1 Movement ecology – Migration and foraging behaviour

The ability to fly makes birds one of the most mobile animals. Their movements occur across a wide range of spatiotemporal scales, extending from daily foraging movements, one-way dispersal movements to seasonal migratory movements (Thorup et al. 2017; Shaw et al. 2020). Migration can be defined as the regular movement of a population to and from a specific region, resulting in distantly separated breeding and wintering grounds, and is characterised by movement patterns that vary within a year but usually not between years (Teitelbaum & Mueller 2019). Many avian migrants demonstrate partial migratory behaviour, i.e. just a fraction of the population migrates whereas the remaining individuals reside in a single habitat for the entire year (Chapman et al. 2011). Factors that influence population size dynamics include events that occur over the full annual cycle and thus, in the case of migrants, events that occur at different localities. Identifying the relative importance of effects, carryover effects and relationships among events at different annual stages (breeding, wintering and migration) is fundamental to the effective conservation and management of species (Robinson et al. 2010; Sedinger et al. 2011; Calderòn et al. 2019). However, these data are rare for most species, often due to the inability to track individuals throughout their annual cycle, resulting in a lack of knowledge about where birds breed and winter (Robinson et al. 2010; González et al. 2020).

Traditionally, mark-recapture techniques, such as marking individuals with leg rings and subsequent sighting or recapture, were used to acquire knowledge on migration flyways (Robinson et al. 2010; Negrier et al. 2020). Also, ratios of isotopes in tissue, genetic markers (reviewed in Robinson et al. 2010) or radar systems (Nilsson et al. 2018; Cui et al. 2020) were used to monitor migratory movements. Rapid advancements in tracking technologies, such as radiotelemetry or satellite transmitter systems, enabled the collection of positioning information on a wide variety of species remotely. With this progress, the movement of animals can be tracked at various spatial and temporal scales over long periods, often without the need for recapture (Robinson et al. 2010; Yoda 2019; Mitchell et al. 2019; Jetz et al. 2022).

The diverse tracking techniques can be used to study various aspects of migration, e.g. orientation, navigation, phenology, flyways, stopover site selection, migratory connectivity and occurrence of niche tracking behaviour (Robinson et al. 2010; Illan et al. 2020). For example, flyways of Turtle Doves were initially identified based on ring-recoveries and sightings, suggesting three flyways between Europe and Sub-Saharan Africa: a Western, a Central and an Eastern one (Fig. 1; Marx et al. 2016). Later on, individual Turtle Doves were equipped with Argos satellite transmitters at their breeding grounds in France to analyse flyways, stopover and African wintering sites in the Sub-Saharan region in more detail (Lormee et al. 2016).

Stock Doves and Woodpigeons are partial migrants. Both species can be roughly divided into individuals from Central and Eastern Europe, which are expected to perform short-distance migration within Europe, and individuals from Western Europe, which are supposed to be residents (Fig. 1; von Blotzheim & Bauer 1994). For instance, a tracking study of

Woodpigeons equipped with Argos transmitters during the wintering period in Portugal showed that these individuals migrate to breeding grounds in Poland or Switzerland (Cohou 2011).

Improving and overcoming deficiencies, like low resolution and accuracy, modern technology, such as high-resolution GPS tracking, provide the level of detail needed to identify and characterise fine-scale space use and movements. The high-frequency movement data enables to link movements with behaviours and thus gain greater insight into the behavioural ecology of species, such as foraging behaviour, representing one of the most important animal behavioural components (Yoda 2019; McDuie et al. 2019; Van Donk et al. 2020).

For example, a radiotracking study suggested a memory-based strategy with a flocking mechanism to be the best fit for explaining observed foraging patterns in Woodpigeons (Kułakowska et al. 2014). Further studies like this may allow to precisely predict spatial and temporal characteristics of foraging habits of avian species like Woodpigeons, taking into account their excellent memory, ability to fly long foraging distances and distinctive flocking behaviour. A precise knowledge of foraging behaviour can be used to assess aspects relevant to species conservation, e.g. predictions of the exposure to pesticides based on known foraging characteristics (Kułakowska et al. 2014; de Montaigu & Goulson 2022). As another example, with leg-ring radio-tag attachments it was shown that in the first weeks after fledging, Turtle Doves forage mainly in the immediate vicinity (≈ 300 m) of their nest, highlighting food availability close to nest areas as a crucial factor for survival in juveniles (Dunn et al. 2016).

1.3.2 Dietary ecology - Reconstruction of diet composition

Worldwide, avian communities inhabiting agroecosystems are threatened as a consequence of agricultural intensification (Crisol-Martínez et al. 2016). In Europe, the mid-20th-century agricultural intensification and the past and ongoing changes in farming practices are expected to have contributed to the decline of several species of farmland birds. The declines are often associated with decreased weed seed availability from farmlands (Gutiérrez-Galán & Alonso 2016; Negrier et al. 2020). Precise knowledge of the diet of a species might be of special interest for designing sound conservation and management strategies (Valentini et al. 2009), such as planning grazing schemes that increase the abundance of relevant plant species to improve food availability (Gutiérrez-Galán & Alonso 2016).

Traditionally, dietary analyses have been conducted mainly by direct observation of foraging behaviour or microhistological analysis of stomach or gut contents and faecal samples. These traditional techniques are often hampered by the difficulty of continuous observation and decomposition by the digestion process impeding identification, respectively. The development of non-invasive molecular techniques has allowed a more precise detection of DNA remains and higher taxonomic resolution of diet items in faecal samples (Valentini et al. 2009; Ando et al. 2013; Crisol-Martínez et al. 2016). The molecular analyses of faeces, i.e. molecular scatology, can be used to reveal significant differences in dietary composition and overlap (potential competition) between bird species (Crisol-Martínez et al. 2016; Dunn et al. 2018; Swift et al. 2018).

With the development of DNA barcoding and next-generation sequencing (NGS) techniques, faecal analysis using high-throughput sequencing (HTC) is nowadays a widespread, non-invasive method to study animal diets (Fig. 3; Pompanon et al. 2012; Ando et al. 2016).

Generally, most species of Columbiformes are considered generalists with broad foraging ranges, often altering their diet components according to the availability of specific food resources (Ando et al. 2016). For instance, Woodpigeons adapt their diet and foraging behaviour due to synurbization by exploiting supplementary feeding at garden bird feeders (Luniak et al. 2004; Dunn et al. 2018). Turtle Doves and Stock Doves feed predominantly on seeds, whereas Woodpigeons also consume leaves, fruits and other plant matter. Animal material, e.g. small invertebrates, is rarely a component of the diet of the columbiform birds (Murton et al. 1964; Browne & Aebischer 2003; Dunn et al. 2018; Negrier et al. 2020).

However, precise data on the diet of Columbiformes are fragmentary and limited (Mansouri et al. 2019; Negrier et al. 2020). Previous studies have demonstrated that the diet of the Turtle Dove changed from mainly non-cultivated (natural) arable plants in the 1960s to seeds of cultivated plants, such as wheat and oilseed rape, in the 1990s (Murton et al. 1964; Browne & Aebischer 2003). The dietary switch may be associated with the reduction in food availability during key periods of the breeding season when seeding natural arable plants have become scarce as a result of agricultural changes (Browne & Aebischer 2004; Dunn et al. 2018). The lack of natural arable plant species potentially deteriorates the condition of nestling Turtle Doves (Dunn et al. 2018).

Moreover, it was hypothesised that the reduced availability of suitable food, both spatially and temporally, contributes to the decline, by causing Turtle Doves to cease breeding earlier and thus produce fewer offspring due to a reduced number of nesting attempts (Browne & Aebischer 2004; Browne et al. 2005). Furthermore, Turtle Doves might be energetically challenged due to the reduction of both diet quality and food availability (Dunn & Morris 2012). This stress could cause weakened immune defense, which in turn could increase susceptibility to infections (Altizer et al. 2006).
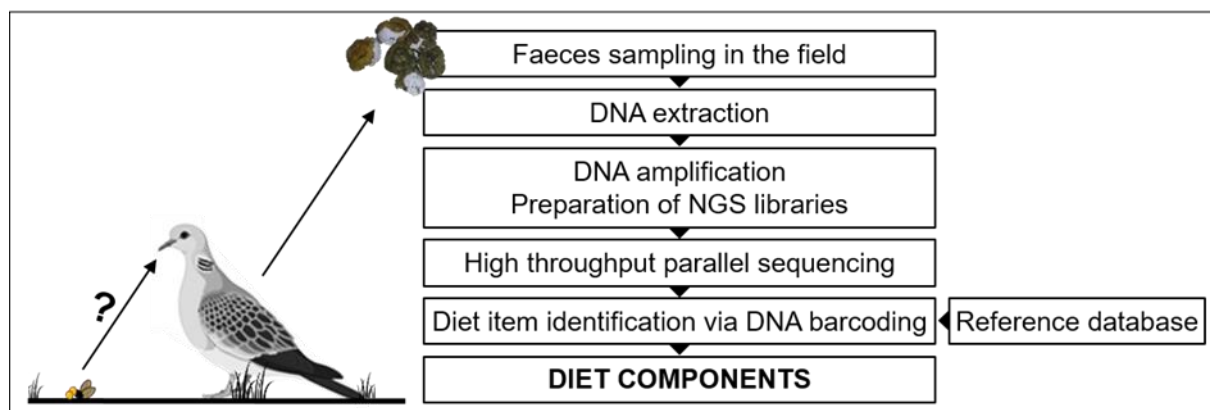

Figure 3 Flowchart diagram showing the main steps (simplified) of a non-invasive, molecular approach for assessing diet composition using faecal samples. Figure modified according to Valentini et al. (2009) and Valkiūnas & Atkinson (2020).

1.3.3 Disease ecology - Avian haemosporidian parasites

Endoparasitic infections are a potential stressor for wild birds. Endoparasites, which can be classified roughly into gastrointestinal parasites and blood parasites, can have effects on their avian hosts ranging from minor to fatal diseases (Kumar et al. 2018; Zahan et al. 2018; Attia & Salem 2022). In recent decades, changing environments, climate change, globalization, human and animal movements and other drivers contributed to the spread of parasites and the emergence of novel pathogens (Rosenberg et al. 2019; Rush et al. 2021). Species of Columbiformes infested with endoparasites are often assumed responsible for the spread of parasites to other avian species (Omer et al. 2015; Attia & Salem 2022). Among others, they can act as a reservoir host for the flagellated protozoan parasite *Trichomonas gallinae*, causing avian trichomonosis (Marx et al. 2017; Martínez-Herrero et al. 2020) or as a reservoir of various avian malaria and malaria-like parasites (Zahan et al. 2018).

Nowadays, it is recognised that avian haemosporidian parasites, the causative agents of avian malaria and related diseases, are among the most pathogenic organisms. These diseases can be responsible for mortality, population declines and even extinctions of poultry and wild birds (Palinauskas et al. 2020). They can cause serious infections in avian hosts with symptoms such as anaemia, weight loss, anorexia, arthritis, hepatosplenomegaly, reduced strength in flight and have long-term negative effects on their reproductive system (Ciloglu et al. 2019). Avian haemosporidian parasites (Apicomplexa) of the genera *Plasmodium*, *Haemoproteus* and *Leucocytozoon* have been documented in a great variety of avian host species all over the world and are one of the most prevalent and diverse groups of avian parasites (Atkinson 1999; Valkiūnas 2005; Galen et al. 2018; Ciloglu et al. 2019; de Angeli Dutra et al. 2021). The life cycles of these vector-borne parasites are obligately heteroxenous, developing in two groups of hosts: vertebrates (here: birds) and vectors (depending on the parasite genus different blood-sucking dipterans). The vector inoculates sporozoites, the infective stage of the parasites, into birds while blood-feeding (Fig. 4). The sexual reproduction process occurs in the vector and thus the birds are intermediate hosts and the dipterans are definitive hosts (Fig. 4; Valkiūnas 2005; Valkiūnas & Atkinson 2020).

Generally, three measures are most commonly considered analysing host-parasite interactions: (i) prevalence refers to the proportion of individuals of a population or group that are infected, (ii) intensity or parasitemia is a measure of how many parasites of one species infect a host individual and (iii) richness is the number of parasite species found in an individual host, a group, or a host species (Herrera & Nunn 2019). While microscopy was the method to identify blood parasites with morphological data before 2000, recent molecular studies have recognised a huge genetic diversity of avian haemosporidian parasites with at least 260 species and over 3600 lineages (Harl et al. 2020; Mandal 2021).

Also, it is known that the avian life-history strategy influences haemosporidian prevalence and richness (Dunn & Outlaw 2019; Fecchio et al. 2021). For example, it is expected that migratory birds might have more prevalent haemosporidian infections and higher parasite richness because they move between breeding and wintering areas and use stopover sites. At these different locations they may encounter a much greater variety of haemosporidian parasites and vectors. In comparison, residents encounter parasites and their vectors in only one ecosystem (Ciloglu et al. 2019; de Angeli Dutra et al. 2021; Fecchio et al. 2021). Even though *Plasmodium* parasites are among the best-studied pathogens, there is still a large gap in our understanding of their diversity, specificity, virulence and development in vertebrate hosts. Even less is known about the other genera *Leucocytozoon* and *Haemoproteus* (Palinauskas et al. 2020). By sampling wild birds, it is possible to define composition up to lineage level and prevalence of haemosporidian parasites in different bird species using a combination of microscopy and molecular tools (Ciloglu et al. 2019; Palinauskas et al. 2020). Accurate detection of avian haemosporidian parasites includes a thorough sampling of avian host species, in particular columbiform species (Zahan et al. 2018). The accurate detection is required to understand host-parasite interactions, effects of coinfections (e.g. with *Trichomonas gallinae*), true diversity and epidemiology of infections and to develop control strategies against these diseases (Ciloglu et al. 2019; Dunn & Outlaw 2019; Thomas et al. 2022).
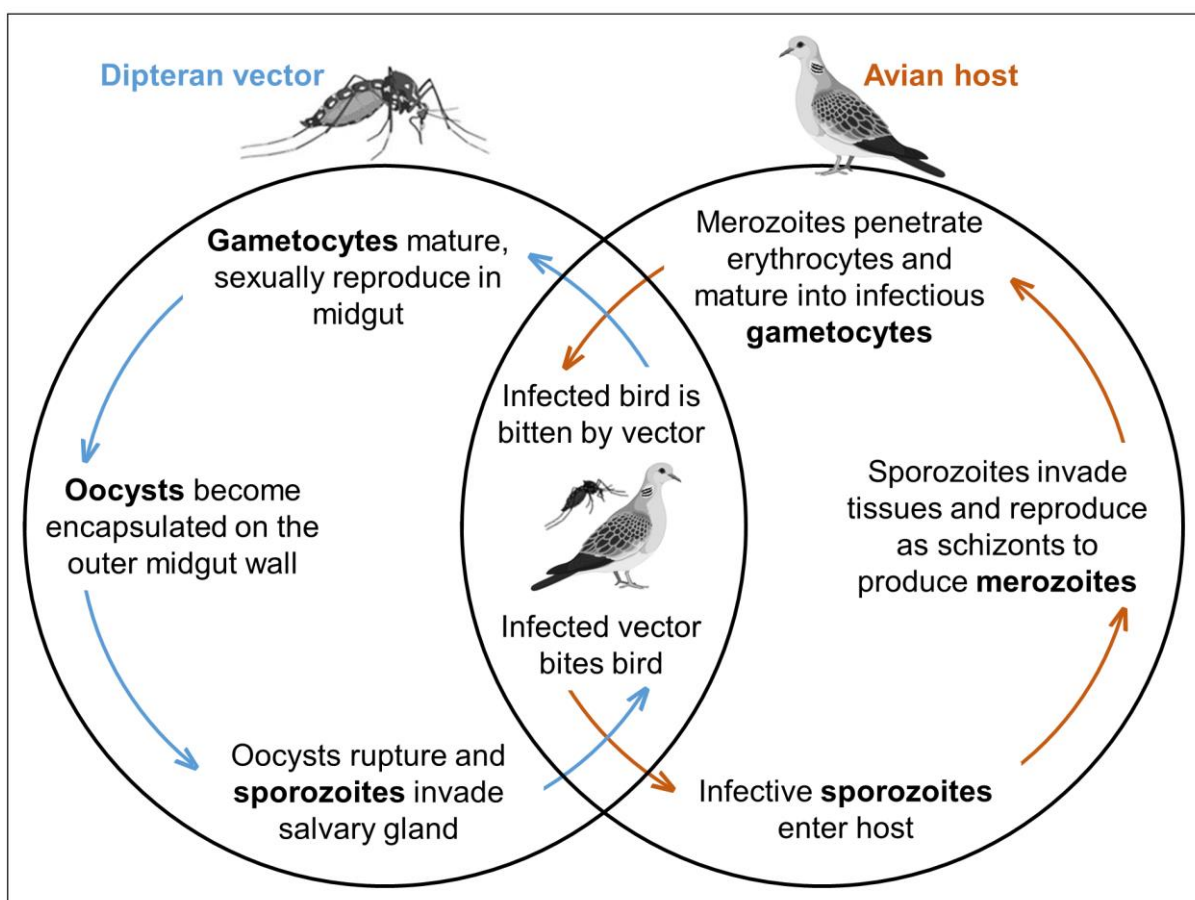

Figure 4 General representation of the life cycle of avian haemosporidian parasites. Figure adapted from Atkinson (1999), Mata (2012) and Valkiūnas & Atkinson (2020).

2 | AIMS AND STRUCTURE OF THE THESIS

The main objective of this thesis was to improve our knowledge about several behavioural-ecological aspects of different migratory species of Columbiformes native to Europe by a comparative approach. Thus, the studies included intend to complement missing basic and fundamental information on the species' ecology. The knowledge acquired through the studies can potentially be used to identify prospective protection strategies, considering interests in both management of game bird species and species conservation. In addition, the data gathered can help to estimate the influence of the different aspects analysed on the population dynamics of the columbiform species. The included aspects can be classified into three broad research topics (Fig. 5 and 6):

- ☐ movement ecology, encompassing migration and foraging behaviour (*chapters 1 to 3*)
- ☐ dietary ecology, focussing on analysing diet compositions (*chapter 4*)
- ☐ disease ecology, in particular, avian haemosporidian parasites (*chapter 5*)

While *chapters 1* to *3* focus on a single species each (*chapters 1* and *2*: Turtle Dove, *chapter 3*: Woodpigeon), *chapters 4* and *5* are community analyses, including several species of wild Columbiformes. Due to the pluralist approach in terms of investigated behavioural-ecological aspects and applied methods (Fig. 6), the present cumulative thesis is structured in five publications, each presented as an individual chapter, with the following specific aims:

CHAPTER 1 | *Feather stable isotopes (δ^2H_f and $\delta^{13}C_f$) identify the Sub-Saharan wintering grounds of turtle doves from Europe*
- Determine the non-breeding, i.e. wintering, regions of the only long-distance migratory species among the Columbiformes native to Europe, namely Turtle Doves
- Analyse whether wintering grounds differ for individuals using different flyways to estimate the migratory connectivity of Turtle Doves across Europe

CHAPTER 2 | *Year-round spatial distribution and migration phenology of a rapidly declining trans-Saharan migrant - evidence of winter movements and breeding site fidelity in European turtle doves*
- Identify migration flyways and phenology as well as wintering areas of Turtle Doves following different flyways on a finer spatial and temporal scale
- Characterise habitat parameters at breeding and wintering sites
- Check for the occurrence of niche tracking or niche switching behaviour with regard to environmental parameters

CHAPTER 3 | *Should I stay or should I fly? Migration phenology, individual-based migration decision and seasonal changes in foraging behaviour of Common Woodpigeons*

- Investigate the migration and foraging behaviour of Woodpigeons from different breeding regions
- Evaluate within-population migratory dimorphism
- Describe habitat characteristics and choice based on land cover data

CHAPTER 4 | *Diet composition of wild columbiform birds: Next-generation sequencing of plant and metazoan DNA in faecal samples*

- Demonstrate how plant and metazoan diet components can be identified based on a non-invasive approach
- Provide a detailed reconstruction of the food items ingested by species from the order Columbiformes
- Determine the potential dietary overlap between the included bird species

CHAPTER 5 | *Prevalence and genetic diversity of avian haemosporidian parasites in wild bird species of the order Columbiformes*

- Examine the overall and species-specific prevalence, diversity and parasitemia of avian haemosporidian parasites in wild columbiform bird species
- Check for potential differences between host species, taking into account the variable behavioural characteristics of each species
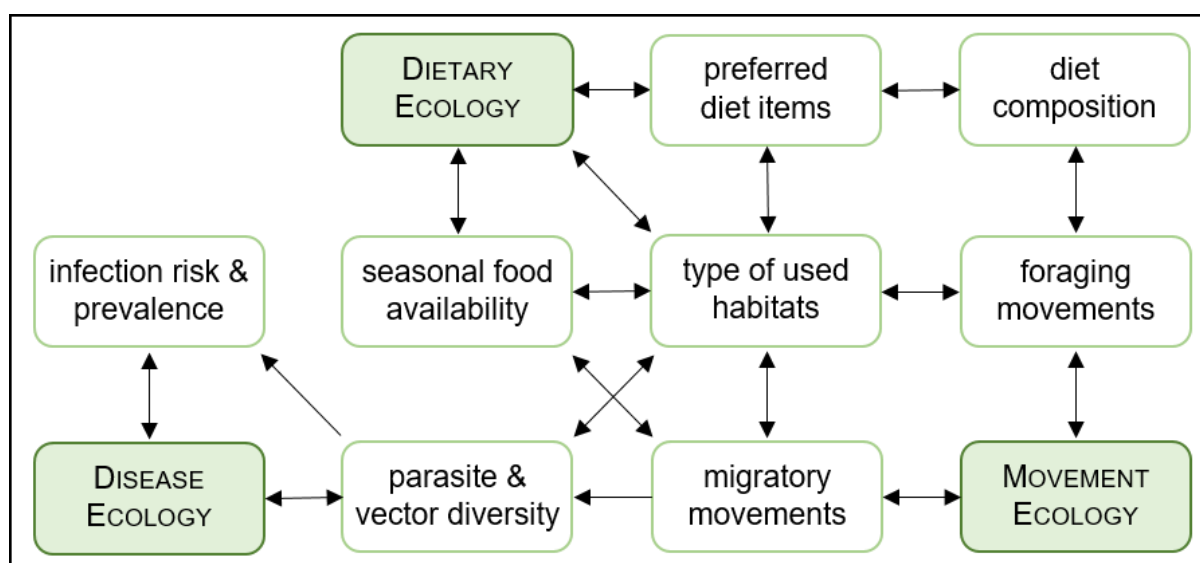- Evaluate the possible negative effects of haemosporidian parasites on host species

Figure 5 Schematic overview of relevant connections and interactions, represented by arrows, between the three broad research topics included in the presented cumulative thesis (dietary ecology: *chapter 4*, disease ecology: *chapter 5* and movement ecology: *chapters 1* to *3*).

3 | CHAPTER OUTLINE

The thesis compromises five studies that provide insight in the above-mentioned main objective, separated into multiple specific aims, presented in the chapters accordingly.

CHAPTER 1 | *Feather stable isotopes (δ^2H_f and $\delta^{13}C_f$) identify the Sub-Saharan wintering grounds of turtle doves from Europe* [PUBLISHED]

Outline – In this publication, analyses of feather-isotope signatures were used to identify the African wintering origins of Turtle Doves. Samples of tenth primary feathers (n = 181), known to grow during the wintering period, have been collected during the breeding and migration period or originated from museum collections. By assigning stable hydrogen (δ^2H_f) and carbon ($\delta^{13}C_f$) isotope values of feathers to multi-isotopic landscapes ('isoscapes') of precipitation stable hydrogen (δ^2H_p) and theoretical vegetation stable carbon ($\delta^{13}C$) in Africa, we presented a first-order estimation of wintering regions of sampled Turtle Doves. Furthermore, it was assessed how these likely wintering quarters, located in the Western and Central Sub-Sahara, overlap with present hunting and protected areas. The use of samples collected at different sites across Europe allowed checking for flyway-specific wintering regions. The probabilistic assignments did not indicate a marked difference between the wintering origin of individuals following the Western (samples from Spain and France) vs. the Central/Eastern flyway (samples from Greece, Malta, Italy and Bulgaria). However, comparisons of the raw δ^2H_f and $\delta^{13}C_f$ values revealed differences, suggesting that individuals potentially have spent the winter in separate regions, had different diets, or occupied different habitats. The advantages and disadvantages of stable isotope analyses, as applied in the study, were discussed. This may provide thought-provoking impulses for research on other long-distance migratory species, in particular for those too small to be equipped with currently available tracking devices.

Contributions – Shared lead author (with M. Marx), including manuscript writing and editing; corresponding author; partial data and statistical analyses of stable isotope data and spatial dataset of protected and hunting areas (shared mainly with M. Marx and K.J. Kardynal)

CHAPTER 2 | *Year-round spatial distribution and migration phenology of a rapidly declining trans-Saharan migrant - evidence of winter movements and breeding site fidelity in European turtle doves* [PUBLISHED]

Outline – This publication focused on the evaluation of satellite tracking data of Turtle Doves to increase our knowledge on migration flyways, migration timing, stopovers, European breeding and African wintering sites. Individuals have been equipped with Argos satellite tags, providing location fixes based on Doppler calculations, during spring migration on Malta (n = 8) and the breeding season in Germany (n = 8). Tracking data combined with environmental habitat parameters showed that environmental parameters are more uniform at the wintering grounds compared to the breeding grounds. This suggests that Turtle Doves

might be even more vulnerable to future changes in their winter ranges than in their breeding ranges. 'Niche tracking' behaviour, i.e. following environmental conditions of similar type throughout the year, was only observed regarding night-time temperatures. Analysis of land cover data on breeding grounds demonstrated the use of a wide range of forest and agricultural landscapes. Home range size at breeding grounds increased with an increasing proportion of agricultural areas. This is probably due to Turtle Doves being forced to fly large distances in intensively farmed areas to reach good quality food resources. Year-round tracking, partly for consecutive years, of individuals revealed behavioural characteristics relevant for planning conservation measures, e.g. breeding site fidelity, prolonged stopovers in Europe, use of multiple wintering sites, evidence for loop migration and a curtailed breeding season. The gathered data were particularly needed for individuals migrating along the Central and Eastern flyway, as no tracking data from there had been analysed before this study.

Contributions – Lead author; partial material collection and bird handling in the field (catching, sampling and tagging); molecular work (sex determination); data and statistical analyses (environmental habitat parameters, tracking and land cover data)

CHAPTER 3 | Should I stay or should I fly? Migration phenology, individual-based migration decision and seasonal changes in foraging behaviour of Common Woodpigeons [PUBLISHED]

Outline – This manuscript dealt with foraging and migratory movements over the annual cycle of Woodpigeons. The analyses were based on ring-recovery and two types of satellite tracking data (Argos and GPS-GSM/GPRS). Individuals (n = 29) with breeding areas located in Germany and Portugal, mainly caught in an urban environment, were equipped with GPS-GSM/GPRS transmitters. Woodpigeons from Lisbon (Portugal) stayed almost exclusively within a large wooded park within the city. Contrary, Woodpigeons from Giessen (Germany) regularly left the city area to forage on surrounding farmland. In general, home ranges were larger for Woodpigeons in Giessen compared to individuals in Lisbon. The results of ringing and tracking data showed a migratory dimorphism: all Woodpigeons breeding in Portugal and the majority of individuals with breeding sites in Germany were residents, but other individuals from Germany were migrants. These migrants wintered either in Germany, but away from their breeding sites, or followed the European sector of the East Atlantic flyway to winter mainly in France and less frequently in Spain, Portugal, Belgium, Netherlands and Denmark. Using the tracking data, it was possible to observe behavioural characteristics on an individual level, revealing a low wintering site fidelity, use of multiple winter sites during one season and switches of migratory strategies (resident vs. migrant) between years. This study, the first to analyse satellite tracking data of Woodpigeons from consecutive years, emphasises their behavioural plasticity. Studying species with such pronounced variation in migratory behaviour might be of value to investigate the effects of ongoing climate change and increasing urbanisation on migratory decisions.

Contributions – Lead author; funding acquisition; partial material collection and bird handling in the field (catching, sampling and tagging); molecular work (sex determination); data analyses (ring-recovery, tracking and land cover data)

CHAPTER 4 | Diet composition of wild columbiform birds: Next-generation sequencing of plant and metazoan DNA in faecal samples [IN PREPARATION]

Outline – In this study, the focus was on a detailed analysis of the diet compositions of different species of Columbiformes by applying next-generation sequencing (NGS) technology as a tool for diet reconstruction through DNA metabarcoding. The use of primer pairs targeting plant nuclear DNA and metazoan DNA, isolated from collected faecal samples (n = 139), provided a complete picture of the food items ingested. Assessing the dietary overlap between the included columbiform species (Turtle Doves, Stock Doves and Woodpigeons) revealed variability in consumed plant taxa (a diverse range of Spermatopsida) among the species. Woodpigeons and Stock Doves showed the highest dietary overlap. Non-metric Multidimensional Scaling was used to visualize the detected differences in plant diet compositions. Animal prey, i.e. metazoan DNA, was present only very rarely. Plant taxa observed in this study, but previously not listed as known food items in other studies, and an evaluation of the proportion of wild vs. cultivated plant species give indications for dietary changes due to urbanization and agricultural intensification. Especially for declining Turtle Doves, identified regional variations may be relevant for the implementation of proposed conservation options, e.g. tailored seed mixtures. The sharp decline of many avian species over the past decades calls for a more thorough knowledge of their dietary requirements. Our study supports the non-invasive approach applied as an accurate method for diet analyses that could be used in other species as well.

Contributions – Lead author; organisation of faecal sample collection with cooperation partners; partial material collection in the field; molecular work (DNA isolation and preparation of NGS libraries); partial data and statistical analyses (shared mainly with J.F. Masello)

CHAPTER 5 | Prevalence and genetic diversity of avian haemosporidian parasites in wild bird species of the order Columbiformes [PUBLISHED]

Outline – Here, the prevalence, diversity and parasitemia of haemosporidian parasites in Columbiformes were presented. Blood samples (n = 259) of different species, predominantly Turtle Dove, Stock Dove and Woodpigeon, were collected in seven countries between 2013 and 2019. Haemosporidian infections were determined through nested PCR, revealing an overall prevalence of 42%. More precisely, the majority of individuals (34%) harboured a single haemosporidian lineage (*Leucocytozoon*: 15%, *Haemoproteus*: 15%, *Plasmodium*: 4%) and 7% had mixed infections. Stock Doves had a lower overall prevalence (4%) than Turtle Doves (49%) and Woodpigeons (62%), potentially because they might be shielded better from dipteran vectors as cavity-nesters.

Overall, 15 distinct mitochondrial cytochrome *b* lineages were detected, including five newly discovered ones. A second PCR assay (One-step multiplex) was applied for improved detection of mixed *Plasmodium*/*Haemoproteus* infections. However, we showed that this assay displays infections of the subgenera *H.* (*Haemoproteus*) at the expected band height of *Plasmodium* infections. Thus, this PCR assay is rather not advisable to examine orders prone to *H.* (*Haemoproteus*) infections. Despite a relatively high observed prevalence and diversity, the parasitemia (obtained by microscopic examination of blood smears) was rather low for most samples, indicating mainly chronic instead of acute infections. The low parasitemia suggests the contribution of haemosporidian infections to population dynamics of sampled hosts might be rather insignificant. Obtained data can constitute an important reference to monitor future changes in parasite ranges and diversity expected as a consequence of climate change.

Contributions – Lead author; partial material collection in the field (blood sampling); examination of blood smears; molecular work (DNA isolation and PCR assays); phylogenetic (medium joining networks and phylogenetic tree reconstruction) and statistical analyses of datasets resulting from Sanger sequencing
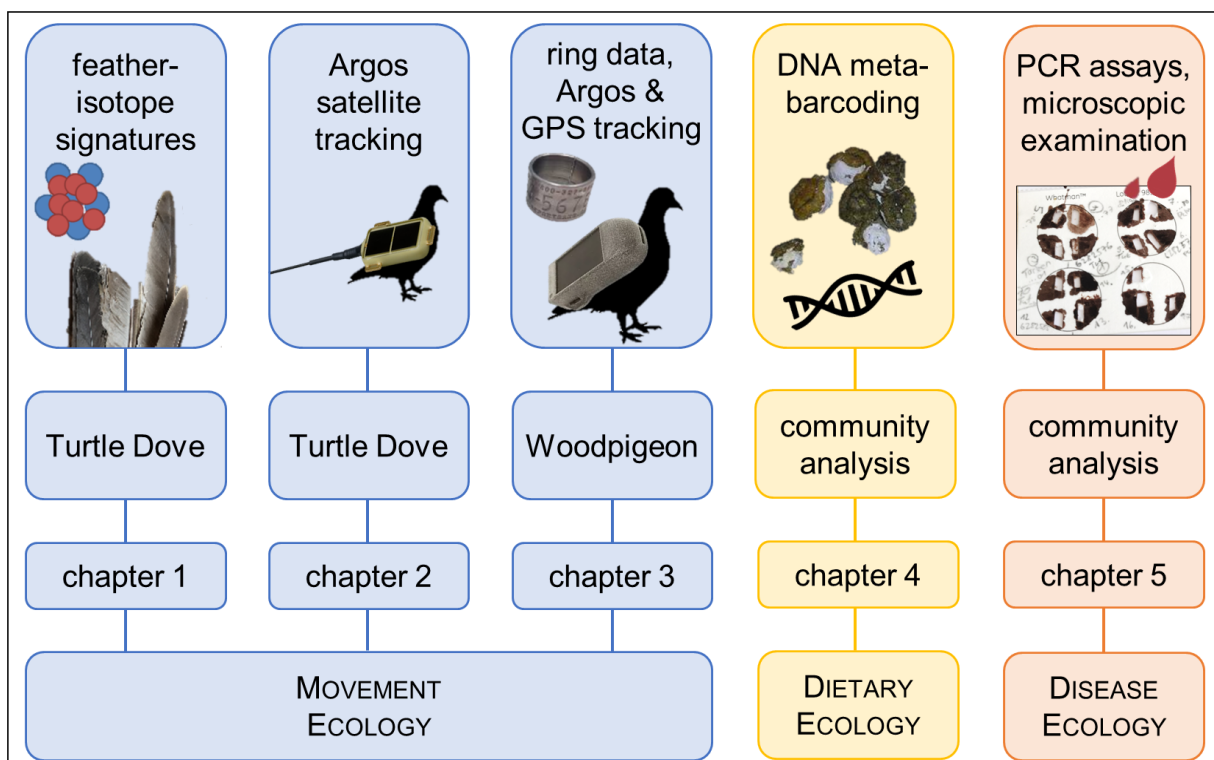

Figure 6 Overview of thesis structure, depicting the three broad research topics with the respective species studied and applied methods (simplified) for each chapter.

4 | GENERAL CONCLUSIONS AND FUTURE OUTLOOK

4.1 General conclusions

The presented cumulative thesis consists of five chapters focusing on behavioural-ecological aspects in the fields of movement, dietary and disease ecology. All chapters together contribute to form a more complete picture of the behaviours exhibited by the studied columbiform species. In particular, because the different fields of ecology are connected and interact with each other (Fig. 5). The presented findings result from the combination of studying single species (*chapters 1* to *3*) and comparative approaches (*chapters 4* and *5*; Fig. 6).

Given the fact that many migratory birds spend most of the year away from their breeding grounds and face seasonally specific threats and limitations with various carryover effects, it is important to consider data from both the breeding and the non-breeding season, i.e. across the entire annual cycle (Hostetler et al. 2015; Briedis et al. 2018; Swift et al. 2020). Studying the migratory movements of Turtle Doves (*chapters 1* and *2*) and Woodpigeons (*chapter 3*) has contributed to a better understanding of the year-round distribution of these two species. The main results obtained under this thematic aspect of my thesis point out that:

- Turtle Doves from the European population migrate along different migration flyways from their European breeding grounds to their African wintering grounds, located in the Western and Central Sub-Sahara. Moreover, we could show that they partly wintered farther south than 10°N (*chapters 1* and *2*), which is often given as a limit in literature. Consequently, also areas farther south than 10°N should be considered as potential wintering areas.

- Turtle Doves following the different flyways are likely to occupy different regions and habitats during the wintering period (*chapters 1* and *2*). This potentially exposes them to different and variable levels of threats during this period in the life cycle, which might be reflected in varying levels of observed population declines across the European breeding grounds (see de Vries et al. 2022).

- In Woodpigeons, which were breeding in Portugal, only resident behaviour was observed. Whereas, the individual-based migratory decision of Woodpigeons with breeding sites in Germany switched between resident and migrant between years. Therefore, they can be classified as facultative partial migrants (*chapter 3*). The annually changing numbers of migrants and residents may provide an explanation for the observed fluctuating numbers of Woodpigeons in their wintering areas (e.g. Lormée & Aubry 2018).

- Woodpigeons show pronounced behavioural plasticity in terms of migration tactics (*chapter 3*). Firstly, this supports the hypothesis that migratory movements in partial migrants are not solely a genetically fixed behaviour (Lundblad & Conway 2020). Secondly, given that Woodpigeons are nowadays one of the most common bird species in many European cities, our results support the general postulation that behavioural plasticity plays an influential role in successful urban colonization processes in birds (Chyb et al. 2021).

- Both migratory Woodpigeons and Turtle Doves show a high breeding site fidelity, i.e. return to the same breeding areas, compared to a lower wintering site fidelity. Also similar in both species is the use of multiple distinct wintering sites during one wintering season by the majority of individuals (*chapters 2* and *3*). Probably these winter movements are linked to the temporally fluctuating availability of food resources.

The dietary and foraging ecology of a species are closely linked to each other, e.g. food availability exerts major influences on foraging distances (Burke & Montevecchi 2009). Therefore, dietary studies can give insights into foraging behaviour and vice versa (Gaglio et al. 2016). Considering the results of the molecular diet analyses (*chapter 4*) and the studies on foraging behaviour based on tracking data (*chapters 2* and *3*) together, the following conclusions can be drawn:

- Turtle Doves and Woodpigeons seem to adapt their foraging areas and distances in breeding areas to the availability of food resources (*chapters 2* and *3*, respectively). This, on the one hand, might highlight an opportunistic or rather plastic nature in both species, but on the other hand, indicates that suitable food resources might not always be available near nesting sites. This forces individuals to forage over longer distances, which might be energetically costly and therefore potentially affect the species negatively (Browne & Aebischer 2001; Masden et al. 2010; Trevail et al. 2019).

- The DNA data from faecal samples showed some differences between the columbiform species in terms of consumed plant species (*chapter 4*). Woodpigeons also consumed plants growing mainly in urban environments, whereas this could not be detected as distinctly in Turtle Doves and Stock Doves. This is also reflected in the tracking studies, demonstrating that much of the foraging of Woodpigeons takes place in urban areas, whereas Turtle Doves very rarely visit urban sites (*chapters 2* and *3*), highlighting the successful adaptation of Woodpigeons to urban environments.

- In general, only certain species, characterized by a combination of specific traits and by certain niche requirements, seem to be capable of coping with the environmental alterations that are imposed by urbanisation (Jokimäki et al. 2016; Patankar et al. 2021). The adapted behaviour in terms of urbanisation of Woodpigeons could be one of the reasons why population numbers of Woodpigeons are not declining despite the continuous increase of urban settlements, which generally constitute a major threat to biodiversity (Seto et al. 2012; Patankar et al. 2021). In contrast, the Turtle Dove has a strong declining population trend in Europe, potentially indicating that this species copes less well with the ongoing urbanisation and associated changes (but see Eddajjani et al. 2022).

- Obtained results point out distinct plasticity in foraging habits, in particular for 'urban' Woodpigeons (*chapter 3*). This is likely an adaptation to the seasonally changing productivity of exploited foraging sites. Moreover, out of the three columbiform species, Woodpigeons had the highest overall diversity in consumed plant families (*chapter 4*).

Many birds living in urban environments adapt their food resources and show a loss in migratory behaviour since there is enough food available in urban areas to support them through the winter (Méndez et al. 2020; Patankar et al. 2021). This could also be the case in Woodpigeons, as most individuals tagged in an urban area in Germany were residents and especially during winter rarely left the city (*chapter 4*). Contrary, Turtle Doves and Stock Doves, which typically do not exploit food resources in urban areas, are forced to migrate to find adequate and sufficient food during winter.

Migration plays a significant role in the ecology and evolution of host species and consequently their parasites (Ishtiaq & Renner 2020). Moreover, migrants are important agents for the distribution of parasites, particularly those lacking free-living stages, and thus disperse only with their hosts and vectors, such as avian haemosporidian parasites (Ricklefs et al. 2017). By analysing avian haemosporidian parasites in columbiform species with different migration strategies (*chapters 2*, *3* and *5*), the results presented in my thesis could:

- Contribute to the debate whether migratory avian species are more often infected by haemosporidian parasites (e.g. Soares et al. 2019; Ciloglu et al. 2020; de Angeli Dutra et al. 2021). Our results support the hypothesis that long-distance migrants harbour a higher diversity of haemosporidian parasites than residents or short-distance migrants. In terms of prevalence, this pattern was only evident for *Plasmodium* and *Haemoproteus* infections. Contrary, *Leucocytozoon* infections were most prevalent in partial migrants, namely Woodpigeons (*chapters 3* and *5*). The detected variation in patterns by parasite genus along with methodological issues raised in the publication emphasises that an accurate detection of avian haemosporidians is crucial for correctly investigating host-parasite interactions and true parasite diversity (Ciloglu et al. 2019).

- Assess the potential impact of blood parasites as a threat to declining Turtle Doves (Fisher et al. 2018). Due to the rather low observed parasitemia, we deem the contribution of haemosporidian infections to the decline of this species to be rather insignificant at the moment (*chapter 5*). However, expected future changes in parasite transmission areas, distribution and diversity associated with global change can pose an upcoming risk (Ishtiaq et al. 2021). My results provide reference information to monitor future changes.

Beyond the contribution to a better general understanding of different behavioural aspects of the included columbiform species, the studies assembled in this thesis provide some insight to draw prospective directive lines for conservation purposes for declining Turtle Doves and management of Woodpigeons:

- For Turtle Doves we demonstrated that prolonged stopovers during autumn migration in Europe overlap with the time of legal hunting activities (*chapter 2*). The sustainability of Turtle Dove hunting in Europe has been discussed during the last several years due to severe population declines (Thomaidis et al. 2022).

Turtle Dove individuals migrating along the Western flyway mainly pass European countries with temporary hunting bans (Fig. 2). However, hunting is still allowed in some countries along the Central and Eastern flyways. The sustainability of harvest and effectiveness of hunting regulations along the Central and Eastern flyway should be re-assessed, similarly as done along the Western flyway (Lormée et al. 2020; Delibes-Mateos et al. 2021; Moreno-Zarate et al. 2021), to develop sustainable hunting concepts.

- In Germany, Woodpigeons can usually be hunted during the winter month (November to February; JagdzeitV 1977). Our results demonstrated that Woodpigeons, which breed in urban areas, rarely leave the urban areas during these months (*chapter 3*). Therefore, shot Woodpigeons are likely mainly local individuals breeding outside the urban area or migrants from more northern European countries, which winter in Germany. Thus, hunting probably has relatively little impact on resident urban Woodpigeons. However, it must be noted that our study represents the situation of only one city in Germany. Woodpigeons in other cities might behave differently, e.g. depending on the type of habitats surrounding the city or food availability within the city.

- In line with Dunn et al. (2021) and Chiatante et al. (2021) we discovered that the home range size of Turtle Doves in breeding areas seems to depend on the availability of food resources connected to certain habitat types. In our case, a higher proportion of agricultural areas led to an increase in home range size (*chapter 2*). Also, the dietary shift from wild plant species to cultivated ones (*chapter 4*) was already observed in other breeding areas and has been proposed to negatively influence Turtle Doves (e.g. Browne & Aebischer 2004; Dunn et al. 2015, 2018). Both, increased home range size and dietary shift, reinforce the importance of the close proximity of suitable nesting sites and feeding areas with accessible seeds throughout the entire breeding season (Browne et al. 2004; Dias et al. 2013; Dunn et al. 2018). To ensure abundant and adequate food resources at the breeding areas, managed feeding areas sown with tailored seed mixtures according to regionally preferred wild plant species could prove to be a successful conservation measure.

- Finally, the obtained results add to the body of research, which highlights the variability of habitats, both breeding and foraging habitats, and food resources exploited by Turtle Doves across European breeding sites. This indicates that the Turtle Dove is not a specialist species, but rather a plastic one, able to adapt to a variety of environmental conditions, i.e. variable habitat compositions. Thus, the 'optimal' Turtle Dove habitat might look very different across regions. This must be considered when planning conservation measures, as it cannot be assumed that a particular measure will be equally effective and useful across different breeding regions, e.g. Western versus Eastern Europe. Therefore, conservation measures should be tailored to regional conditions.

4.2 Future outlook

Not all pertinent questions dealing with behavioural aspects of the included species of Columbiformes and, moreover, the resulting consequences of specific behaviours for populations dynamics, could be covered and answered comprehensively within the scope of my thesis. Some of the included analyses could be examined in more depth with a better data basis. For instance, the analyses of habitat requirements would benefit from an improved understanding on the relationships between specific landscape components and bird abundance (cf. Saâd et al. 2021). This could be achieved if more detailed information were available on landscape elements, such as the presence of small-scale elements like hedgerows or field margins, and on land management practices, e.g. the use of agrochemicals (cf. Marx & Quillfeldt 2018). A thorough identification of the landscape elements that are crucial in explaining abundance patterns can be helpful to promote specifically these elements through measures and thus enhance potential habitats. In the particular case of Turtle Doves, a closer look at habitat requirements in their wintering areas is needed, as their sensitivity to changes in their wintering quarters has already been suggested (Eraud et al. 2009). However, a significant knowledge gap on threats in the wintering areas remains (Fisher et al. 2018).

To be able to draw more direct causal links in future research, it should be striven to assess more concrete connections between different behavioural aspects (Fig. 5). For example, assessing if the availability of specific food resources influences the migratory decision in partial migrants. Another research question could be whether individuals weakened by a suboptimal diet are more likely to be affected negatively by pathogens.

During research for this thesis, I attempted to equip Stock Doves with transmitters, but tracking them was not successful due to different reasons, such as the breaking off of transmitter antennas or low recapture success. Future studies should take a step forward by expanding applied tracking methods, such as using transmitters with GPS accuracy on all species. Moreover, they should include individuals of all columbiform species, using the same location to ascertain habitat segregation or potential competition on particular resources, e.g. nesting sites or certain food resources (cf. Benghedier et al. 2020; Floigl et al. 2022; Squalli et al. 2022). It should be noted that the presented studies only cover small sub-areas of the complete distribution ranges of the respective species and that different behavioural patterns should be expected in other areas, especially since our studies have already determined regional differences. For example, there are hardly any studies from the Eastern part of the breeding distribution range (Eastern Europe and parts of Asia) of Turtle Doves.

To recapitulate, the outcomes of the studies incorporated in my thesis show how research into different behavioural aspects not only provides insights into the general biology of species and species communities but can also provide information relevant for appropriate management and conservation actions (cf. Hays et al. 2019; Saâd et al. 2021). However, to actually help declining species in practice, it will be particularly important to close the prevailing science-practice implementation gap in the future.

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CHAPTER 1

- 30 -

FEATHER STABLE ISOTOPES (δ^2H_f AND $\delta^{13}C_f$) IDENTIFY THE SUB-SAHARAN WINTERING GROUNDS OF TURTLE DOVES FROM EUROPE

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ORIGINAL ARTICLE

Feather stable isotopes (δ^2H_f and $\delta^{13}C_f$) identify the Sub-Saharan wintering grounds of turtle doves from Europe

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Abstract

Conservation of migratory birds requires knowledge of breeding and nonbreeding ranges and the connections between them. European turtle doves (*Streptopelia turtur*) are Palearctic-African long-distance migrants with wintering areas in the Sub-Saharan belt that are classed as vulnerable due to strong population declines. However, detailed non-breeding locations of individuals from different migratory flyways are unknown. To identify wintering regions of turtle doves, we measured stable isotopes of feathers grown on the wintering grounds and used a dual-isotope (hydrogen (δ^2H_f) and carbon ($\delta^{13}C_f$) probabilistic assignment to analyse origins of individuals migrating through the western and central/eastern flyways. The most probable wintering areas for turtle dove samples from both flyways were in the western and central Sub-Sahara. However, we found differences in δ^2H_f and $\delta^{13}C_f$ values between turtle doves following different migratory routes (western vs central/eastern flyway). This result suggests a higher likelihood of origins in the central Sub-Sahara for central and eastern migrants, while turtle doves using the western flyway originated primarily in the western Sub-Sahara, highlighting the importance of both regions for the future conservation of turtle doves from European breeding populations. The establishment of migratory connectivity of populations requires sampling from birds from the European as well as Asian continent; however, we provide important results that can be used to test hypotheses regarding population declines resulting from factors experienced over the full annual cycle for some populations.

Keywords *Streptopelia turtur* · Assignment to origin · Feather isoscape · Deuterium · Carbon-13 · Migratory connectivity

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Introduction

Migratory birds occupy geographically disparate regions during different parts of the annual cycle, and long-distance migrants typically spend the majority of their life away from their breeding sites (Kelly et al. 2005). Nonetheless, most studies have focused on factors affecting conditions during the breeding period, but research to understand how events outside the breeding season impact survival of migrants are still insufficient (e.g., Prochàzka et al. 2008; Morrison et al. 2013; Briedis et al. 2018; Tobolka et al. 2018). Therefore, research into the identification of areas used during the non-breeding period and the spatial linkages between breeding and non-breeding grounds, or 'migratory connectivity', is of crucial importance for the effective conservation of migratory birds (Prochàzka et al. 2008).

European turtle doves (*Streptopelia turtur*, subspecies *S. t. turtur*, henceforth, turtle doves) are the only long-distance migrating species of European breeding columbids and winter in the African Sahel between 10 and 20° N (Glutz von Blotzheim 1980; Geroudet 1983; Cramp 1985). Turtle doves have undergone a rapid and steep decline of ~ 80% across the European breeding range since 1980, and the species is currently listed as 'vulnerable' on the IUCN's red list (Fisher et al. 2018; PECBMS 2021). Agricultural intensification in Europe, which includes the removal of hedges and extensive use of agro-chemicals, may lead to increased predation risk, a shortened breeding season, and decreased productivity (i.e., lower number of breeding attempts and fledged young per season), is suspected to be the main driver of the turtle dove decline (Browne and Aebischer 2004; Browne et al. 2005). Environmental conditions experienced on migration and on the non-breeding grounds can also affect the individual physical condition and population dynamics of migratory avian species (e.g., sedge warbler *Acrocephalus schoenobaenus*, Peach et al. 1990; white stork *Ciconia ciconia*, Kanyamibwa et al. 1990; Schaub et al. 2005; Baillie and Peach 1992; Robson and Barriocanal 2011; European sand martin *Riparia riparia*, Szép 1995; turtle dove, Eraud et al. 2009). Unsustainable legal and illegal hunting activities along the migration routes are further contributing to the turtle dove decline (Fisher et al. 2018; Lormée et al. 2020; Moreno-Zarate et al. 2021), with eight countries in the EU-27 reporting hunting bags totaling over 1.4 million birds (Hirschfeld et al. 2019). Furthermore, turtle doves face multiple threats on the Sahelian non-breeding grounds (henceforth winter grounds), including reduced survival resulting from food shortages, scarcity of roosting sites and freshwater, as well as an increased human disturbance (e.g., agricultural intensification, hunting; Zwarts et al. 2009). Therefore, identifying the main wintering areas of turtle dove populations is important in order to develop appropriate management and conservation strategies over the full annual cycle (Hobson et al. 2009a; Eraud et al. 2013; Hanane 2017).

Turtle doves use three main migratory flyways (western, central, and eastern) to move between breeding and wintering grounds with regular mixing between the central and eastern routes but no or limited mixing between western and other routes (Marx et al. 2016). Mirroring the three migratory flyways, previous studies suggested three different wintering regions in the western, central, and eastern Sub-Sahara, which may indicate the presence of strong migratory connectivity in turtle dove populations (Glutz von Blotzheim 1980; Zwarts et al. 2009). The strength of migratory connectivity has important consequences for the ecology and conservation of migratory species (see Webster et al. 2002) but it is not well described for turtle doves. Turtle dove populations using the western flyway mainly winter in western Africa, namely in Senegal, Gambia, Guinea Bissau, Conakry Guinea, Burkina Faso, and Mali (Morel 1987; Morel and Morel 1988; Carvalho and Dias 2001, 2003 according to Fisher et al. 2018; Aebischer 2002; Eraud et al. 2013; OFB 2021). Populations following the central route may winter in Sudan and Ethiopia, possibly reaching as far west as Mali, Burkina Faso, Ghana, and Nigeria (Zwarts et al. 2009; Schumm et al. 2021). Migratory movements of populations breeding in the eastern part of the European range remain poorly documented (Bankovics 2001; Fisher et al. 2018). Generally, suitable wintering habitats appear to be featured by abundant food and water availability as well as by large trees or patches of woodland as roosting sites (Zwarts et al. 2009). In addition, studies indicate that turtle doves move among several habitats during winter and move up to hundreds of kilometers between sites (Morel 1986; Eraud et al. 2013; Lormée et al. 2016; OFB 2021).

Currently, the efficacy of extrinsic markers, such as geolocators or satellite tags, to define main wintering areas of turtle dove populations is hampered by the small sample size of tracked individuals (Eraud et al. 2013: five geolocators; Lormée et al. 2016: three satellite tags; Schumm et al. 2021: 16 satellite tags; OFB 2021: 26 satellite tags; RSPB 2021: seven satellite tags). Turtle doves have been equipped with satellite transmitters and geolocators at their breeding grounds or during migration, but only a fraction of these tracked individuals reach their wintering quarters (e.g., five out of 16, Schumm et al. 2021), and few geolocators were retrieved back containing full data (five out of 64, Eraud et al. 2013). In addition, low recovery rates of ringed birds from the winter quarters (Zwarts et al. 2009; Marx et al. 2016) and difficulty in distinguishing between the subspecies *S. t. turtur* and *S. t. arenicola* on potentially shared wintering ranges complicate determining a complete picture of the wintering destinations (Hanane 2017). However, information from previous research, ringing, and tracking studies

are useful to help define plausible wintering areas for other methods, including probabilistic assignment to origin using stable isotopes (Hobson et al. 2012).

In this study, we used intrinsic isotopic markers in metabolically inert feathers grown during the wintering period to identify African non-breeding origins of turtle doves sampled during migration and breeding periods in Europe. Feathers indirectly provide environmental isotopic information from the African wintering habitats where they were grown. Within a predetermined wintering range, feather isotope information can be used to assign turtle doves from different breeding or flyway origin to wintering areas (Hobson et al. 2009a). For this purpose, we assigned stable hydrogen (δ^2H_f) and carbon ($\delta^{13}C_f$) isotope values of turtle dove feathers to multi-isotopic landscapes ("isoscapes") of precipitation stable hydrogen (δ^2H_p), and theoretical vegetation stable carbon ($\delta^{13}C$) in Africa (Bowen et al. 2005; Still and Powell 2010; Hobson et al. 2012). With this approach, we provide (I) a first approximation of the wintering regions used by turtle dove populations from different countries of origin in Europe, (II) an estimation for differences in δ^2H_f, and $\delta^{13}C_f$ among the western and the central/eastern flyway, and (III) an assessment of the current overlap between turtle dove winter quarters and existing protected areas.

Material and methods

Moult cycle of turtle doves

Generally, adult turtle doves start moult in July while on the breeding grounds (Demongin 2016). They renew the first to occasionally fourth primary feathers (i.e., P1–P4), then suspend moult until their arrival on the wintering areas around September, where they renew their remaining primary feathers (P5–P10). Hence, the outer primary feathers are grown on the wintering grounds (Morel 1986; Demongin 2016).

Feather sampling

Feather samples of adult turtle doves from the subspecies *S. t. turtur* were collected from migrating individuals, rescued birds from wildlife recovery centers, or breeding birds. Individuals were either hunted, rescued, trapped (e.g., whoosh or mist nets, cage traps), or they originated from museum (Natural History Museum Berlin) collections (Table 1). Adding samples from museum collections assumes continuity in precipitation isotope patterns through time: Feather δ^2H values of samples originating from museum did not vary significantly relative to values of the remaining sample of respective countries, Germany, Italy, and Spain (*t*-test: $t = 2.39$, df $= 5.46$, $P = 0.058$). In order to increase the general sample size, and as the aim of the analysis was to provide a good

first-order estimation of wintering regions vs information on a small geographic scale, we deem the inclusion of samples originating from museums valuable. Moreover, our inclusion of an appropriate error term in isotopic assignments (see below) renders our inferences conservative. Samples were collected during spring and autumn migration as well as during the breeding season in June and July (Marx et al. 2016). A small sample of approximately 1 cm^2 was cut from the vane of the tip of the tenth primary feather (P10), i.e., outermost primary, for stable isotope analysis. In total, we collected 181 adult feather samples from seven European countries, 121 from the western flyway, 55 from the central/eastern flyway, and five samples without a reliable classification to one of the flyways (Table 1). The central and eastern European flyways were combined, as ringing data showed that several individuals crossed between those two flyways (Marx et al. 2016).

Stable isotope analyses

Stable isotope analyses were conducted at the Stable Isotope Laboratory of the Leibniz Institute for Zoo and Wildlife Research (IZW). Feather samples were washed with a 2:1 chloroform/methanol solution for 24 h and then dried for 48 h under a fume hood. A feather subsample of 0.55 ± 0.05 mg was placed into a tin capsule for carbon (C) stable isotope analyses. The tin capsules were combusted in an elemental analyzer (Flash EA 1112 Series, Thermo Fisher Scientific, Bremen, Germany) and measured with a Delta V-Advantage mass spectrometer (Thermo Fisher Scientific) connected continuous-flow mode.

Stable isotope values are given in the delta notation (δ) as parts per thousand (‰) deviation from the ratio of international standards (for C: VPDB). Secondary laboratory standards of known $^{13}C/^{12}C$ values of tyrosine (-24.0‰) and leucine (-30.3‰) were used for calibration and drift correction. The within-run accuracy of $\delta^{13}C$ measurements was always better than 0.2‰ for laboratory standards. For stable hydrogen (H) analyses, 0.27 mg ± 0.1 mg were loaded into silver capsules (IVA Analysetechnik e.K., Meerbusch, Germany). Loaded samples in folded capsules were stored in 96-well microtiter plates loosely covered with the lid in order to allow exchange with ambient air moisture. Then, trays were placed in a compartment drier at 50 °C for at least 24 h to speed up equilibration and remove extra moisture. Afterwards, samples and standards were loaded into the carousel of a Zero Blank autosampler (Costech Analytical Technologies Inc., Italy) and flushed with dry helium for a minimum of 1 h. Samples were then dropped one by one into the elemental analyzer (EA; HT Elementanalysator HEKAtech GmbH, Wegberg, Germany), which operated at 1350 °C and contained a silicon carbide (Sic) tube filled halfway with glassy carbon chips and including a carbon/

Table 1 Mean tenth primary feather (P10) isotope values (δ^2H_f and $\delta^{13}C_f$ in ‰) of European turtle doves sampled in seven countries across Europe at different periods

Country	Flyway	Sample location	Sample type	Collection period (year)	N	Mean δ^2H_f	Mean $\delta^{13}C_f$
Bulgaria		Kavarna	Hunt	AM (2014)	5	−83.89	−19.16
Bulgaria total	Central/East				5		
France		Auvergne	Live capture	BS (2014)	19	−66.37	−20.84
France		Chize	Live capture	BS (2014)	20	−68.33	−20.93
France		Marne	Live capture	BS (2014)	20	−65.13	−19.95
France		Oleron	Live capture	BS (2014)	20	−60.24	−20.27
France total	West				79		
Germany		Berbisdorf	Museum	BS (1934)	1	−78.95	−22.73
Germany		Dahme	Museum	BS (1981)	2	−59.35	−20.66
Germany		Villingen	Live capture	BS (2014)	1	−62.09	−10.90
Germany		Ottenheim	Museum	BS (1890)	1	−21.69	−10.43
Germany total	Unknown				5		
Greece		Soufli	Hunt	BS/AM (2013)	20	−59.80	−19.51
Greece total	Central/East				20		
Italy		Rieti	Wildlife recovery center	BS (2014)	1	−39.96	−12.07
Italy		Sardegna	Museum	SM/BS (1908)	1	−32.64	−8.53
Italy		Ventotene island	Live capture	SM (2014)	16	−60.69	−18.41
Italy total	Central/East				18		
Malta		Comino	Live capture	SM (2014)	2	−65.73	−14.49
Malta		undefined	Hunt	SM/AM (2014)	10	−55.72	−17.34
Malta total	Central/East				12		
Spain		Ibiza	Museum	SM (1930)	1	−47.99	−11.86
Spain		Monfrague	Hunt	AM (2013)	41	−75.79	−21.94
Spain total	West				42		
Overall total					181		

BS breeding season, *SM* spring migration, *AM* autumn migration

water trap. The obtained H_2 sample pulse was then introduced into the stable isotope ratio mass spectrometer (Delta V advantage, ThermoFisher Scientific, Bremen, Germany) via an interface (Finnigan Conflo III, ThermoFisher Scientific, Bremen, Germany). Samples were analysed together with three in-house keratin standards, which had previously been calibrated to the USGS42 standard (Soto et al. 2017). Measured stable isotope values are reported in the delta notation (δ) as parts per thousand (‰) deviation from V-SMOW. The precision of δ^2H measurements was always better than 2‰. We calculated the stable isotope value of the non-exchangeable portion of the hydrogen in samples based on laboratory keratin standards with known stable isotope values of the non-exchangeable portion of hydrogen: sheep wool from Sweden SWE-SHE (−111.6‰), sheep wool from Spain ESP-SHE (−61.5‰), and goat wool from Tanzania AFR-GOA (−26.4‰), standards that were calibrated directly to KHS and CBS laboratory keratin standards (−54.1 and −197.0‰, respectively).

We chose not to include nitrogen isotopic values ($^{15}N/^{14}N$) in our analyses. While $\delta^{15}N$ has been regularly included in studies dealing with main diet and trophic relationships of marine or aquatic birds, as $\delta^{15}N$ patterns there are relatively well understood and/or constant over large geographic areas (Hobson 2011), the use of $\delta^{15}N$ measurements in tracing origins of animals, particularly terrestrial animals, is relatively rare (Hobson and Wassenaar 2019). This is because $\delta^{15}N$ values in plant and animal tissues can vary even locally as values are heavily influenced by anthropogenic sources of nitrogen, in particular by agricultural inputs, including fertilizers, sewage, and agricultural animal waste, and by atmospheric deposition via fossil fuel burning (Cruz et al. 2012; Hobson et al. 2012; McMahon et al. 2013; Hobson and Wassenaar 2019).

Assignment to wintering areas

To delineate likely African winter areas of turtle doves, we applied a spatially-explicit multi-isotope likelihood assignment method (Royle and Rubenstein 2004; Wunder 2007; Hobson et al. 2009b). To accomplish this, and following Hobson et al. (2012), we used African isoscapes reflecting

the (1) amount-weighted growing seasonal surface precipitation (δ^2H_p) of Bowen et al. (2005) and (2) a theoretical spatial distribution of plant $\delta^{13}C$ (Still and Powell 2010). We converted the δ^2H_p isoscape into a feather δ^2H (δ^2H_f) isoscape using the calibration equation for common wood pigeons (*Columba palumbus*) from Hobson et al. (2009a; $4.73 + 0.78* \delta^2H_p$) because we lacked a similar one for turtle doves. However, both species have relatively similar diets (Dunn et al. 2018) and are migratory therefore, we deemed use of this equation reasonable. To account for plant-feather $\delta^{13}C$ isotope discrimination, we first conducted separate assignments using discrimination factors of $+1$ and $+2$ ‰ added to the plant $\delta^{13}C$ isoscape (see Hobson et al. 2012) but found no difference in the spatial distribution between the resulting depictions. Because of this lack of difference and since turtle doves are entirely granivorous, whereas wood pigeons also take leaves and other plant matter (Dunn et al. 2018), we used a discrimination factor of $+1.5$ ‰ to derive a feather $\delta^{13}C$ isoscapes ($\delta^{13}C_f$). We then used digital range maps from BirdLife International and NatureServe (2011), which were extended southwards to about 4° N based on winter range information from tracking data, to restrict the assignments to the known turtle dove winter range by 'clipping' the calibrated feather (δ^2H_f, $\delta^{13}C_f$) isoscapes.

To assess the likelihood that a georeferenced location (i.e., raster cell) within the feather isoscape of the turtle dove winter range was a potential area of origin, we used a multivariate normal probability density function (mvnpdf):

$$fx\left(x^i y^i | \mu_{HC}, \sigma_{HC}, \rho_{HC}\right) = 2\pi^{-1/2k} | \sum |^{-1/2k} e^{[-1/2(y-\mu_x i/\sum^{-1}(y-\mu_x i)]}$$

where fx represents the spatially explicit probability density function for x^i indicating the geographic location of origin given a feather of unknown provenance (y^i) with isotopic composition (δ^2H_f, $\delta^{13}C_f$). Subscripts HC indicate the expected mean (μ), standard deviation (σ), and correlation (p) of δ^2H_f and $\delta^{13}C_f$, respectively, for a feather grown at that location and k represents the number of isotopes. The estimated mean isotopic composition was estimated from raster cells in the calibrated isoscapes for δ^2H_f and $\delta^{13}C_f$ at each location (x^i). Thus, the parameter μ_x^i represents a vector of means for each location (x^i) in the isoscape:

$$\mu = [\mu\delta^2H_f, \cdot\mu\delta^{13}C_f].$$

The variance–covariance matrix ($|\sum|$) of the two-isotope matrix is represented as.

$$\sum = \begin{bmatrix} \sigma^2_{\delta^2H} & \sigma^2_{\delta^2H,\delta^{13}C} \\ \sigma^2_{\delta^2H,\delta^{13}C} & \sigma^2_{\delta^{13}C} \end{bmatrix};$$

where diagonal elements represent expected variance for the given isotope and off-diagonal elements represent covariance between pairs of isotopes. We assumed that covariance among isoscapes was constant (Royle and Rubenstein 2004).

All cells in the upper 67% (i.e., 2:1 odds ratio) of the resulting probability surfaces from assignments for each individual were defined as likely (1) origins, and all others were considered unlikely (0). Thus, assignments conducted for feather samples resulted in a spatially explicit binary surface for each individual, which was summed (i.e., 'stacked') across assignments for all individuals in the sample to represent potential origins for the entire sample set but also per sampled country. Manipulation of digital files and assignment to origin analyses were conducted using several packages including 'raster' 2.5–8 (Hijmans 2016), 'mvnmle' 0.1–11.1 (Gross and Bates 2018), 'maptools' 0.9–9 (Bivand and Lewin-Koh 2019), and 'rgdal' 1.2–13 (Bivand et al. 2019) in the R statistical computing environment 3.5.3 (R Core Team 2019). As stable isotope values of δ^2H_f were normally distributed (Shapiro–Wilk: $W = 0.991$, $p = 0.326$) but $\delta^{13}C_f$ were not (Shapiro–Wilk: $\delta^{13}C_f$: $W = 0.861$, $P < 0.005$), we applied parametric and non-parametric statistics where appropriate.

To assess the overlap between existing protected areas as well as hunting areas and winter quarters of turtle doves according to the dual-isotope multivariate probabilistic assignment, we clipped the obtained assignment with a spatial dataset of protected areas (mix of polygons and points) from UNEP-WCMC (2021). The dataset was limited to 'internationally designated sites' (including UNESCO-MAB Biosphere Reserves and Ramsar Sites), 'nationally designated sites' (national parks, nature reserves, faunal reserves, wildlife sanctuaries, and reserves, and forest reserves), and 'bird sanctuaries'. The 'hunting areas' include the UNEP-WCMC categories hunting area, hunting zone, controlled hunting area, and hunting reserve.

Results

Wintering areas of sampled turtle doves

From the probabilistic assignment to origin analyses, wintering areas for pooled turtle dove samples were likely in the western and central Sub-Sahara. The most likely wintering sites in the western Sub-Sahara were in western Africa: Senegal, Gambia, Guinea-Bissau, Guinea, Sierra Leone, northern Ivory Coast, western Burkina Faso, south-western Mali, and in the central Sub-Sahara Togo, Benin, Nigeria, and North-Cameroon. No turtle doves were assigned to wintering areas in the eastern part of the Sub-Saharan Sahel region (Fig. 1). Similar to the results for pooled turtle dove samples, the assignments by flyway (Fig. 2) as well as by country (Online Resource 1) highlighted similar wintering areas as indicated above.

Fig. 1 Assignment to likely wintering origin (moulting areas of winter-grown primary feathers) of European turtle doves ($n = 181$) sampled in seven different European countries (labeled and shaded grey) predicted from a multivariate normal probability distribution function based on tenth primary feather (P10) δ^2H and δ^{13}C isotope assignments of individual birds. Assignment probabilities of individuals (0 to 1) were summed according to the maximum value obtained in a pixel during the assignment process for the overall sample set representing the percent of individuals potentially originating from a cell in the isoscape. The assignment is restricted to a hitherto described turtle dove wintering range (outline; in red in the online version). (**a**) Overlap of likely wintering areas with internationally and nationally protected areas as well as bird sanctuaries, and (**b**) overlap with hunting areas. Information on protected and hunting areas was based on a dataset (mix of polygons and points) from UNEP-WCMC (2021)
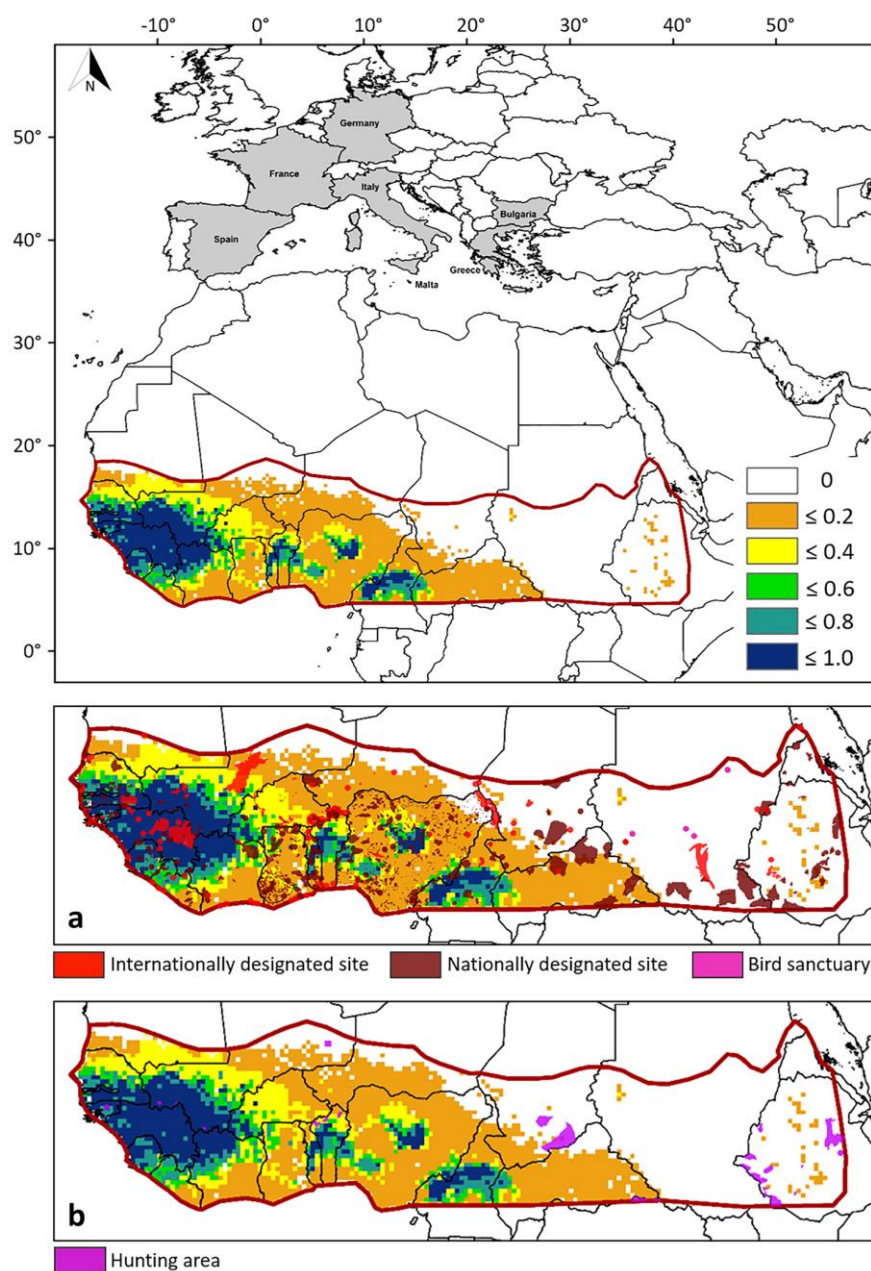
Differences in δ^2H$_f$ and δ^{13}C$_f$ values between flyways

Whereas the assignment depictions (Fig. 2) do not indicate a marked difference between the wintering grounds of turtle doves following the western (samples from Spain and France) vs the central/eastern flyway (samples from Greece, Malta, Italy, and Bulgaria), comparisons of the raw δ^2H$_f$ (t-test: $t = 2.77$, df $= 77.94$, $P = 0.007$), and δ^{13}C$_f$ values (Mann–Whitney: $W = 4337$, $P = 0.001$) show differences between flyways. Feathers of turtle doves following the central/eastern migratory flyway compared to those of individuals using the western flyway had significantly higher δ^2H$_f$ (means $\pm$ SD: -60.87 ± 2.51‰ vs -68.52 ± 1.16‰, respectively) and δ^{13}C$_f$ (means: -18.25 ± 0.67‰ vs -20.91 ± 0.38‰) values (Fig. 3).

Fig. 2 Assignments to likely wintering origin (moulting areas of winter-grown primary feathers) of European turtle doves following (**a**) the western flyway (*n* = 121) sampled in two different European countries (grey shaded) and (**b**) the central/eastern flyway (*n* = 55) sampled in four different countries (grey shaded) predicted from a multivariate normal probability distribution function based on tenth primary feather (P10) δ^2H and δ^{13}C isotope assignments of individual birds. Assignment probabilities of individuals (0 to 1) were summed according to the maximum value obtained in a pixel during the assignment process for (**a**) individuals following the western flyway and (**b**) turtle doves following the central-eastern flyway representing the percent of individuals potentially originating from a cell in the isoscape. The assignments are restricted to a hitherto described turtle dove wintering range (outline; in red in the online version)
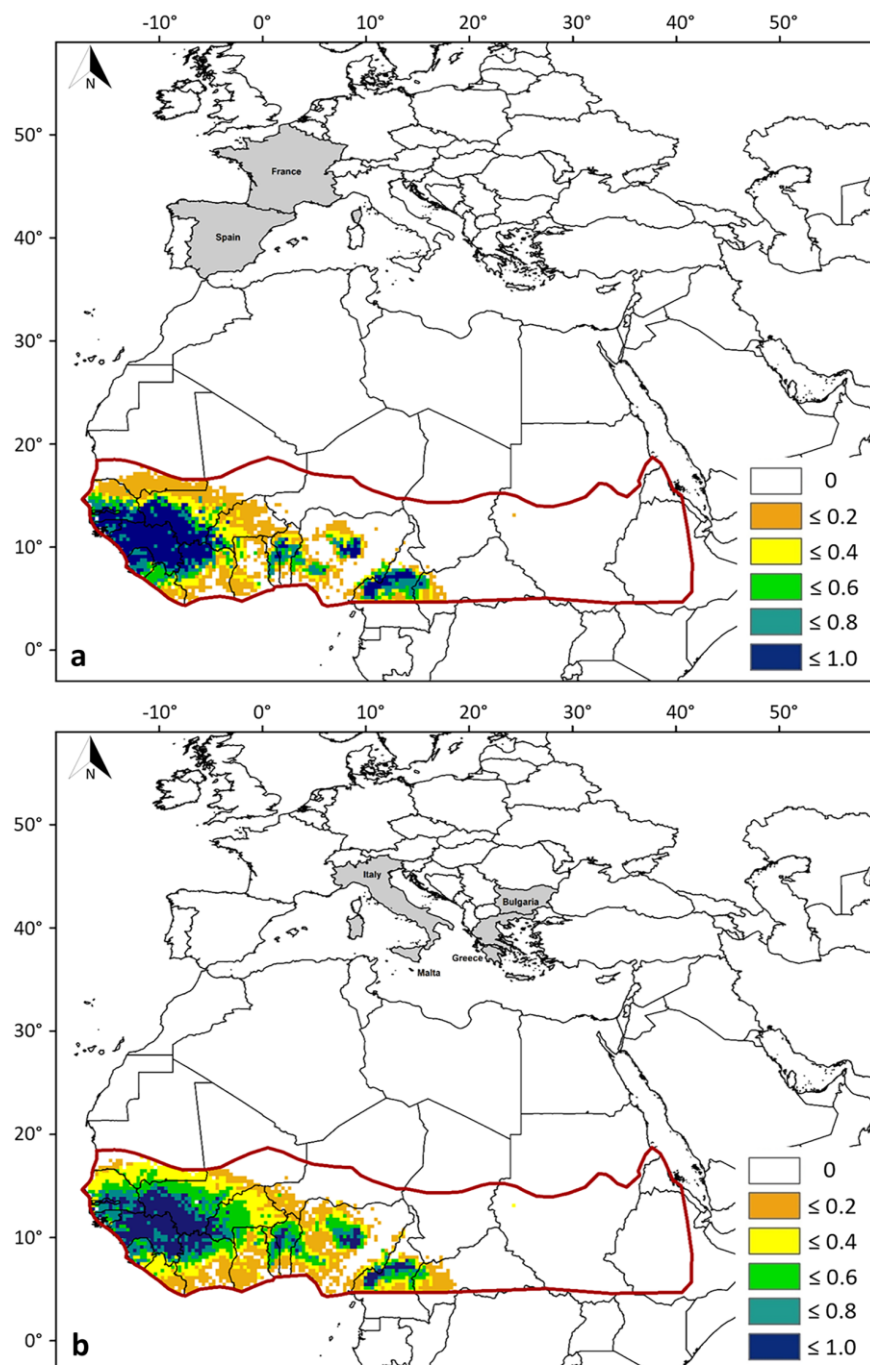
Overlap of assigned wintering grounds with protected areas

International biosphere reserves and Ramsar sites overlapping with the most likely wintering grounds of turtle doves (based on the probabilistic assignment) were mainly located in Guinea and Senegal and to a smaller extent in Mali and Sierra Leone. No internationally protected areas were situated in Benin, Ivory Coast, Cameroon, and Nigeria in areas of a high probability origin (Fig. 1a). Most of the larger national protected areas and bird sanctuaries were located in the central and eastern part of our preselected area,
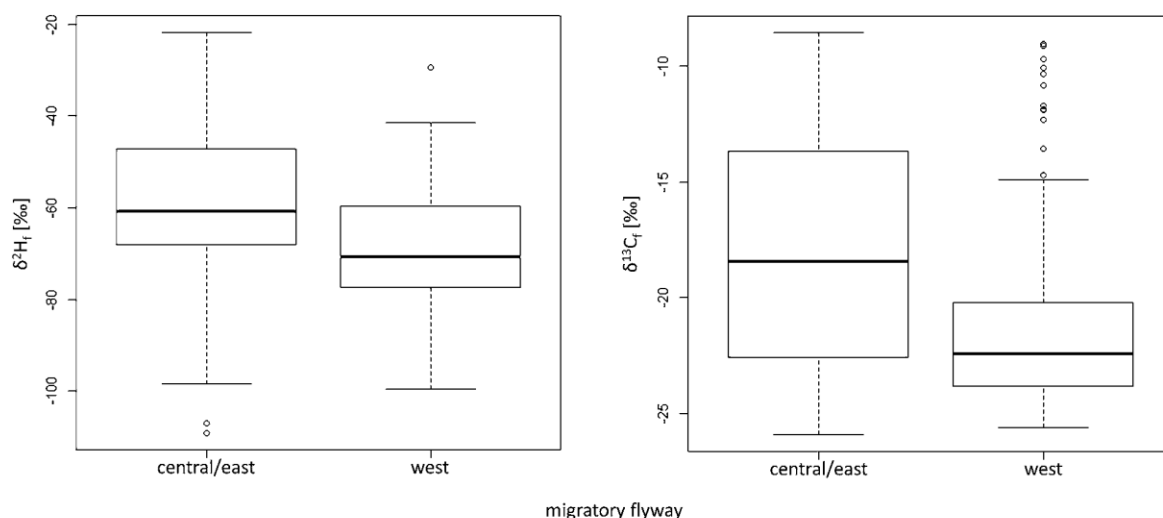
Fig. 3 Boxplots for $\delta^2 H_f$ and $\delta^{13} C_f$ values from the tenth primary (P10) feather samples of turtle doves moulted during the overwintering period at African winter grounds grouped by central/eastern ($n = 55$) and western ($n = 121$) flyways. The boxes represent the range in which 50% of the data occur (inter-quartile distance from 25% quartile to 75% quartile). Whiskers show extreme values, and the median is shown as a black line within the boxes. Circles highlight outliers and extend 1.5 times beyond the inter-quartile distance

where wintering, according to our results, is rather unlikely. Smaller national protected areas overlapped with the most likely wintering locations in many countries (e.g., Senegal, Mali, and Niger) but were also scarce or absent in others, such as Benin, Cameroon, and Ivory Coast (Fig. 1a). Some hunting areas were located in areas of the high probability of European turtle dove origins, which are among the most likely wintering grounds, in Guinea-Bissau and Mali (Fig. 1b). However, only a few hunting areas were included in the UNEP-WCMC dataset.

Discussion

The main objective of our investigation was to determine general and flyway-specific wintering regions of turtle doves sampled on European breeding areas and migration following the western and central/eastern migratory routes. Previous assessments of wintering regions and connectivity were based on a relatively small number of reported sightings, which may include discrimination errors of subspecies (*S. t. turtur* vs *S. t. arenicola*), or recaptures of ringed birds and tracked individuals. Yet, the decline of European populations of turtle doves over the past decades calls for a more thorough understanding of migratory connectivity between summer and wintering ranges (Fisher et al. 2018). Based on our dual-isotope geographic assignment, we identified regions in the western and central sub-Saharan belt to be

the most likely wintering grounds of turtle doves originating from Europe.

Stable isotopes

Feather hydrogen ($\delta^2 H_f$)

While assignments to the wintering origin based on deuterium values only did not show a clear difference for the different flyways (Online Resource 2), turtle doves using the western migration flyway and those using the central/eastern migration flyway differed in the raw $\delta^2 H_f$ values of the tenth primary feather (P10) grown on the wintering range. This potentially indicates that turtle dove populations from western vs central/eastern Europe may spend the boreal winter in different areas, for example, lower $\delta^2 H_f$ values in European hoopoes sampled along the central/eastern flyway suggest more easterly wintering areas for these individuals (Reichlin et al. 2013). However, the geographic precision of this assignment was limited due to large confidence intervals and a shallow slope, and the difference in deuterium values in body feathers for the longitudinal $-10°$ to $+15°$ range was relatively minor (Reichlin et al. 2013).

Feather carbon ($\delta^{13} C_f$)

Similar to differences in $\delta^2 H_f$, our results demonstrate higher raw $\delta^{13} C_f$ values in turtle doves using the central/eastern flyway vs western flyway. The relative abundance of stable

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carbon isotopes depends on the relative contribution of plant biomass with contrasting photosynthetic pathways, such as Calvin cycle (C3), Hatch-Slack cycle (C4) or Crassulacean acid metabolism (CAM) (O'Leary 1981; Rubenstein and Hobson 2004), whereby C3 plants, mainly trees, and shrubs, are typically isotopically depleted in ^{13}C in relation to ^{12}C compared to C4 plants, which are mostly grasses (Marshall et al. 2007). While $\delta^{13}C_f$ values may provide minor additional information concerning E–W delineations of individuals, they can indicate possible habitat segregation. For instance, regardless of the photosynthetic pathway, more xeric environments typically have higher $\delta^{13}C$ values compared to mesic regions (Reichlin et al. 2013). Therefore, higher $\delta^{13}C_f$ values in turtle doves using the central/eastern migration route suggest that they occupied more xeric regions than birds using the western flyway. However, differences in $\delta^{13}C_f$ values may also reflect diet and microhabitat (Veen et al. 2014), and differences in the diet of individuals following the different migration routes might indicate different areas used for wintering with different plant species (C3 vs C4) consumed by doves. Much of the variation in $\delta^{13}C$ values of animal tissues can be ascribed to the differential use of C4/CAM and C3 plant-based food (Lajtha and Michener 2007; Procházka et al. 2010), but there are also local habitat-specific $\delta^{13}C$ signatures on small latitudinal gradients as a result of the water-use efficiency of the dominant C3 plant species (see Marra et al. 1998; Paxton and Moore 2015). Higher $\delta^{13}C_f$ values in birds following the central/eastern vs the western migration route may be an indicator for winter occupancy of lower-quality habitats (Marra et al. 1998) and/or differences in the proportion of consumed food plants. While some studies analysed the diet of turtle doves on their European breeding grounds (e.g., Murton et al. 1964, 1965; Browne and Aebischer 2003; Dunn et al. 2018), very few have addressed the diet composition during the wintering period. Observations on some wintering localities indicate that rice (*Oryza*, C3 plant) and wild fonio (*Panicum,* C4 plant) may be the main food sources for wintering turtle doves (e.g., Morel 1986; Curry and Sayer 1979; Zwarts et al. 2009). Detailed studies are needed to gain a more precise picture of the dietary composition on the wintering grounds.

Differences in δ²Hf and δ¹³Cf values

Derived from the probabilistic assignments based on both δ^2H and $\delta^{13}C$ values (Fig. 2; based on deuterium values only, Online Resource 2), no obvious difference in the derived probability of origin during winter of individuals sampled along the western or the central/eastern flyway was found. Therefore, based solely on the assignments, we cannot conclude that the Sub-Saharan wintering range of turtle doves varies with their flyway and cannot differentiate geographic

wintering ranges by flyway. Such assignments are, however, dependent on the structure of the underlying isoscape and further insight is still possible by examining each isotope individually. When comparing raw values of δ^2H_f and $\delta^{13}C_f$, both isotopes suggest that individuals from different migratory flyways spent the winter in different Sahelian regions, had different diets, or used different habitats. Several studies already identified populations of bird species that differ in their wintering regions or habitats on the basis of isotopic analysis of feathers and used these results to estimate migratory connectivity (e.g., aquatic warbler *Acrocephalus paludicola*, Pain et al. 2004; reed warbler *Acrocephalus scirpaceus*, Procházka et al. 2008; sand martin *Riparia riparia* and barn swallow *Hirundo rustica*, Szép et al. 2009; blue swallow *Hirundo atrocaerulea*, Wakelin et al. 2011).

Migratory connectivity links breeding and non-breeding grounds of a species and ranges in strength from weak to strong (Webster et al. 2002). Strong connectivity occurs when most individuals from one breeding population migrate to the same wintering location or region, whereas weak connectivity occurs when individuals from a single breeding population move to several different regions to winter or from several breeding areas to a single non-breeding area (Webster et al. 2002; Rubenstein and Hobson 2004). Zwarts et al. (2009) investigated migratory connectivity of bird species based on recoveries and recaptures in Africa between 4 and 35° N. From their analysis, the west–east distribution in Africa of birds breeding in different longitudinal zones in Europe showed hardly any overlap for turtle doves, indicating strong migratory connectivity to broad regions (Zwarts et al. 2009).

Consistent with this result, isotope values of turtle doves in this study suggest a relationship between migration route (west vs central/eastern flyway) and African winter moulting grounds (western part of the Sahel vs central part of the Sahel, respectively), but this was not supported by the assignment to origin analyses, which could be interpreted as being contradictory. Although differences in feather stable isotope values between flyways were significant, they are small relative to the variation in the associated feather isoscapes. Despite the heterogeneity of sample origin regarding collection period and sample location (see Table 1), based on the moult cycle, all sampled tenth primary feathers had been grown during the previous winter and, therefore, reliably contained the isotopic signature of wintering grounds. However, sample sizes for western ($n = 121$) and central/eastern flyway ($n = 55$) were not equally distributed, which could have caused a bias towards the western part of the Sahara. In general, there has been a strong bias of studies towards turtle doves using the western flyway, whereas detailed knowledge of turtle doves migrating via the central/eastern flyway as well as a stopover and wintering sites of central and eastern turtle dove populations remains limited

(Bankovics 2001; Fisher et al. 2018). Therefore, our findings for individuals using the central/eastern flyway, even if they originate from a comparably small sample size, add valuable new information.

The use of stable isotopes and museum samples present several challenges for assessing turtle dove migratory connectivity. Our analysis could potentially have been more precise if individuals using the central and eastern flyways were examined separately. However, as tracking and ringing data indicate a loop-migration pattern between the central and eastern flyway (Marx et al. 2016; Schumm et al. 2021), separate analyses potentially could result in incorrect assignment to a flyway for some individuals. Also, for individuals with breeding sites in western Europe, such as France, it was described that individuals migrate more easterly during spring than during autumn (Eraud et al. 2013). To our knowledge, however, no switch of flyways, i.e., from western to central flyway, has been observed so far. The inclusion of museum specimens (1890 to 2014; Table 1) could also potentially bias our results due to changes in distributional patterns in breeding and wintering areas and hotspots of natal origin because declines are expected to cause range retractions towards available and optimal habitats (Thomas et al. 2008; Sirami et al. 2009; Burgess et al. 2020). Contraction towards high-quality habitats may be reflected by varying levels of observed decline across the European breeding area, e.g., a strong population decline of 97% (1967 to 2015) in the UK or > 90% (1984 to 2015) in the Netherlands, compared to less pronounced declines of 37% (1996 to 2018) in Spain or of 54% (1998 to 2015) in Austria (see De Vries et al. 2021). Furthermore, habitat modifications through time resulting from agricultural expansion may result in changes to the $\delta^{13}C$ isoscape or diet potentially influencing the isotope values between modern and historic samples (Lemenih et al. 2005; English et al. 2018; Arias-Ortiz et al. 2021). However, feather isotope values of samples from museums did not vary significantly relative to region-specific samples. Finally, our aim was to provide a first-order estimation of wintering regions and not an assignment on a small geographic scale or analysing changes over time, so we deem the use of museums samples reasonable.

Another study that investigated breeding ground provenance of migrating turtle doves, including some of the sampled individuals from this study, showed relatively coarse and broad possible distribution ranges using δ^2H_f only, and no spatially precise breeding localities could be assigned (Marx et al. 2020). Therefore, a precise estimation of the connectivity between breeding and wintering areas was not possible in this study, as we do not know the exact breeding areas of individuals sampled during migration. Nevertheless, the use of stable isotopes can provide insight into the migratory connectivity of species, especially when involving the use of informed priors in a Bayesian framework (see Hobson

and Wassenaar 2019). Combining probabilistic information based on other methods, such as analysis of ringing or tracking data, can indeed inform evidence-based, long-term, and effective conservation measures for threatened species. Such a combined approach is urgently needed to confirm the possible strong migratory connectivity for turtle doves, as turtle doves originating from different breeding grounds may be subject to several different or variable levels of similar threats across the full annual cycle. For example, a breeding population from one country can experience little or no decline while another is decreasing drastically due to factors experienced at different stopover or wintering sites (e.g., habitat loss, unsustainable harvesting; Weber et al. 1999; Runge et al. 2014).

Conclusions and perspectives

Our results highlight the potential importance of the western and central Sub-Sahara as a wintering region for turtle doves migrating through western and central Europe and thus partly support previously described wintering ranges (see Glutz von Blotzheim 1980; Carvalho and Dias 2001, 2003; Aebischer 2002). However, this and previous studies focused on the analyses of flyways within the European-African migration system (Zwarts et al. 2009; Marx et al. 2016), and little information is available from the eastern Sahel (Zwarts et al. 2009). While our results did not assign any turtle doves to the eastern part of the Sahel, some sightings of turtle doves (e.g., Sudan: Hartmann 1863; Nikolaus 1987) suggest that this region also hosts wintering individuals. Hence, it is possible that the eastern sub-Saharan region is important for turtle dove populations breeding in Asia (e.g., Russia, Kazakhstan, Turkmenistan, Uzbekistan; BirdLife International 2021), being part of the Asian-African migratory system (Cramp 1985), or for birds from the central/eastern European flyway, breeding farther east than turtle doves sampled in this study, e.g., Ukraine, Turkey. A longitudinal gradient of non-breeding African areas, with western and central regions being occupied by European populations and the eastern region being occupied by Asian populations, has also been observed in other migrating species whose breeding distribution ranges from western Europe to middle and eastern Asia (Trierweiler et al. 2014; Sarà et al. 2019). However, to confirm whether Asian turtle dove populations winter in eastern sub-Saharan Africa, stable isotope studies are similar to our approach and ideally combined with tracking studies (e.g., Jiguet et al. 2019; Monti et al. 2021) are needed to investigate their flyways and wintering destinations.

The present study provides a good first-order estimation of wintering regions of turtle doves from European populations but still has some limitations due to potential winter movements of turtle doves (Eraud et al. 2013; Lormée et al. 2016) that could not be accounted for. Furthermore, our analysis

did not provide information on inter-annual wintering site fidelity, which may be important for the planning and maintenance of protected areas. For instance, we show that based on the probabilistic wintering ranges, large parts of the most likely wintering grounds do not overlap with the protected area network in Africa. Turtle dove conservation management should consider hunting regulations over the species' entire range. Whereas much attention has been given to the impact of illegal hunting during migration in Europe on population declines, very little consideration has been given to hunting in African wintering areas (Hirschfeld et al. 2019; Lormée et al. 2020), where the resources to enforce hunting laws are much more limited. It is a common practice to shoot turtle doves at roost and drinking sites in some countries, e.g., Senegal and Mali. These hunting activities at roosting sites are likely to affect survival not solely through direct mortality via shooting but also by scaring away turtle doves from safe and suitable feeding and roosting sites (Zwarts et al. 2009).

In general, defining wintering regions is important, as the causes of population decline in European-breeding migrants are associated with the region and habitats in which they winter (Ockedon et al. 2012). A significant part of the variance in the annual survival of turtle doves was explained by environmental conditions encountered by birds on their wintering grounds (Eraud et al. 2009). Nevertheless, a substantial knowledge gap on conditions and threats that turtle doves face on the wintering grounds remains. This gap needs to be filled urgently in order to understand the factors leading to the turtle dove decline (Fisher et al. 2018). Wintering conditions are likely to deteriorate further in the future, as in the sub-Saharan region where agricultural landscapes are changing rapidly (Cour 2001; Sissoko et al. 2011; Walther 2016). Increasing human pressure in this region has resulted in a reduction of preferred habitat (i.e., woody vegetation) and migratory birds have suffered particularly severe declines (Walther 2016). Turtle doves are susceptible to previous and ongoing changes, such as increased cultivation of the Sahel and Sudan zone, overgrazing and cutting of trees, overuse of pesticides, suppression of woodland within farmland, and the homogenization of cropland (Lutz 2007; Fisher et al. 2018; Mansouri et al. 2020). Moreover, similar to other migratory species, turtle doves are particularly at-risk due to global climate change, as they must adapt their breeding and migration timing to asynchronous changes in suitable conditions across broad, spatiotemporal scales (Fraser et al. 2019).

Declarations

Ethics approval All applicable institutional and/or national guidelines for the care and use of animals were followed.

Consent to participate All authors voluntarily agree to participate in the elaboration and publication of this manuscript.

Consent for publication All authors declare that they participated in the study and the development of the manuscript, as well as read the final version and give consent for the article to be published in EJWR.

Conflict of interest The authors declare no competing interests.

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CHAPTER 2

YEAR-ROUND SPATIAL DISTRIBUTION AND MIGRATION PHENOLOGY OF A RAPIDLY DECLINING TRANS-SAHARAN MIGRANT – EVIDENCE OF WINTER MOVEMENTS AND BREEDING SITE FIDELITY IN EUROPEAN TURTLE DOVES

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ORIGINAL ARTICLE

Year-round spatial distribution and migration phenology of a rapidly declining trans-Saharan migrant—evidence of winter movements and breeding site fidelity in European turtle doves

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Abstract

Populations of migratory bird species have suffered a sustained and severe decline for several decades. Contrary to non-migratory species, understanding the causal mechanisms proves difficult (for migratory bird species) as underlying processes may operate across broad geographic ranges and stages of the annual cycle. Therefore, the identification of migration routes, wintering grounds, and stopover sites is crucial for the development of relevant conservation strategies for declining migrant bird species. We still lack fundamental data of the non-breeding movements for many migratory species, such as European turtle doves *Streptopelia turtur*, a trans-Saharan migrant. For this species, knowledge of non-breeding movements is mainly based on ringing data that are limited by a low recovery rate in Africa, and tracking studies with a strong bias towards individuals breeding in France. We used Argos satellite transmitters to obtain detailed year-round tracks and provide new insights on migration strategies and winter quarters, of turtle doves breeding in Central and Eastern Europe. The tracking data along with analysis of land cover data confirm previously assumed use of multiple wintering sites and the use of a wide range of forest and agricultural landscapes at the breeding grounds. Tracking data in combination with environmental parameters demonstrated that most environmental parameters and niche breadth differed between breeding and wintering grounds. "Niche tracking" was only observed regarding night-time temperatures. Furthermore, we provide evidence for breeding site fidelity of adult individuals and for home range size to increase with an increasing proportion of agricultural used areas.

Significance statement

The European turtle dove, a Palearctic-African migrant species, is one of the fastest declining birds in Europe. The rapid decline is presumed to be caused mainly by habitat modification and agricultural changes. Here, we represent data on migration strategies, flyways, and behavior on European breeding and African non-breeding sites of turtle doves breeding in Central and Eastern Europe equipped with satellite transmitters. Our results confirm the use of different migration flyways and reveal an indication for "niche switching" behavior in terms of environmental factors during the different annual phases. The migratory behaviors revealed by the tracking approach, e.g., prolonged stopovers during autumn migration in Europe overlapping with time of hunting activities, stopovers in North Africa during spring migration, or evidence for loop migration, are important protection-relevant findings, particularly for the Central-Eastern flyway, for which no tracking data has been analyzed prior to our study.

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Introduction

Twice every year an estimated number of more than two billion birds, belonging to the Palearctic–African migration system, migrate between Europe and sub-Saharan Africa

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(Hahn et al. 2009). In general, these migrants travel between their European breeding and sub-Saharan non-breeding grounds crossing the Mediterranean Sea and the Sahara desert via several broad-scale migration corridors and flyways, formed by specific geographical structures and ecological barriers. Migration within this system strongly funnels along two major flyways, namely the Western flyway, over the Iberian Peninsula crossing the strait of Gibraltar to Northwest-Africa, and the Eastern flyway via the Balkan Peninsula and the Middle East (Briedis et al. 2020). A third migration route via the Apennine peninsula and across the strait of Sicily is the Central flyway (Marx et al. 2016).

One of the bird species migrating from Europe to the African Sahel zone is the European turtle dove *Streptopelia turtur* (hereafter turtle dove). It is the smallest member of the European Columbiformes and the only long-distance migrant among them. Formerly a widespread and common breeding bird species over a large part of the European Continent, Western Asia, and Northern Africa (Glutz von Blotzheim and Bauer 1987), the turtle dove has faced population declines over the past decades and is now listed as "Vulnerable" by the IUCN (BirdLife International 2019). In Europe, numbers have decreased by around 80% between 1980 and 2017 (PECBMS 2020). The major reasons for the population decline are presumed to be habitat modification and agricultural intensification at the breeding and wintering areas as well as potentially also on stopover sites used during migration (Browne and Aebischer 2004; Eraud et al. 2009; Fisher et al. 2018). Unsustainable legal and illegal hunting activities along the migration routes are further contributing to the decline (Fisher et al. 2018; Lormée et al. 2019).

Analyses of ring recoveries confirm that turtle doves migrate along all three aforementioned flyways. Ringing studies further found evidence for a migratory divide in Europe with Western European populations of turtle doves using the Western flyway and Central and Eastern European populations migrating along the Central or the Eastern flyway (Dimaki and Alivizatos 2014; Marx et al. 2016). However, sampling turtle dove populations across Europe did not reveal any genetic structure that would support discerned populations according to this migratory divide, but rather one large, panmictic population (Calderón et al. 2016). Furthermore, only 1.6% (14 out of 897) ring recoveries came from Sub-Saharan Africa (Marx et al. 2016), indicating that there is still a lack of knowledge regarding the wintering grounds and the exact flyways of turtle doves.

Advances in tracking techniques have started to shed more light onto the migration routes of turtle doves (Eraud et al. 2013; Lormée et al. 2016). However, so far there has been a strong bias of studies towards turtle doves breeding in France, which use the Western flyway, whereas detailed knowledge based on tracking of individuals migrating on the Central and Eastern flyway remains very limited.

As the seasonal movement patterns are not solely influenced by the internal state of organisms and biological factors, but also by external factors, i.e., the environment and underlying context (Nathan et al. 2008), we compared the environmental conditions at the European breeding and the sub-Saharan non-breeding region of turtle doves by selecting different environmental variables to describe and characterize the individual habitats of tracked birds. The environmental factors determining the distribution of migrants may differ between breeding and non-breeding areas (Ponti et al. 2020), depending on if migrants move in geographical space to track their favored environmental conditions to remain in a specific subset of preferred niche space, so-called "niche tracking" (van der Graaf et al. 2006; Tingley et al. 2009; Gómez et al. 2016). Alternatively, they may change their environmental niche ("niche switching") between periods of the annual cycle. If different aspects of seasonal movements reflect conservatism in ecological characteristics vs. seasonal changes, the conserved patterns may greatly inform related issues, such like habitat choice and timing of migration (Nakazawa et al. 2004).

We present findings of a satellite tracking study on turtle doves equipped with Argos transmitters during spring migration on Malta, located on the Central flyway, and during breeding season in two states of Germany, with hitherto unknown assignment to the possible flyways. In addition to the description of the different annual phases (breeding, spring and autumn migration, stopover, and wintering) of each tracked bird, we analyzed the favored environmental conditions at the breeding and wintering sites in order to test for niche overlap in the seasonal niches.

Material and methods

Bird capture and transmitter deployment

From 2016 to 2020, turtle doves were caught during stopover on their return migration in spring along the Central flyway on the Maltese islands ($n = 8$) using mist nets. In 2019 and 2020, turtle doves were caught during breeding time in two states of Germany (Central Germany: Hesse ($n = 5$) and Eastern Germany: Brandenburg ($n = 3$)) using drop traps baited with a mix of cereal seeds (Table 1). It was not possible to record data blind, as our study involved individually marked animals in the field.

The sex was determined by molecular analysis based on collected feather or blood samples (Griffiths et al. 1998) and by characteristics of plumage (Demongin 2016). Birds were individually fitted with an Argos satellite tag (Solar 5 g PTT, Microwave Telemetry, USA), providing location fixes based on Doppler calculations, fixed as a wing-loop backpack using a 2-mm-wide Teflon ribbon (Ecotone, Poland)

Table 1 Individual information of European turtle doves *Streptopelia turtur* equipped with Solar 5 g PTT Argos satellite tags during spring migration on Malta and during breeding period in Germany

Bird ID	Duty cycle [ON/OFF]	Deployment date [dd.mm.yyyy]	Catching location [Lat, Long]	Sex	Body mass [g]	Device weight [% of the birds' body mass]	End data transmission[a] [dd.mm.yyyy]	Data transmission [days]
#161046	10 h/48 h	13.05.2016	Malta 35.95, 14.38	NA	137	3.6	05.08.2017	450
#161047	10 h/48 h	21.04.2017	Comino 36.01, 14.34	NA	136	3.7	30.04.2017	9
#161048	10 h/48 h	22.04.2017	Comino 36.01, 14.34	f	118	4.2	27.04.2018	370
#161049	08 h/15 h	22.04.2017	Comino 36.01, 14.34	m	142	3.5	20.09.2017	151
#161050	08 h/15 h	23.04.2017	Comino 36.01, 14.34	m	129	3.9	14.08.2020	1209
#181091	10 h/48 h	13.06.2019	Hesse 50.49, 08.92	f	160	3.1	25.09.2020	470
#181090	10 h/48 h	24.06.2019	Brandenburg 51.92, 14.33	m	160	3.1	26.09.2019	94
#181092	10 h/48 h	25.06.2019	Brandenburg 51.92, 14.33	m	161	3.1	09.05.2020	319
#181089	10 h/48 h	25.06.2019	Brandenburg 51.92, 14.34	f	158	3.2	20.10.2019	117
#200345	08 h/15 h	01.05.2020	Comino 36.01, 14.34	f	125	4.0	02.05.2020	1
#200348	08 h/15 h	04.05.2020	Comino 36.01, 14.34	f	132	3.8	22.06.2020	49
#200349	08 h/15 h	05.05.2020	Comino 36.01, 14.34	m	180	2.8	01.09.2020	119
#200351	08 h/15 h	05.06.2020	Hesse 50.44, 08.55	m	148	3.4	30.10.2020	147
#200352	08 h/15 h	07.06.2020	Hesse 50.44, 08.55	f	155	3.2	07.10.2020	122
#200353	08 h/15 h	08.06.2020	Hesse 50.49, 08.92	f	149	3.4	16.09.2020	100
#200350	08 h/15 h	13.06.2020	Hesse 50.44, 08.55	m	173	2.9	12.10.2020	121

[a]Transmission of locations stopped without known reasons or transmission manually terminated due to stable positions from the same (unhospitable) location for consecutive weeks

harness, following the method described by Lormée et al. (2016). The overall weight of the tracking device was below the 5% of the birds' body masses threshold, recommended in literature (Fair et al. 2010). Satellite tags deployed were programmed with a standard duty cycle of 10 h ON/48 h OFF or with a modified duty cycle of 8 h ON/15 h OFF (Table 1). All birds were released immediately after tagging at the location of capture.

The transmitters of the individuals #161047 and #200345 stopped recording 9 days and 1 day after tagging, respectively, while both birds were still on stopover on the Maltese Islands. These two individuals were probably killed by poachers. Individual #200348 sent data for 49 days. However, within that time, she did not settle at a breeding site but crossed the sea between Sicily and Libya twice until

data transmission stopped over the Mediterranean Sea (Supplementary Fig. 1n). These three turtle doves were excluded from all further analyses, resulting in a final data set of 13 tracked turtle doves.

Handling of tracking data

All location data as received from Argos of all location classes (LC: 3, 2, 1, 0, A, B) were automatically uploaded to Movebank (movebank.org) in their original projection (WGS84). We applied the "Douglas Filter" (Filter Method: Best Hybrid, Douglas et al. 2012) to remove erroneous data and afterwards checked for possible remaining outliers manually. These filtered location data were used when plotting the data in QGIS 2.18 (QGIS.org 2016). Tracks

were displayed by using the "Points2One" plugin in QGIS (Kapusta 2015).

Locations obtained before deployment were used to estimate accuracy of the locations. The deviation of these locations ($n = 63$) was on average 2 km, ranging from 0.1 to 10 km.

To determine the different phases (i.e., breeding, migration, stopover, and wintering) in the annual cycle of the individuals, we used an approach similar to that described in Lormée et al. (2016): Clear switches in the pattern of the location data defined the onset and end of the different phases. Breeding phase was the period when an individual spent at least 45 days between April and September in one distinct area (Glutz von Blotzheim and Bauer 1987; Marx et al. 2016). We defined the pre-migratory movements as movements to a distinct site, onwards called post-breeding site, where the period (minimum of 10 days) after likely nesting was spent before the onset of migration (Pagen et al. 2000; Vitz and Rodewald 2007). The onset of molt migration or autumn migration was specified as soon as movements > 100 km in direction to the wintering grounds, e.g., southwards, south westerly or south easterly, occurred. Molt migration is the temporal overlap in the molt and migration life history stages (Tonra and Reudink 2018). Since there is no uniform pattern whether molt migration occurred, as some individuals presumably molt the first inner primaries on or near their breeding sites, while others stop during autumn migration to molt en route (Demongin 2016; Pillar et al. 2016), the term "autumn migration" used throughout the paper includes possible molt migration (Pillar et al. 2016; Table 2). A stopover site was defined as consecutive set of locations overlapping spatially for at least three days during migration period and being at least 100 km away from the breeding site. Molt of the first inner primaries at stopover sites was assumed if the individuals staged after leaving the breeding site for at least 21 days (Mallet-Rodrigues 2012) in Europe before October (Demongin 2016). These sites are referred to as "stopover molt sites" (Pillar et al. 2016). If an individual stayed for at least 14 consecutive days in one distinct area south of 20° N (Glutz von Blotzheim and Bauer 1987; Eraud et al. 2013), this was specified as the start of the wintering period.

Epanechnikov kernels (95% and 50% kernel utilization distributions "KUD"; Epanechnikov 1969) of filtered localizations received during the breeding and wintering period were calculated in R with the function the "kernelUD" in the package "adehabitatHR" (Calenge 2015). We used a generic grid of 200 cells and the smoothing parameter was estimated with a href parameter. The area covered by the individual

birds was estimated with the R package "sp Classes and Methods for Spatial Data" (Pebesma 2020).

To characterize the land cover in the occupied habitats the 95% KUDs were clipped in QGIS 2.18 with raster land cover data and the percentages of different land covers classes in the 95% KUDs were calculated. For European breeding grounds, land cover data were based on Corine Land Cover CLC 2018 v.2020_20u1 (Copernicus Land Monitoring Service 2021) and for African wintering grounds on ESA CCI Land Cover S2 prototype LC 20 m map of Africa 2016 (ESA CCI Land Cover project 2021).

Only complete periods in the life cycle of the birds were used in the detailed analyses of durations. To determine whether migratory movements of the turtle doves occurred during the night or day, we calculated for each location fix of complete autumn ($n = 7$) and spring tracks ($n = 5$), when the morning and evening civil twilight had been by using the function "crepuscule" (R package maptools, Bivand et al. 2020). With the "crepuscule" function we estimated when the geometric center of the sun was 6° below the horizon in the morning (civil dawn) and in the evening (civil dusk). Night-time was defined as the period of time between civil dusk and the consecutive civil dawn (Zúñiga et al. 2016). Fixes included in this analysis were at least 1 h, but not more than 6 h, apart from each other and only considered when individuals were actively migrating ($n = 189$ pairs of fixes). If the direct distance between two consecutive fixes was more than 25 km (mean flight speed during migration is approx. 50 km/h; Lormée et al. 2016), active migratory movement was presumed ($n = 51$ pairs of fixes) and classified as night-time, daytime, or between (i.e. one of the fixes during night- and the other one during daytime). From the fixes during active migratory movement ($n = 51$ pairs of fixes), the mean flight speed was calculated.

To compare environmental habitat parameters at breeding and wintering grounds, nine habitat parameters were obtained through the Environmental Data Automated Track Annotation System (Env-DATA, Dodge et al. 2013; interpolation: bilinear) on Movebank for filtered positions of those turtle doves from which we obtained locations at both wintering and breeding grounds ($n = 5$; Table 2). Locations received during winter movements (i.e., movement between different wintering sites), stopovers, and active migration were excluded. The environmental data included parameters from MODIS land: net photosynthesis (PsnNet), gross primary productivity (GPP), total evapotranspiration, enhanced vegetation index (EVI), daily land surface temperature day and night, and vegetation index (NDVI) as well as the parameters elevation (ETOPO1) and human population density adjusted (SEDAC GPW V3 and GRUMP V1 GRUMP

Table 2 Details of the migration schedule of 13 European turtle doves *Streptopelia turtur* equipped with satellite tags from 2016 to 2020

Bird ID	Spring migration				Breeding	Autumn migration			Wintering
	Year	Duration in days [dd.mm to dd.mm]	No. stopover Africa [country:days]	No. stopover Europe [country:days]	Duration in days [dd.mm to dd.mm]	Duration in days [dd.mm to dd.mm]	No. stopover Europe [country:days]	No. stopover Africa [country:days]	Duration in days [dd.mm to dd.mm]
#161046	2016	[STᵃ–22.05]			116 [22.05–14.09]	64 [14.09–16.11]	0	4 [LY:12, ML:6, NE:13; 4]	156 [16.11–20.04]
	2017	23 [20.04–12.05]	1 [LY:9]	0	[12.05–ETᵃ]				
#161048	2017	[ST–18.05]			114 [18.05–08.09]	16 [08.09–23.09]	1 [GR:3]	0	213 [23.09–23.04]
	2018	[23.04–ET]							
#161049	2017	[ST–20.06]		2 [IT:18; 16]	63 [20.06–21.08]	[21.08–ET]	1 [HU:25ᶜ]		
#161050	2017	[ST–05.05]			117 [05.05–29.08]ᵇ	18 [29.08–15.09]	1 [IT:4]	1 [NE:6]	207 [15.09–09.04]
	2018	23 [09.04–01.05]	1 [TN:15]	0	134 [01.05–11.09]ᵇ	7 [11.09–17.09]	0	0	209 [17.09–13.04]
	2019	20 [13.04–02.05]	1 [TN:8]	0	131 [02.05–09.09]ᵇ	14 [09.09–22.09]	0	1 [MA:6]	205 [22.09–13.04]
	2020	21 [13.04–03.05]	1 [DZ:10]	0	[03.05–ET]				
#181091	2019				[ST–01.09]	35 [01.09–05.10]	2 [FR:12, ES:6]	0	206 [05.10–27.04]
	2020	50 [27.04–15.06]	3 [MR:6, MA:16; 4]	0	76 [15.06–29.08]	[29.08–ET]	2 [FR:5;14]		
#181090	2019				[ST–27.08]	[27.08–ET]	1 [HU:13]	1 [LY:6]	
#181092	2019				[ST–13.09]	22 [13.09–04.10]	1 [HU:3]	2 [LY:6, NE:3]	206 [04.10–26.04]
	2020	[26.04–ET]							
#181089	2019				[ST–05.08]	[05.08–ET]	2 [SK:11, HU:12]	2 [TN:5, DZ:6]	
#200349	2020	[ST–09.05]			[09.05–ET]				
#200351	2020				[ST–05.09]	[05.09–ET]	1 [FR:15]		
#200352	2020				[ST–29.08]	[29.08–ET]	2 [FR:7, IT:20]	1 [TN:5]	
#200353	2020				[ST–01.09]	[01.09–ET]	1 [ES:9]		
#200350	2020				[ST–31.07]	[31.07–ET]	2 [GE:24ᶜ, ES:18]	1 [DZ:14]	

ᵃ*ST*, start of data transmission; *ET*, end of data transmission

ᵇIncluding time spent at the post-breeding site (see Supplementary Fig. 2)

ᶜStopovers at stopover molt sites (see Supplementary Fig. 1f, k)

2000). These parameters were selected as they likely influence the habitat selection of turtle doves and had a sufficient data coverage for both breeding and wintering sites.

Statistical analyses were conducted using R 3.4.1 (R Development Core Team 2018). Means ± SE for the environmental parameters for each individual are given in Table 3. To compare the aforementioned environmental habitat parameters at the different stages (breeding and wintering), a principal component analysis (PCA) was performed for every single individual as well as for all individuals together. The PCA extracted two significant components PC1 and PC2. Habitat niche plots were created from the two dimensions of the habitat (PC1 and PC2) using kernel densities function calculated in R with the function "kde2d" in the MASS package (Venables and Ripley 2002).

Results

Transmitters operated for 291 days on average (*n* = 13 turtle doves, Table 1), resulting in 19,482 filtered location fixes during 18 breeding (hereof seven complete) and seven complete wintering periods, 12 migration cycles in spring (hereof five complete), and 15 in autumn (seven complete) as well as 39 stopovers of which 2 are likely stopover molt sites (Fig. 1; Table 2).

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Table 3 Habitat variables at breeding and wintering grounds of tracked European turtle doves. Habitat variables (mean ±SE) were obtained through the Environmental Data Automated Track Annotation System (Env-DATA) on Movebank linked to Argos location fixes (n = number of filtered Argos locations)

	#161046		#161048		#161050		#181091		#181092	
	Breeding (n = 651)	Wintering (n = 432)	Breeding (n = 926)	Wintering (n = 720)	Breeding (n = 2948)	Wintering (n = 3759)	Breeding (n = 523)	Wintering (n = 611)	Breeding (n = 297)	Wintering (n = 549)
PsnNet [kg C m^{-2}]	0.02 ± 0.01	< 0.00	0.02 ± 0.01	< 0.00	0.03 ± 0.02	< 0.00	0.03 ± 0.01	< 0.00	0.02 ± 0.01	< 0.00
GPP [kg C m^{-2}]	0.03 ± 0.01	< 0.00	0.03 ± 0.02	< 0.00	0.05 ± 0.02	< 0.00	0.04 ± 0.02	< 0.00	0.03 ± 0.01	< 0.00
Evapotranspiration [kg m^{-2}]	13.2 ± 4.2	0.4 ± 0.4	15.6 ± 9.4	0.6 ± 1.0	28.7 ± 14.6	0.5 ± 0.5	27.9 ± 10.6	1.8 ± 2.4	14.6 ± 4.5	0.5 ± 0.5
EVI	0.31 ± 0.05	0.13 ± 0.02	0.33 ± 0.08	0.15 ± 0.03	0.49 ± 0.13	0.14 ± 0.02	0.44 ± 0.08	0.18 ± 0.03	0.33 ± 0.03	0.11 ± 0.01
Surface temperature day [°C][a]	36.5 ± 5.2	34.5 ± 5.9	33.7 ± 4.1	35.7 ± 5.7	31.3 ± 6.1	39.0 ± 4.9	29.2 ± 3.3	35.6 ± 5.5	28.2 ± 5.2	35.0 ± 5.4
Surface temperature night [°C][a]	19.7 ± 3.1	18.4 ± 2.5	17.8 ± 3.5	18.2 ± 3.1	17.8 ± 3.9	19.2 ± 4.7	14.8 ± 2.8	17.3 ± 3.7	15.7 ± 4.3	17.6 ± 4.2
NDVI	0.45 ± 0.06	0.18 ± 0.04	0.51 ± 0.10	0.25 ± 0.05	0.68 ± 0.15	0.22 ± 0.04	0.68 ± 0.08	0.29 ± 0.06	0.64 ± 0.05	0.15 ± 0.03
Elevation [m amsl]	136.2 ± 84.3	343.7 ± 10.3	45.2 ± 35.6	297.2 ± 4.3	401.1 ± 156.4	239.7 ± 72.7	189.9 ± 33.5	291.7 ± 118.4	73.4 ± 8.1	307.5 ± 41.7
Population density [person/km²]	237.4 ± 170.2	89.6 ± 34.9	408.1 ± 219.1	27.2 ± 26.1	175.2 ± 140.9	22.3 ± 16.6	229.5 ± 46.8	18.9 ± 14.0	58.0 ± 62.1	35.7 ± 15.0

[a] Unit changed from Kelvin to °C

Breeding period and post-breeding areas

The turtle doves arrived at their breeding grounds between 1. May and 20. June (median 18. May, $n = 10$) and spent a total of 63–134 days (median 107.3 days, $n = 7$) at the breeding grounds (Table 2). Breeding grounds of individuals tagged during spring migration on Malta were located in Italy (#161046, #161050, and #200349), Bulgaria (#161048), and Slovakia (#161049; Supplementary Fig. 1).

Individuals ($n = 8$) tagged at the beginning of the breeding season in Germany stayed in the area where they were caught during the ongoing breeding season (Supplementary Fig. 1). The home range (95% KUD) used by the turtle doves ($n = 18$ breeding sites from 13 individuals) was on average 496 ± 335 km² (min 263 km² to max 1554 km²) and the core area (50% KUD) was 39 ± 29 km² (min 14 km² to max 121 km²). Land cover in the 95% Epanechnikov kernels varied per breeding area and individual. In total 35 of 44 Corine Land Cover classes occurred in the breeding areas (Supplementary Table 1). Land cover classes that were present in every single turtle dove breeding habitat were non-irrigated arable land ($29.4 \pm 15.5\%$ of the 95% KUD), broadleaved forest ($20.9 \pm 19.5\%$), discontinuous urban fabric ($4.6 \pm 2.4\%$), and industrial or commercial units ($1.0 \pm 0.3\%$; Fig. 2). The size of home ranges (95% KUD) increased with a higher percentage of agricultural areas (Supplementary Table 1) as land cover in the 95% kernel, while there was no significant relation between home range size and the proportion of forest and seminatural areas (GLM: agricultural areas: $F_{1,10} = 5.47$, $p = 0.041$; forest and seminatural areas: $F_{1,10} = 3.53$, $p = 0.090$). The same applied for the size of core areas (GLM: agricultural areas: $F_{1,10} = 5.02$, $p = 0.049$; forest and seminatural areas: $F_{1,10} = 3.87$, $p = 0.078$).

Turtle doves for which we had data from the breeding grounds for consecutive years ($n = 3$; #161046, #161050, and #181091) showed high site fidelity, i.e., returned to the exact same breeding area (Supplementary Fig. 1a, b, d).

The male individual #161050 performed short pre-migratory movements (< 1 day) into a defined post-breeding site around 20–30 km north-easterly to its breeding site (Supplementary Fig. 2), where it stayed for 39, 18, and 38 days in 2017, 2018, and 2019, respectively, before starting the autumn migration. The other birds, stayed in their breeding areas until they started autumn migration, but individuals #161049 and #200350 moved to stopover molt sites for a prolonged period in more southern latitudes within Europe (Table 2, Supplementary Fig. 1f, k), indicating a stopover molt migration for these individuals.

Autumn migration

Turtle doves left their breeding areas between 31. July and 14. September (median 30. August, $n = 15$). The overall
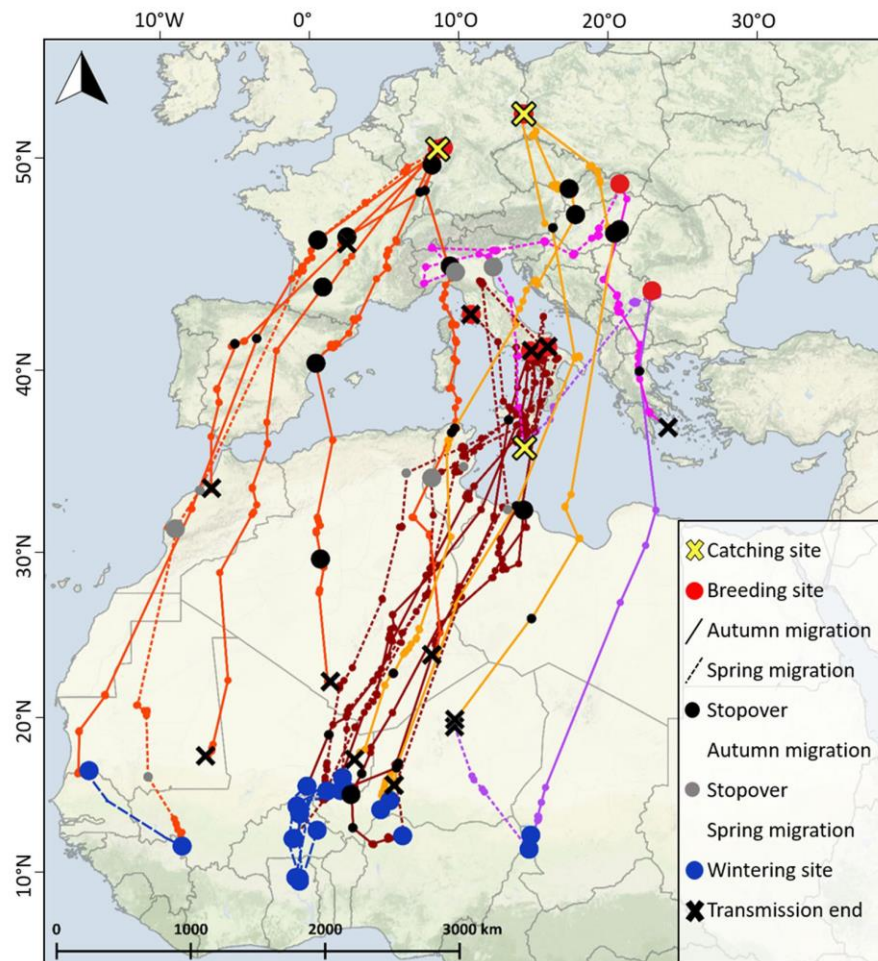
Fig. 1 Satellite tracks of 13 European turtle doves *Streptopelia turtur* during migration between European breeding (red circles) and African wintering grounds (blue circles). Tracks are given in different colors corresponding to different countries individuals had their breeding sites in: orange = Germany (dark orange = Hesse, Central Germany; light orange = Brandenburg, Eastern Germany); dark red = Italy; pink = Slovakia: purple = Bulgaria. Detailed tracks for single individuals can be found in Supplementary Fig. 1. Autumn migration is shown as solid line and spring migration as dashed line. Location fixes (based on Doppler locations) received during migration are shown as circles in the background of the track lines. Winter movements are displayed in dashed, blue lines. Black circles correspond to stopover sites during autumn migration and gray circles to stopover sites during spring migration. Circle size corresponds to stopover duration: Small circle ≤ 10 days and big circle > 10 days. Background colors indicate the terrain and gray lines indicate national borders (background map: Stamen terrain (map tiles by Stamen Design: http://maps.stamen.com; data by OpenStreetMap: www.openstreet map.org))

duration of the autumn migration including stopovers was 7–64 days (median 25.1 days, $n = 7$), with a total stopover duration between 0 and 35 days (median 11.9 days, $n = 7$). Stopovers were taken in 57.1% of autumn migrations in Europe (median 4 days, $n = 7$) and in 57.1% in Africa (median 8 days, $n = 7$, Table 2, Supplementary Fig. 3). However, considering the data of all tracked individuals, i.e., partial tracks included, several birds (3 of 4 individuals in the Western flyway and 4 of 8 in the Central-Eastern flyway) made prolonged post-breeding stopovers (> 10 days)

in Europe after leaving their breeding site (Table 2; Fig. 1). Prolonged stopovers were mainly made in Europe (58.8% of stopovers; 10 of 17 stopovers) and less often in Africa (23.1%; 3 of 13 stopovers).

Of the 12 turtle doves four individuals (#181091, #200351, #200353, and #200350), all with breeding grounds in Hesse, started in south-westerly direction. The two females #181091 and #200353 crossed the Mediterranean Sea at or close to the strait of Gibraltar, while the two males #200351 and #200350 crossed the Mediterranean Sea

Fig. 2 Proportional occurrence [%] of Corine Land Cover classes (Copernicus Land Monitoring Service 2021) in 95% Epanechnikov kernels of satellite-tracked European turtle doves *Streptopelia turtur* at different breeding grounds. **a** Bulgaria (#161048), **b** Slovakia (#161049), **c** Southern Italy (#161046 and #161050), **d** Central Italy (#200349), **e** Central Germany (#181091, #20035, #200352, #200353, and #200350), **f** Eastern Germany (#181090, #181092, and #181089). Only land cover classes accounting for a fraction of more than 1% are shown. All remaining classes < 1% have been summed up to "O: others." Further details to the other classes can be found in Supplementary Table 1

DUF: discontinuous urban fabric	AC: annual crops associated with permanent crops	SC: sclerophyllous vegetation
ICU: industrial or commercial units	CCP: complex cultivation patterns	TW: transitional woodland/shrub
NAL: non-irrigated arable land	LPA: land principally occupied by agriculture	IM: inland marshes
V: vineyards	BLF: broad-leaved forest	WC: water courses
FBP: fruit trees & berry plantations	CF: coniferous forest	WB: water bodies
OG: olive groves	MF: mixed forest	O: others
P: pastures	MH: moors and heathland	
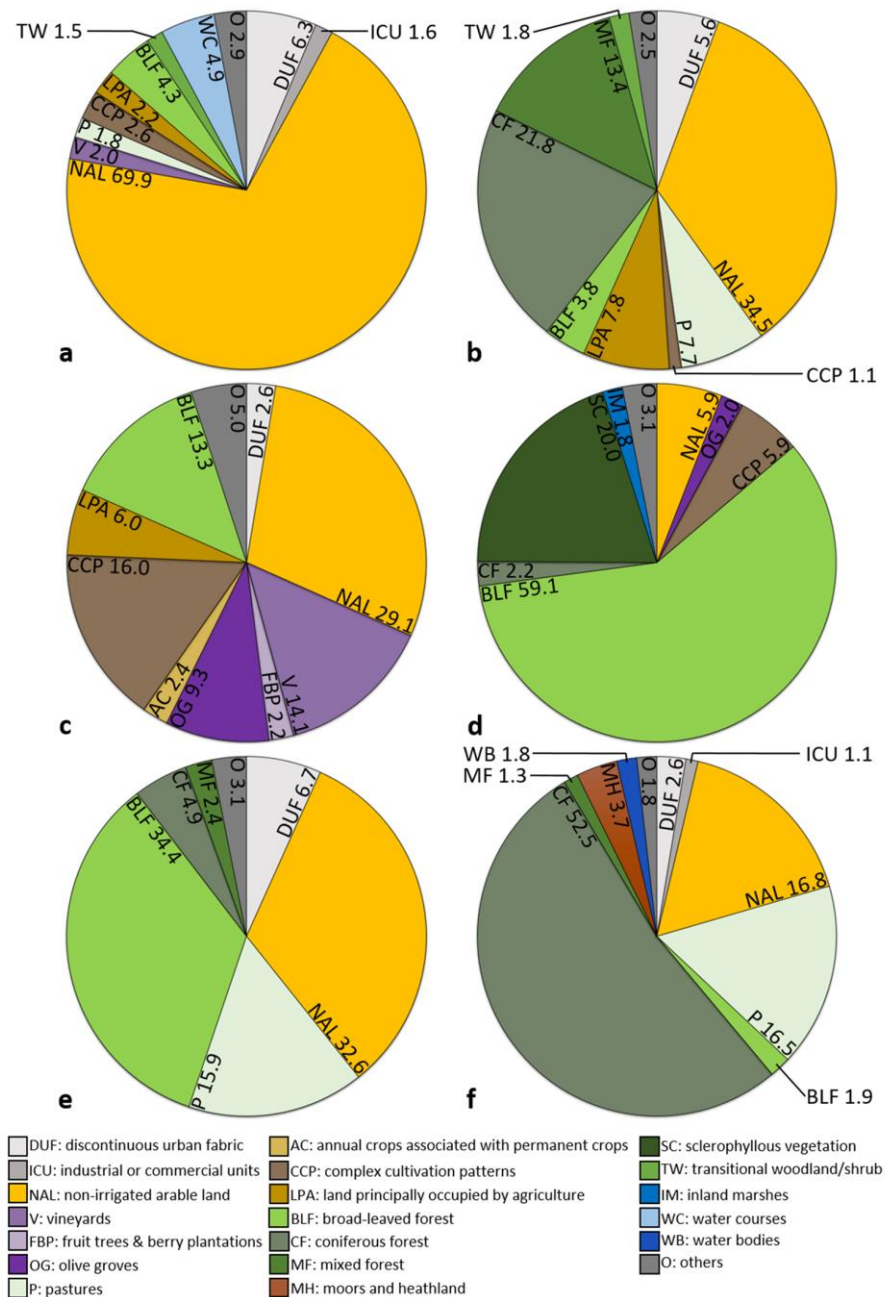
further east and therefore had a longer sea crossing (Supplementary Fig. 1j, k). The remaining turtle dove with breeding ground in Hesse (#200352) migrated south over Corsica and Sardinia. Individuals breeding in Italy (#161046 and #161050) migrated along the Central flyway. Turtle doves with breeding grounds in Brandenburg (#181090, #181092, and #181089) migrated first in a south-easterly direction to stopover sites in Eastern Europe and from there changed in

a more south-westerly direction. Turtle doves breeding in Eastern Europe (#161048 and #161049) started their autumn migration in a southern direction over mainland and islands of Greece (Fig. 1, Supplementary Fig. 3).

Overall, turtle doves in our study were largely nocturnal migrants. The majority of location fixes (81.8%) during active migratory movements were recorded during the night, compared to 9.1% during daytime and 9.1% between.
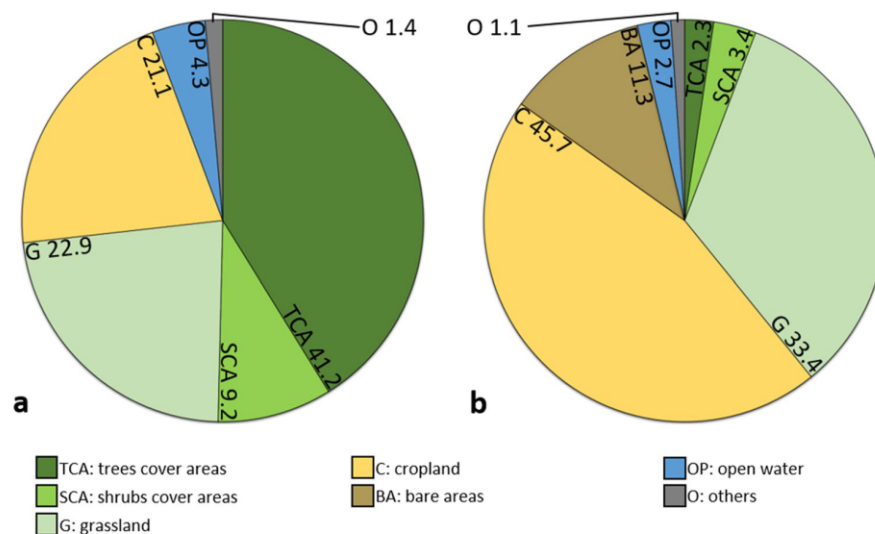
Fig. 3 Proportional occurrence [%] of ESA CCI Land Cover classes (ESA CCI Land Cover project 2021) in 95% Epanechnikov kernels of satellite-tracked European turtle doves *Streptopelia turtur* at different wintering grounds. **a** Western part of Western Africa. Individual #181091 using the Western flyway and wintering in Senegal and South-Western Mali. **b** Central and Eastern parts of Western Africa. Individuals #161046, #161048, #161050, and #181092 using the Central-Eastern flyway and wintering in Cameroon, Nigeria, Niger, Mali, Burkina Faso, and Ghana. Only land cover classes accounting for a fraction of more than 1% are shown. All remaining classes < 1% have been summed up to "O: others." Further details to the other classes can be found in Supplementary Table 2

The mean flight speed during the active migration was 45.7 ± 12.8 km/h.

We lost most of our tagged individuals during autumn migration (61.5%, 8 of 13 individuals) compared to the other annual stages (wintering 0%, spring migration: 15.4% and breeding: 23.1%).

Wintering period

Five of our satellite-tracked turtle doves arrived at their wintering grounds. The birds spent 156–213 days (median 200.3 days, $n = 7$) wintering after arriving between 15. September and 16. November (median 2. October, $n = 7$). Turtle doves overwintered in Western and Central Africa south of the Sahara (Figs. 1 and 3). While one individual (#161046) spent the entire wintering period at one wintering site (Supplementary Fig. 1b), the other four individuals used multiple (2–6) distinct wintering sites with a southward shift during the wintering period. The 95% KUD of wintering sites used by the turtle doves ($n = 20$ wintering sites) was on average 65 ± 154 km^2 (min 18 km^2 to max 510 km^2) and the 50% KUD was 5 ± 21 km^2 (min 1 km^2 to max 67 km^2). From one individual (#161050), we have locations from the wintering period for consecutive years. While its wintering duration is quite consistent (Table 2), the wintering localities varied between the wintering periods (Supplementary Fig. 1a).

A mix of crop- and grassland, likely used for foraging, and tree and shrub covered areas, presumably used as resting and roosting sites, mainly characterized land cover at the wintering sites. Open water was available at all wintering areas (Fig. 3, Supplementary Table 2). Remarkably, we observed a much higher proportion of tree cover area (41.2%) at the wintering sites of individual #181091 (migrating along the Western flyway) compared to the other four turtle doves (2.3 ± 1.9% tree cover), which used the Central-Eastern flyway and spend the wintertime in the Eastern parts of Western Africa.

Spring migration

The turtle doves started their spring migration between 9. April and 5. May (median 18. April, $n = 7$). The duration of the spring migration including stopovers was 20–50 days (median 27.4 days, $n = 5$). Stopovers lasted 8–26 days (median 13.6 days, $n = 5$). African stopover sites were located in Mauritania, Morocco (Western flyway) and Algeria, Libya, and Tunisia (Central-Eastern flyway). None of the five individuals of which we have a complete spring migration track staged in Europe (Table 2, Supplementary Fig. 3). However, #161049 from which we have a partial track of its spring migration made two stopovers in Italy, which lasted 18 and 16 days, before reaching its Slovakian breeding ground (Supplementary Fig. 1f). Individual #161046 showed overshooting behavior during spring migration, i.e., first flying further north before returning to its breeding ground (Supplementary Fig. 1b). The three

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Fig. 4 Habitat niches of satellite-tracked European turtle doves *Streptopelia turtur* ($n = 5$) for wintering (blue) and breeding (red) grounds. Obtained from Argos-positions and kernel densities of principal component scores of environmental parameters ($n = 9$) obtained through the Environmental Data Automated Track Annotation System (Env-DATA) on Movebank. PC1 (eigenvalue 5.34) was determined mainly by the five variables (PsnNet, NDVI, EVI, GPP, and Evapotranspiration) and PC2 (eigenvalue 1.37) mainly by the surface temperature at night
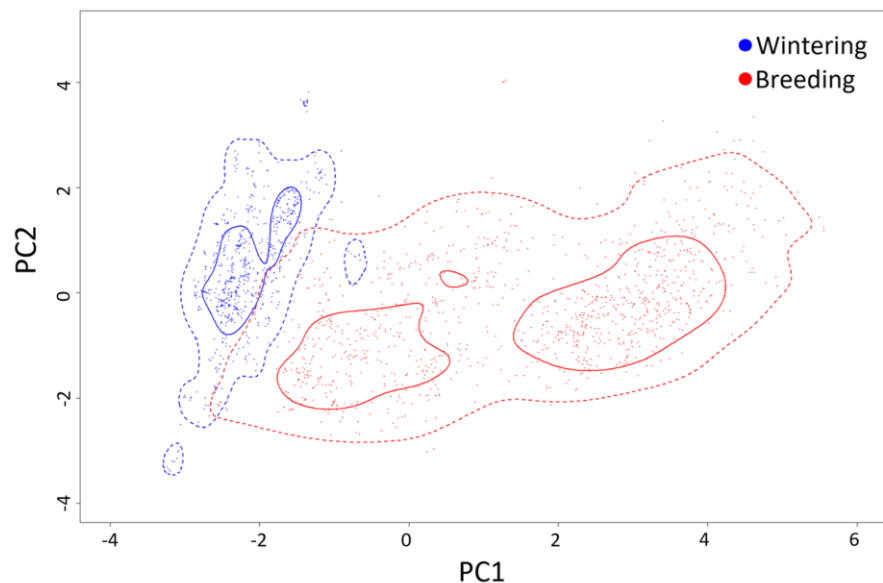
individuals we have complete spring migration tracks following the Western (#181091) and Central flyway (#161046 and #161050) for autumn migration followed a very similar route for spring migration (Fig. 1). The partial migration tracks of individuals #161048 and #161049 following the Central flyway for spring migration and a flyway further to the east during autumn migration, suggesting a clockwise loop migration (Supplementary Fig. 1c, f).

Similarly to autumn, turtle doves migrated mainly during night-time (77.8%). The mean flight speed during the active spring migration (50.7 ± 12.6 km/h) was not significantly faster than during autumn migration (independent t-test: $t = -0.53$, $df = 38$, $p = 0.601$).

Niche description approach

Environmental parameters differed between breeding and wintering grounds (Table 3). The PCA for all individuals combined extracted two significant components: PC1 (eigenvalue 5.34) was determined mainly by the five variables, which are related to vegetation and biomass production (PsnNet, NDVI, EVI, GPP, and Evapotranspiration) and PC2 (eigenvalue 1.37) mainly by the surface temperature at night (Fig. 4, Supplementary Table 3).

The significant components extracted by the PCA differed slightly when single individuals were analyzed separately. PC1 was characterized for all single individuals mainly by PsnNet, NDVI, EVI, GPP, and Evapotranspiration. For the individuals #161046, #161048, and #181092 additionally by elevation and for individual #181091 also by the population density and surface temperature during the day. PC2 of all individuals was mainly determined by the surface

temperature at night and for the individuals #161046, #161048, and #181092 additionally by the daytime surface temperature and for #181091 by elevation (Supplementary Table 3).

When comparing the ecological niches based on the tested parameters, turtle doves showed a change in environmental conditions represented by PC1 between the winter and breeding season, with the 95% kernels of the single individuals and combined data of the PCs hardly overlapping and the 50% kernels not overlapping (Fig. 4, Supplementary Fig. 4), indicating different occupied niches in breeding and wintering sites with respect to vegetation and biomass production. However, PC2 (temperature, especially at night-time) did not differ remarkably.

Discussion

Year-round data from our satellite transmitters allowed us to trace the timing and route followed by turtle doves from breeding grounds in Italy, Germany, Bulgaria, and Slovakia to the sub-Saharan wintering regions and vice versa, and to compare parameters of the breeding and wintering sites.

Breeding and wintering areas

The turtle dove breeding season starts immediately after arrival on the breeding grounds (Browne and Aebischer 2001). Assuming a total brood duration of around 45 days (Glutz von Blotzheim and Bauer 1987) and considering the average number of days (107) tracked individuals spent at the breeding areas, not more than two broods are possible.

For some individuals spending even fewer days (#161049: 63; #181091: 76) in the breeding area, only one brood was possible. One of the main findings comparing the British population during the 1960s and 1990s was that turtle doves curtailed their breeding season, which ties in with a reduction of nesting attempts and productivity per pair (Browne and Aebischer 2001, 2003, 2004). Our results indicate that the time spent in breeding areas may have shortened for turtle doves all over Europe.

For the first time, mean size of home ranges and core areas (496 and 39 km^2, respectively) could be calculated based on satellite tracking data. Glutz von Blotzheim and Bauer (1987) state that turtle doves often move 3–6 km or more from their nesting site for foraging. Even greater foraging distances, sometimes > 10 km, were recorded (Browne and Aebischer 2001). Home ranges based on 100% minimum convex polygons (MCPs) of radio-tagged turtle doves in Britain were between < 1 and 11.30 km^2 (Browne and Aebischer 2001) and based on 90% MCPs 0.86 ± 0.16 km^2 (Dunn et al. 2020). Our calculated home range areas seem far larger than these ones. Differences might be due to varying calculation methods: On one hand, radio-transmitters are constrained by line-of-sight range between transmitter and receiver, easily leading to missed fixes during foraging trips. On the other hand, satellite data have larger error ranges due the Doppler method, possibly leading to the larger size of calculated home ranges. In addition, we calculated the KUD sizes based on fixes received during the entire time individuals were at their breeding grounds, while Dunn et al. (2020) calculated home ranges derived solely from fixes during incubation and chick stage. As habitat use of turtle doves differs during the breeding season (Browne and Aebischer 2001; Mansouri et al. 2019), different foraging areas used over the seasonal progress may have added up in our calculation.

It is suggested that individual turtle doves are not site-faithful (Browne and Aebischer 2001; Dunn and Morris 2012). In contrast, all our turtle doves returning to the breeding grounds ($n = 3$) returned to the same breeding site occupied in the previous year. For #161050, this was the case for four consecutive years. Tracking results therefore propose that adult turtle doves are highly faithful to their breeding sites.

In general terms, turtle doves nest in trees or bushes in a landscape characterized by a patchy habitat mosaic of open land, nearby to wooded areas and an adjacent water source (Lutz 2007; Fisher et al. 2018). Habitat selection patterns and habitat requirements were investigated by numerous studies mainly based on observational absence and presence data (Supplementary Table 4). These studies show that turtle doves occur over a wide range of forest and agricultural landscapes, depending on the availability of certain habitat types at the regional level, and that nesting and feeding

habitats can be very diverse, depending on their nature (agricultural or natural), location, and time (Hanane 2012; Dias et al. 2013; Mansouri et al. 2019). Unlike the aforementioned studies, based on predetermined areas, e.g., grid squares, we checked the land cover in the actually used habitats according to the satellite tracking data. Our results support that habitat composition varies between different locations, e.g., preponderance of coniferous forest in Brandenburg, broad-leaved forest in Hesse or olive groves in Italy (Fig. 2, Supplementary Table 1). Our land cover analysis showed that land cover types suitable for nesting activities (e.g., forests, olive groves, or shrubs) and areas most likely used for foraging (e.g., non-irrigated arable land, pastures, crop cultivations, or heathland) were present in every home range. This reinforces the assumption that the close proximity of suitable nesting and feeding areas is a key requirement for good quality habitats (Browne et al. 2004; Dias et al. 2013). Our findings indicate that a higher proportion of agricultural areas within the home range leads to an increase in home range size. This is in line with Dunn et al. (2020), associating small home ranges with a high proportion of non-farmed habitats and Chiatante et al. (2020), reporting that areas with a high proportion of crops were avoided. As large areas of the intensively farmed arable landscape are not suitable for feeding, those breeding turtle doves with a high proportion of intensively farmed arable land within their home range are forced to forage over large distances to reach good quality food resources. It is likely that the long distances covered affect the adults' body condition throughout the breeding season, and hence may negatively influence their overall breeding performance (Browne and Aebischer 2001). It must be noted that land cover categories used in the aforementioned studies and in our study mainly describe landscape types, but do not consider management procedures. Breeding numbers of turtle doves show an overall decline particularly from the 1970s onwards (Fisher et al. 2018). While there was no major land cover type change in Europe between 1950 and 2000 (Gerard et al. 2010), many agricultural and silvicultural management procedures have been modified drastically (Baessler and Klotz 2006; Dallimer et al. 2009; Wesche et al. 2012; EEA 2020). Therefore, future studies should take into account information about agricultural and silvicultural management, such as the use of herbicides, conventional or organic farming, timing of harvest, understorey, or forest margin management, to be able to draw a more precise picture of turtle dove habitat requirements.

At their winter quarters, turtle doves are also susceptible to agricultural changes, e.g., increased cultivation, overgrazing, and cutting of trees (Lutz 2007; Fisher et al. 2018). It was shown that the overwinter survival of adult turtle doves is strongly related to the cereal production at the winter quarters (Eraud et al. 2009). Suitable wintering habitats appear to

be defined by an abundant food supply, an accessible water source and large trees or patches of woodland for roosting. If one of these key factors is absent, the habitat will typically only be used temporarily (Zwarts et al 2009). Previous tracking confirmed that turtle doves wintering in West Africa make movements of several hundreds of kilometers during the wintering season (Eraud et al 2013; Lormée et al. 2016). Our results confirm the use of more than one wintering site for the majority of tracked individuals (4 of 5) with a predominantly southward shift during the wintering period (Fig. 1). It is likely that the winter movements are linked to the availability of food resources, i.e., tracking food resources that become temporally available by the maturing and harvesting of cereal crops in different regions (Eraud et al. 2013; Lormée et al. 2016). Average sizes of 95% and 50% KUDs (65 and 5 km^2, respectively) were very similar with the size of winter sites (95% MCPs: 60 and 87 km^2, 50% MCPs: 2 and 3 km^2) calculated by Lormée et al. (2016). The habitat mosaic used at wintering sites consisted predominantly of crop- and grassland as well as a more varying proportion of areas covered by trees or shrubs. The proportion of tree and shrub covered areas appears to be higher for individuals wintering in the Western part compared to the ones in Central and Eastern part of Western Africa (Fig. 3). However, this pattern should be verified with more individuals in order to derive possible connections between, e.g., survival, body condition, or migration performance and overwintering region and differing land cover types there.

Niche tracking versus niche switching

We still lack a general understanding whether seasonal migration occurs in order to track a specific niche between summer and winter distribution ranges, i.e., migrants following a fixed set of environmental conditions throughout the annual cycle (Zurell et al. 2018). The PCA in our analysis extracted ecological habitat parameters related to vegetation and biomass production to mainly determine PC1. This fits the suggestion that Afro-Palearctic migrating landbirds track the vegetation green-up in spring and depart before vegetation senescence in autumn (Briedis et al. 2020). The extracted parameters for PC1 might also be interpreted as a proxy for food availability, what would correspond to the observed winter movements, which are most likely connected to the tracking of different available food resources over time (Eraud et al. 2013; Lormée et al. 2016). PC2, that was more constant for both periods, was mainly determined by temperature (Supplementary Table 3). For breeding grounds, it was shown already, that mainly climate variables, in particular "minimum temperature in January" and "precipitation of the warmest quarter," shape distribution models of turtle doves (Marx and Quillfeldt 2018) and that distribution is linked to an isotherm of a minimum of 16–19 °C in

July (Fisher et al. 2018). Plotting the habitat niches (Fig. 4) shows that the environmental parameters are more widely dispersed for data from the breeding grounds compared to more uniform parameters at the wintering grounds, indicating differences in the niche breadth for both seasons and a more pronounced intraspecific difference in individual habitat choice at the breeding compared to wintering sites. This matches the fact that turtle doves occur over a wide range of forest and agricultural landscapes at European breeding grounds, but winter along a relatively narrow and more uniform, with regard to climate and vegetation, latitudinal band along the Sahel and Sudan savannah.

The main conclusion to be drawn from the niche tracking approach is that habitat requirements and preferences determined at breeding sites cannot be assumed for wintering sites and vice versa but need to be investigated separately due to the apparent observed niche switching in turtle doves. A narrower niche breadth, during the wintering compared to the breeding season, might suggest that turtle doves might be more vulnerable to future changes, such as land cover conversion or climate changes, in their winter than in their breeding ranges.

Migration and stopovers

Like previous studies (Murton 1968; Lormée et al. 2016), our data clearly show that turtle doves are mainly nocturnal migrants. The migration durations shown by our tracked birds are similar to other studies (autumn migration: 21.3 and 22 days; spring migration: 28.3 and 20–21 days, Eraud et al. 2013; Lormée et al. 2016, respectively). Even if the mean duration did not vary remarkably between spring and autumn (27 and 25 days, respectively), the duration of autumn migration was more variable inter-individually (7–64 vs. 20–50 days), but also intra-individually (#161050: spring migration: 20–23 days and autumn migration 7–18 days, Table 2). As migration consists of flight and refueling periods, the total migration duration is determined by flight speed as well as variables reflecting fuel deposition performance. The latter, e.g., total stopover duration, are expected to have a much stronger impact than flight behavior on the duration of migration (Houston 2000; Nilsson et al. 2013; Schmaljohann 2018). Our results show a similar mean speed flight during active spring and autumn migration (approx. 46 vs. 51 km/h, respectively) as well as a similar stopover duration (12.4 vs. 11.9 days).

On spring migration, turtle doves were expected to stop over in the southern border area of the Sahara to refuel prior to crossing the desert enabling them to cross the Sahara, North Africa, the Mediterranean Seas as well as much of Southern Europe without additional stopovers (Zwarts et al. 2009). Only individual #181091 showed that behavior, staging in Mauritania, while the remaining individuals possibly

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may have fueled at their wintering sites already. Instead, we found that all complete spring migration tracks included a stopover in North Africa (Fig. 1). This is in line with the results of Eraud et al. (2013) and Lormée et al. (2016), showing that birds staged before crossing the Mediterranean Sea. As adult turtle doves have completed the flight-feather molt at that time, it is likely that these stopovers in North Africa are used to refuel before heading further north (Eraud et al. 2013), emphasizing the importance of these stopover sites for successful arrival at breeding grounds.

Contrary to the clear pattern during spring migration, the stopover pattern during autumn migration showed higher inter- and intraspecific variation. Importantly, in relation to current efforts on adaptive hunting management, the autumn migration of the majority of individuals (58.3% and excluding the individuals with breeding sites in Italy even 70.0%) included prolonged stopovers (> 10 days) in Europe, e.g., in France and Spain (Western flyway) or for the Central-Eastern flyway Slovakia and Hungary, representing the most important country for autumn stopovers (Supplementary Fig. 3). These stopovers as well as autumn migration movements match timewise with the legal hunting activities in the respective European countries (Fisher et al. 2018).

It is likely that the molt of the first inner primaries took place at the post-breeding and stopover molt sites (Demongin 2016) along with building up reserves for migration. A common suggestion for explaining this shift in habitat on the breeding area is that post-breeding adults might seek out for more abundant food resources and denser vegetation for cover, as they may be more vulnerable because of compromised flight capabilities while they molt (Vitz and Rodewald 2007; Tonra and Reudink 2018). Turtle dove #161050 was the only carrying out an autumn migration without any stopover but staged during the other years (Table 2). Also the migration routes of #161050 were not exactly the same over the years (Supplementary Fig. 1a). A larger inter-individual variation in autumn than in spring migration was also found in other tracked bird species (Alerstam et al. 2006; Vardanis et al. 2011; Stanley et al. 2012). This individual variation in migration routes may indicate that the birds navigate mainly by other means, e.g., responding to variation in environmental conditions, than a detailed route recapitulation based on the recognition of landmarks (Vardanis et al. 2011). Clearly more turtle dove tracks are needed to statistically confirm that the species might be quite flexible in space, i.e., flexibility in migration route.

By tagging different individuals in the same year as well as from the same breeding sites, we can show diverse movement patterns for individuals sharing a common breeding site. Individuals #181089, #181090, and #181092 all tagged at the same forest in Brandenburg started the autumn migration with a difference of up to over 1 month (05.08, 27.08, and 13.09.2019, respectively) and followed different

migration routes (Fig. 1). This was particularly notable in differing longitudes at which birds arrived at the African continent (ranging from 9.5, 13.8 to 18.0°O). Likewise, three individuals from one capture site in Hesse (#200350, #200351, and #200352) showed a similar variability in time for migration onset (31.07, 29.08, and 05.09.2020, respectively) and migration routes (Fig. 1). In other bird species, individuals from one breeding area or colony also follow different migration routes (Bächler et al. 2010; Schmaljohann et al. 2012; Trierweiler et al. 2014; Wellbrock et al. 2017). The fact that different individuals from the same breeding site performed diverse movement patterns during autumn migration suggests that several suitable areas for overwintering coexist, assuming turtle doves taking different migration routes also spend the winter period in different sub-Saharan areas as suggested by our tracking results and ringing data (Marx et al. 2016). This indicates a rather weak linkage between breeding and non-breeding grounds, i.e., a rather weak migratory connectivity. A rather weak migratory connectivity is in line with the non-existent genetic structuring across flyways (Calderón et al. 2016).

In general, our results confirm the three main migration routes previously suggested based on mark-recovery data (Dimaki and Alivizatos 2014; Marx et al. 2016). However, compared to ring recoveries, the tracking data provide a more detailed picture of the routes. Thus, we show that not all individuals following the Western migration route fly along the strait of Gibraltar but cross the Mediterranean Sea already further east by leaving from the Spanish mainland, indicating that turtle doves do not necessarily avoid larger sea crossings. Moreover, the expected course of the Central flyway through Italy and Malta was not taken by #200352, which instead crossed the sea further west through Corsica and Sardinia (Fig. 1).

The hypothesis for a loop migration pattern, i.e., using a flyway lying west or east of the spring route for autumn migration, in turtle doves hitherto assumed based on geolocator data (Eraud et al. 2013) is partly supported by our data. In particular, the partial tracks of #161048 and #161049 indicate a clockwise loop migration, i.e., an Eastern route for autumn migration and a Central route for spring migration. However, #181091, following the Western route, #161046 and #161,050, following the Central route for both migratory directions, provide no evidence for a consistent loop migration pattern. These findings together with the ringing studies, which demonstrated a regular mixing between the Central and Eastern flyway (Marx et al. 2016), indicate that loop migration might be more common for turtle doves following the Eastern flyway in autumn than for individuals following the Western or Central flyway. Two main likely factors may result in loop migration patterns in some turtle doves: regional variations in habitat availability and foraging conditions during the two seasons (Tøttrup et al. 2008;

Stach et al. 2016) and adaptation to prevailing wind patterns (Patchett and Cresswell 2020; Lisovski et al. 2021), such as different flight altitudes in relation to trade winds and antitrades during spring and autumn migration in the Sahara (Bruderer et al. 2018).

Turtle doves spend about two-thirds of the year away from their breeding grounds, at stopover sites, on active migration and in the wintering grounds, highlighting the importance of these periods in the life cycle when considering conservation efforts. Less strictly defined migration flyways, some individuals carrying out loop migration and an apparent rather weak migratory connectivity result in an overall observed pattern of migration occurring in a broad front instead of funneling at specific sites. Consequently, turtle doves should be considered as one (panmictic) population, spending time in many different countries during migration, demanding concerted conservation actions across all relevant countries to provide protection along all flyways in order to protect the entire population of turtle doves breeding in Europe.

Data availability Data generated and/or analyzed during the current study are included in the published article and its supplementary information files.

Declarations

Conflict of interest The authors declare no competing interests.

Ethics approval All applicable institutional and/or national guidelines for the care and use of animals were followed. Capture, handling and tagging was carried out under license number BR029/16 of the Wild Bird Regulation Unit (WBRU) and the Maltese Environment and Resources Authority (ERA) and under licenses of the regional council Hesse (license number TVA-51/2017), including an ethical approval according to the national animal protection law (TierSchG §7a), and the state office for occupational safety, consumer protection and health, Brandenburg (license number 2347–11-2018).

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CHAPTER 3

SHOULD I STAY OR SHOULD I FLY? MIGRATION PHENOLOGY, INDIVIDUAL-BASED
MIGRATION DECISION AND SEASONAL CHANGES IN FORAGING BEHAVIOUR OF
COMMON WOODPIGEONS

Yvonne R. Schumm, Juan F. Masello, Valerie Cohou, Philippe Mourguiart, Benjamin Metzger, Sascha Rösner, Petra Quillfeldt

ORIGINAL ARTICLE

Should I stay or should I fly? Migration phenology, individual-based migration decision and seasonal changes in foraging behaviour of Common Woodpigeons

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Abstract

Migration is used by many species as a strategy to deal with a seasonally changing environment. For some species, migration patterns can vary across different or even within the same breeding area. The Common Woodpigeon *Columba palumbus*, an abundant and widespread Palearctic species, exhibits three migratory strategies (strictly migratory, partially migratory and resident) across its European breeding grounds. Based on ring recoveries and satellite tracking data, we investigated the migration and foraging behaviour of Woodpigeons breeding in Southwestern Europe (Portugal) and Central Europe (Germany). We found that individuals could be classified as residents (Portugal) or partial migrants (Germany), with migrating individuals following the European sector of the East Atlantic flyway, and mainly wintering in France. In addition to general data on migration phenology, we provide evidence for different migration strategies (migration of varying distances or resident behaviour), low wintering site fidelity and the use of multiple wintering sites. Furthermore, tracking data provided information on migratory behaviour in consecutive years, clearly showing that individuals may switch migratory strategies (resident vs. migrant) between years, i.e. are facultative partial migrants. While individuals from Portugal mainly stayed within a large park ('green urban area') year-round, Woodpigeons from the city of Giessen (Germany) regularly left the urban area to forage on surrounding farmland (with an average distance covered of 5.7 km), particularly from July to September. Overall, our results highlight the behavioural plasticity in Woodpigeons in terms of foraging and migration strategies within and amongst individuals as well as populations.

Keywords *Columba palumbus* · Non-breeding partial migration · Ringing data · Satellite tracking · Wintering sites

Introduction

Animal movements are performed in a variety of ways, ranging from daily foraging movements, one-way dispersal movement, nomadism, to seasonally predictable round-trip migratory movements (Shaw 2020). In some bird species, strictly migrant to strictly resident wintering behaviours can occur (Chambon et al. 2018). However, in others, within-population migratory dimorphism exists, with some individuals migrating between habitats whilst others remain resident in a single habitat, so-called partial migration (Chapman et al. 2011a). Whether individuals in a population migrate can be determined genetically, i.e. fixed across lifetime, or depend on condition (e.g. age, sex, and personality) and the environment (e.g. resources, temperature and predation) or on a combination of these factors. Moreover, migratory behaviour may change within an individual's life

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(Lundberg 1987; Ogonowski and Conway 2009; Nilsson et al. 2016). For instance, individual Western Burrowing Owls *Athene cunicularia hypugaea* change their migratory tendency from one year to the next (Ogonowski and Conway 2009) and White-Winged Snowfinches *Montifringilla nivalis nivalis* adopt a partial migratory strategy, likely correlated to winter temperatures (Resano-Mayor et al. 2020).

The Common Woodpigeon *Columba palumbus* (henceforth Woodpigeon) is an abundant and widespread Palearctic bird species, which breeds throughout Europe (von Blotzheim and Bauer 1994; Saari 1997), where all of the aforementioned wintering behaviours appear. Woodpigeons breeding in Western Europe are mainly residents. Most Central European individuals are expected to be partial migrants, whereas populations from Eastern Europe and Fennoscandia are strictly migratory (Rouxel and Czajkowski 2004). For migrating Woodpigeons, three flyways in Europe were reported. Woodpigeons from Northern and Eastern Europe use the East Atlantic flyway, stretching along the coasts of the Baltic and North Seas to Atlantic coastal regions of South-West France and further to Spain and Portugal. Birds breeding in Hungary, the Czech Republic and Southern Germany use the Mediterranean flyway and winter in the West Mediterranean region, including Italy and Southern France, and particularly in Corsica and Sardinia. Individuals from South-Eastern Russia, Eastern Ukraine and birds breeding further east are likely to follow the Black Sea flyway (von Blotzheim and Bauer 1994; Bankovics 2001; Sruoga et al. 2005; Boere and Stroud 2006; Hobson et al. 2009; BirdLife International 2010; Butkauskas et al. 2013, 2019; Cavina et al. 2018).

Until recently, these migratory routes were mainly determined on the basis of available ring recovery data (Saari 1979; Bankovics 2001; Švažas 2001; Fiedler et al. 2004). However, ring recovery data are limited by poor recovery rates, particularly in Eastern and Northern Europe, and are biased due to different degrees of harvesting activity across Europe (Fiedler et al. 2004; Butkauskas et al. 2019). Furthermore, intensive ringing of Woodpigeons started only recently, in the last few decades (Negrier et al. 2020). Alternative approaches, such as hydrogen isotope analysis of feathers (Hobson et al. 2009) and genetic methods (Sruoga et al. 2005; Grosso et al. 2006; Butkauskas et al. 2013, 2019), were used to check for geographically-based divergence amongst Woodpigeon populations and to designate their flyways. Studies on genetics indicate a high genetic variability in Woodpigeons across Europe (Sruoga et al. 2005; Butkauskas et al. 2013, 2019), detecting the largest genetic distances amongst breeding Woodpigeons sampled in Central Europe and Portugal. Genetically similar were breeding individuals sampled in Germany and migratory Woodpigeons harvested in Northwest France (Sruoga et al. 2005). However, data from cytochrome *b* sequences did not support the existence of a geographically based divergence between populations (Grosso et al. 2006). Results from stable isotope analysis suggest that individuals harvested in Spain were primarily migrants from more northerly areas in Europe, and

Woodpigeons taken in Corsica were from Eastern Europe (Hobson et al. 2009). While the aforementioned approaches provide important information on a population level, some questions, particularly on individual level, such as migration timing and routes, fidelity to wintering sites and wintering behaviour, remain outstanding.

Once a typical woodland species, inhabiting deciduous, mixed and coniferous forests, an increase in population size had resulted in an expansion to urban areas across the European breeding grounds (Tomiałojć 1976; Slater 2001; Bea et al. 2011; Schuster 2017). Currently, the Woodpigeon is one of the most common bird species in many European cities and towns (Bea et al. 2011; König et al. 2015; Sakhvon and Kövér 2020). Nevertheless, individuals from urban areas usually fly to agricultural areas to feed upon farmland, i.e. perform foraging movements outside the actual city area (Slater 2001). In temperate, seasonal environments, such as Germany, the non-breeding season, i.e. winter, is often characterized by a deterioration of abiotic factors, e.g. shorter day length, lower temperatures and lack of some food sources, which might promote migration to more benign areas (Nilsson et al. 2011). However, in some regions of Germany, winter records of Woodpigeons increased since the year 2000. Yet, it is not clear whether rising numbers of resident birds or an influx of migrating individuals from more northerly areas cause this observed increase, in particular as it is challenging to observe individual Woodpigeons due to their possible extensive activity range (Schuster 2017).

The present study aims to update and improve our knowledge on Woodpigeon migration strategy as well as foraging behaviour throughout the annual cycle by analysing and comparing (a) ringing recoveries, (b) Argos satellite tracks from individuals tagged during the winter in France and Portugal, as well as (c) GPS tracks from individuals tagged at their breeding sites in Germany and Portugal. Based on these three datasets, the following hypotheses were investigated:

1. Woodpigeons breeding in Germany are partial migrants
2. Migrating individuals from Central Europe follow the East Atlantic flyway
3. Foraging movements and habitat use of Woodpigeons vary throughout the annual cycle and differ depending on the breeding region (Portugal vs. Germany)

Material and methods

Analysis of ring recoveries

The EURING Data Bank provided long-term ringing recoveries of Woodpigeons from Europe (EURING Data Bank extract 8th May 2020; du Feu et al. 2009). Recoveries of birds ringed from July 1929 until October 2019 were analysed ($n = 11{,}842$ recoveries). For the final analysis, only records of Woodpigeons ringed

during the breeding season in Germany (defined as 01 April – 30 September; von Blotzheim and Bauer 1994) and recovered during the wintering season within Germany (01 November – 30 February; Fiedler et al. 2004) or in any other European country (01 October – 30 March; von Blotzheim and Bauer 1994) and individuals ringed during the wintering season at any location and recovered during the breeding season in Germany were selected. Records with a time span of more than 5 years between ringing and recapture and an accuracy of date worse than 6 weeks were discarded. This resulted in a final data set of 315 ring recoveries belonging to Woodpigeons with breeding sites in Germany. The individual records were visualized as straight lines via mapping in QGIS 2.18 (QGIS Development Team 2016).

To indicate the main migration corridor, an analysis of line density kernels of mark-recovery lines for individuals wintering outside of Germany (n=216) was performed using the line density tool under Spatial Analyst in ArcGIS 10.7.1 (ESRI, Redlands California). Furthermore, kernel densities of ring recovery positions outside of Germany during the wintering time were calculated in R (R Core Team 2018) with the package 'adehabitatHR' (Calenge 2015) in order to illustrate main wintering sites. We used a generic grid of 100 cells and the bandwidth href (ad hoc method) as the smoothing parameter. No further analyses were conducted on the data set of Woodpigeons recovered within Germany during wintering time due to the low sample size of recovery records (n=99).

Analysis of Argos data

Data from 12 Woodpigeons, equipped with Argos transmitters (PTT non-solar or solar tags, Microwave telemetry, Inc., USA) in France (n=11) and Portugal (n=1) during the wintering season, were analysed (Table 1). These 12 Woodpigeons, caught from 2003 to 2014, comprised a sub dataset

of a larger project by GIFS France (Groupe d'Investigations sur la Faune Sauvage).

Argos transmitters deployed as backpacks were programmed with a duty cycle of 10 h ON/48 h OFF for 12 g transmitters and 10 h ON/24 h OFF for 18 g transmitters. Only location data as received from Argos of location classes (LC) 3, 2, and 1, which were afterwards checked for possible outliers manually (n=29 locations removed), were used for analysis. The filtered locations were plotted in QGIS in their original projection (WGS84) and likely migration tracks were displayed by using the 'Points2One' plugin (Kapusta 2015). To determine the different phases in the annual cycle, i.e. breeding, migration, stopover and wintering, we used a similar approach as described in Lormée et al. (2016). Clear switches in the pattern of the location data together with movements of at least 100 km from the wintering or breeding site defined the onset of spring or autumn migration, respectively. A stopover site was defined as consecutive set of locations overlapping spatially for a minimum of 3 days during the migration period.

To estimate breeding and wintering site fidelity for individuals providing data for consecutive years (n=5), repeatability of site utilization (based on longitude) was calculated as described in Lessells and Boag (1987). To avoid overrepresentation of individuals with multiple wintering sites, only the site occupied first was chosen for these birds. Migration phenology was specified by calculating the mean between all tracked individuals. If there was a time gap in the transmission of consecutive locations, the mean was selected and if the time gap was more than 14 days, the data were excluded from phenology analysis.

Analysis of GPS data

Between June 2018 and March 2021, we captured 21 Woodpigeons in Hesse, Germany (20 in the city of Giessen:

Table 1 Individual information of Common Woodpigeons *Columba palumbus* equipped with Argos transmitters during the wintering period in France and Portugal

Transmitter ID	Transmitter type [weight]	Deployment date [dd mm yyyy]	Capture location [Lat, Long]	Age	Sex	Weight [g]	End data transmission [dd mm yyyy]	No. locations
#39369	PTT non-solar [20 g]	05.02.2003	FR [43.833, −0.211]	Ad	–	560	17.08.2003	13
#90094	PTT solar [18 g]	04.02.2009	FR [43.730, −0.622]	Ad	–	520	07.01.2010	131
#90097	PTT solar [18 g]	05.02.2009	FR [43.995, −0.097]	Ad	–	580	15.09.2009	73
#90099	PTT solar [18 g]	11.02.2009	PO [38.111, −8.358]	Ad	–	580	11.10.2012	372
#104633	PTT solar [12 g]	17.02.2011	FR [43.730, −0.622]	Ad	–	520	18.04.2011	124
#104638	PTT solar [18 g]	17.02.2011	FR [43.730, −0.622]	Ad	–	–	20.07.2011	244
#104639	PTT solar [18 g]	17.02.2011	FR [43.730, −0.622]	Ad	–	530	04.09.2011	71
#104640	PTT solar [18 g]	18.02.2011	FR [43.995, −0.097]	Ad	–	530	28.05.2011	77
#113891	PTT solar [12 g]	16.02.2012	FR [43.833, −0.211]	Ad	m	550	11.07.2013	731
#123147	PTT solar [18 g]	13.02.2013	FR [44.102, −0.572]	Ad	m	520	02.03.2014	548
#133559	PTT solar [12 g]	29.11.2013	FR [44.571, 0.507]	Ad	f	465	07.05.2014	518
#141869	PTT solar [12 g]	07.11.2014	FR [43.760, 0.140]	Ad	f	500	28.01.2016	1033

Table 2 Individual information of Common Woodpigeons *Columba palumbus* equipped with GPS-GSM/GPRS transmitters on their breeding sites in Hesse (Germany) and Lisbon (Portugal)

Transmitter ID	Transmitter type[a]	Deployment date [dd mm yyyy]	Capture location [Lat, Long]	Age	Sex	Weight [g]	End data transmission [dd mm yyyy]	Migration
180777	OT-15-2GC	13.06.2018	DE [50.576, 8.690]	Ad	f	460	12.02.2020	Yes (FR)
180786	OT-15-2GC	29.06.2018	DE [50.568, 8.672]	Ad	f	555	31.05.2021[b]	No
180781	OT-15-2GC	13.12.2018	DE [50.566, 8.675]	Ad	f	580	17.01.2020	No
182891	OT-15-2G	27.02.2019	DE [50.569, 8.673]	Ad	f	550	15.04.2021	No
182890	OT-15-2G	19.03.2019	DE [50.587, 8.677]	Ad	f	535	31.05.2021[b]	No
180784	OT-15-2GC	03.05.2019	DE [50.571, 8.674]	Ad	f	635	31.05.2021[b]	Yes (FR)
191391	OT-15-2G	14.06.2019	DE [50.571, 8.671]	Ad	f	475	31.05.2021[b]	Yes (DE)[d]
190758	OT-15-3G	18.06.2019	DE [50.839, 8.677]	Ad	f	510	31.10.2020	Yes (FR)
191390	OT-15-2G	10.07.2019	DE [50.576, 8.690]	Ad	m	530	31.05.2021[b]	No
191392	OT-15-2G	22.07.2019	DE [50.569, 8.673]	Ad	m	560	31.05.2021[b]	No
191389_A	OT-15-2G	29.07.2019	DE [50.568, 8.672]	Ad	f	540	29.02.2020	No
190213	OT-15-2G	30.07.2019	DE [50.576, 8.690]	Ad	f	540	31.05.2021[b]	Yes (FR)
180778	OT-15-2G	31.07.2019	PO [38.722, -9.193]	Ad	m	390	31.05.2021[b]	No
180779_A	OT-15-2G	31.07.2019	PO [38.722, -9.193]	Ad	f	428	14.04.2020	No
180780_A	OT-15-2G	01.08.2019	PO [38.722, -9.193]	Juv[c]	m	398	16.11.2019	No
180782_A	OT-15-2G	14.08.2019	PO [38.722, -9.193]	Ad	f	385	20.09.2020	No
180783	OT-15-2G	14.08.2019	PO [38.722, -9.193]	Ad	f	395	31.05.2021[b]	No
180785_A	OT-15-2G	14.08.2019	PO [38.722, -9.193]	Juv[c]	m	385	23.11.2019	No
190759	OT-15-3G	24.04.2020	DE [50.572, 8.672]	Ad	f	480	08.04.2021	No
190760	OT-15-3G	10.06.2020	DE [50.571, 8.671]	Ad	f	515	04.12.2020	No
190761	OT-15-3G	12.06.2020	DE [50.571, 8.671]	Ad	m	540	31.05.2021[b]	No
190762	OT-15-3G	12.06.2020	DE [50.571, 8.671]	Ad	f	520	04.04.2021	No
190763	OT-15-3G	12.06.2020	DE [50.571, 8.671]	Ad	f	545	09.03.2021	Yes (DE)[d]
190764	OT-15-3G	16.06.2020	DE [50.571, 8.671]	Juv[c]	m	430	31.05.2021[b]	Yes (DE)[d]
190765	OT-15-3G	18.06.2020	DE [50.571, 8.671]	Ad	f	500	27.09.2020	No
190766	OT-15-3G	18.06.2020	DE [50.571, 8.671]	Ad	m	450	16.02.2021	No
180779_B	OT-15-2G	25.06.2020	PO [38.722, -9.193]	Ad	f	498	31.05.2021[b]	No
180780_B	OT-15-2G	30.06.2020	PO [38.722, -9.193]	Ad	m	498	31.05.2021[b]	No
180785_B	OT-15-2G	25.08.2020	PO [38.722, -9.193]	Ad	m	464	31.05.2021[b]	No
191389_B	OT-15-2G	31.08.2020	PO [38.722, -9.193]	Juv[c]	f	380	31.05.2021[b]	No
180782_B	OT-15-2G	31.03.2021	DE [50.569, 8.673]	Ad	f	525	31.05.2021[b]	-

[a]All individuals were equipped with OT-15 (OrniTrack-15 solar powered GPS-GSM/GPRS) transmitters, but differ in models: 2G or 3G model

[b]Data of still functioning transmitters was included until 31.05.2021

[c]Juv = hatched during current calendar year

[d]Individual used another distinct site during the non-breeding, i.e. wintering season, than during the breeding season, but migratory movements occurred within the same country (see Fig. S6)

50°35′ N, 8°40′ E and one in the forest of Caldern: 50°50′ N, 8°39′ E. Most (*n* = 19) of these were captured during the breeding season (mid-March – August), and only two during winter (Table 2). Furthermore, 10 Woodpigeons were captured in Lisbon, Portugal (38°43′ N, 9°10′ W) prior to the migratory season (June – August, Table 2). We determined the age of each bird by plumage examination (Demongin 2016) and the sex by molecular analysis (Griffith et al. 1998, Table 2). Individuals were fitted with an OrniTrack-15 solar powered GPS-GSM/GPRS transmitter

(Ornitela, Lithuania), fixed as a backpack using a 4-mm-width Teflon ribbon harness. OrniTrack-15 transmitters were programmed to take a GPS-position every 5 min if the battery was more than 75%, every 30 min (battery > 50%) and every 4 h (battery > 25%). No GPS-fixes were received during the night (GPS set to sleep from a sun angle of − 6° from dusk to dawn). GPS data were checked for erroneous locations by applying a speed filter to omit all data points exceeding 30 m/s (Bruderer and Boldt 2001). Migration tracks were displayed in QGIS in WGS 84 projection as

described for Argos data. Breakdown in the different phases of the annual cycle, site fidelity and migration phenology were evaluated as delineated for the Argos tracking data.

Movements and habitat use

The habitat use and foraging movements between individuals of the cities Giessen, Germany ($n = 19$; #190758 individual from forest and #190759 individual from Herborn were excluded as they were not comparable due to the different types of occupied habitat) and Lisbon, Portugal ($n = 10$) were compared. Data from Argos transmitters were not considered for kernel utilization distributions (KUD) analysis due to partly large time gaps between consecutive localizations. To estimate the area used by the individual Woodpigeons, Epanechnikov kernels (95% and 50% KUD; Epanechnikov 1969) were calculated in R with the function *kernelUD* in the package 'adehabitatHR' (Calenge 2015) and the R package 'sp Classes and Methods for Spatial Data' (Pebesma 2020) with a generic grid of 100 cells ($n = 370$) or 500 cells ($n = 49$) and the smoothing parameter was estimated with a href parameter (ad hoc method). KUDs were calculated of GPS positions from wintering and breeding sites per month. Months for which localizations were only partially available (e.g. in month of capturing) were excluded. To characterize the land cover in the occupied home ranges (95% KUD), the KUDs were superimposed and subsequently clipped in QGIS with Corine Land Cover CLC 2018 v.2020_20u1 raster land cover data (Copernicus Land Monitoring Service 2021).

For Woodpigeons with breeding sites in the city of Giessen ($n = 19$), the distance travelled between city habitats and agricultural (foraging) sites outside the city was estimated by calculating the distance between the mean coordinates of the monthly 95% KUD polygon parts in the city and the agricultural area in R with the function *distm* in the 'geosphere' package (Hijmans et al. 2019). The mean coordinates were used also for circular statistics of foraging flight direction in Oriana 4 (Kovach Computing Services, Anglesey, Wales, https://www.kovcomp.co.uk/oriana/).

Results

Migratory behaviour and movements

Ring recoveries

A 28% of the ring recoveries from Woodpigeons ringed in Germany during the breeding season and recovered during wintering period were found within Germany, while 72% outside Germany. Most recoveries out of Germany were from France (85%). Ring recoveries within Germany

indicate resident or short-distance migrating Woodpigeons (Fig. S1). Migrating Woodpigeons leaving Germany wintered near the German border (Netherlands, Belgium, Denmark, East and South-East France) or flew greater distances to South-Western France, Spain and Portugal (Fig. 1).

According to the ring recoveries, all individuals followed the western European part of the East Atlantic flyway. Line density kernels showed a south-westerly migration direction with the majority of Woodpigeons ending their migration in France (Fig. 1b). Density kernels of positions of ring recoveries during wintering time indicate South-Western France (regions: Occitanie and Nouvelle-Aquitaine) as the wintering region for the majority of migrating Woodpigeons with breeding sites in Germany. The majority of ring recoveries (90%) in all countries were due to hunting activities (Table S1).

Argos tracking data

Woodpigeons equipped during the non-breeding season in France and Portugal ($n = 12$) departed for spring migration on average on 13 March (21 February – 21 March) and arrived at the breeding sites on 07 April (20 March – 14 May; Fig. 2, S2 and S3). On average, one stopover (range $0 - 4$) lasting 8.9 ± 4.5 days ($n = 20$ stopovers) was made during spring migration. Breeding sites were located in the German federal states Bavaria ($n = 4$), Baden-Württemberg ($n = 1$), Rhineland-Palatinate ($n = 1$), Thuringia ($n = 1$), Lower Saxony ($n = 1$), North Rhine-Westphalia ($n = 1$) as well as in Switzerland ($n = 1$; Fig. S2).

For five Woodpigeons, data transmission lasted beyond the first breeding season after transmitter deployment. Migrating individuals ($n = 4$) departed from the breeding sites on 11 October (26 September–18 October) and arrived at the wintering sites on 29 October (22 October–10 November) with 0.5 stopovers on average (range 0–2) which lasted 7.0 ± 0.0 days ($n = 2$).

In the tagging year, only individual #141869 (Table 1) used several distinct wintering sites ($n = 3$; Fig. S2). However, most individuals (83%) were tagged in February (Table 1), which is rather at the end of the wintering period; therefore, the use of multiple wintering sites in the year of tagging might be underestimated in the Argos data set. For migrating birds of which we have data for a second wintering period ($n = 4$), 75.0% used two distinct wintering sites (Fig. S2). We observed high repeatability of breeding site utilization (Longitude: $r = 1.0$, Anova: $F_{3,4} = 162{,}009.0$, $p < 0.01$), whereas a low repeatability of location for the wintering sites (Longitude: $r = 0.008$, Anova: $F_{8,9} = 1.0$, $p = 0.484$) between two consecutive years.

GPS tracking data

GPS tracking data revealed three migratory strategies of Woodpigeons (Table 2; Fig. S4). 4 out of 21 individuals tagged in Hesse

Fig. 1 Ring recoveries of Common Woodpigeons *Columba palumbus* with breeding sites in Germany spending the wintering period outside of Germany. **a** Validated ring recoveries are represented as a line between ringing and recovery site. Different colours correspond to the respective country in which the wintertime was spent. **b** Line density kernels for Woodpigeon ring recoveries. Kernel densities of ring recovery positions outside of Germany during the non-breeding period are displayed as dashed line (50% kernel) and dotted line (95% kernel). Background colours indicate the terrain (Background map: Stamen terrain (map tiles by Stamen Design: maps.stamen.com; data by OpenStreetMap: www.openstreetmap.org))
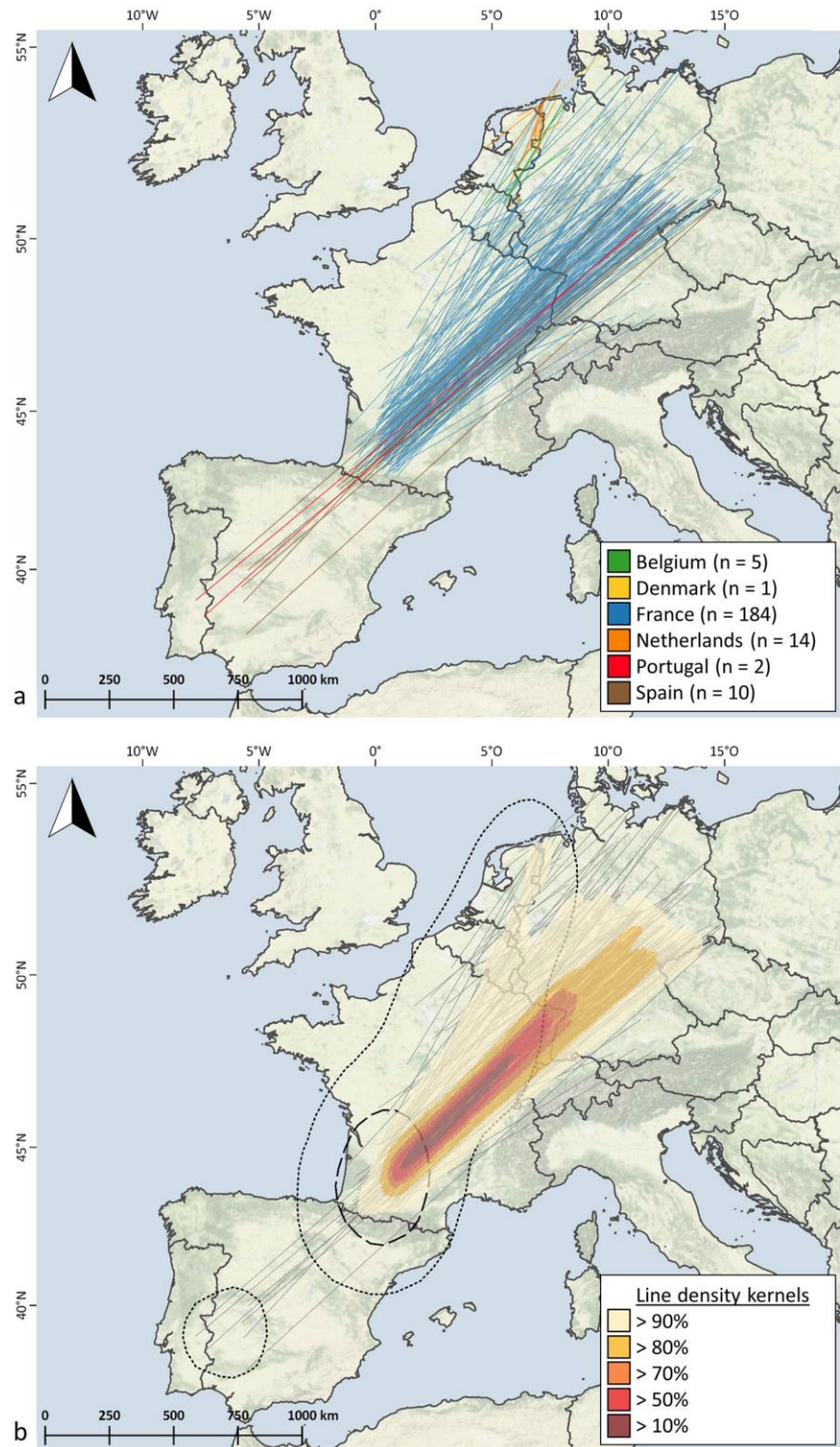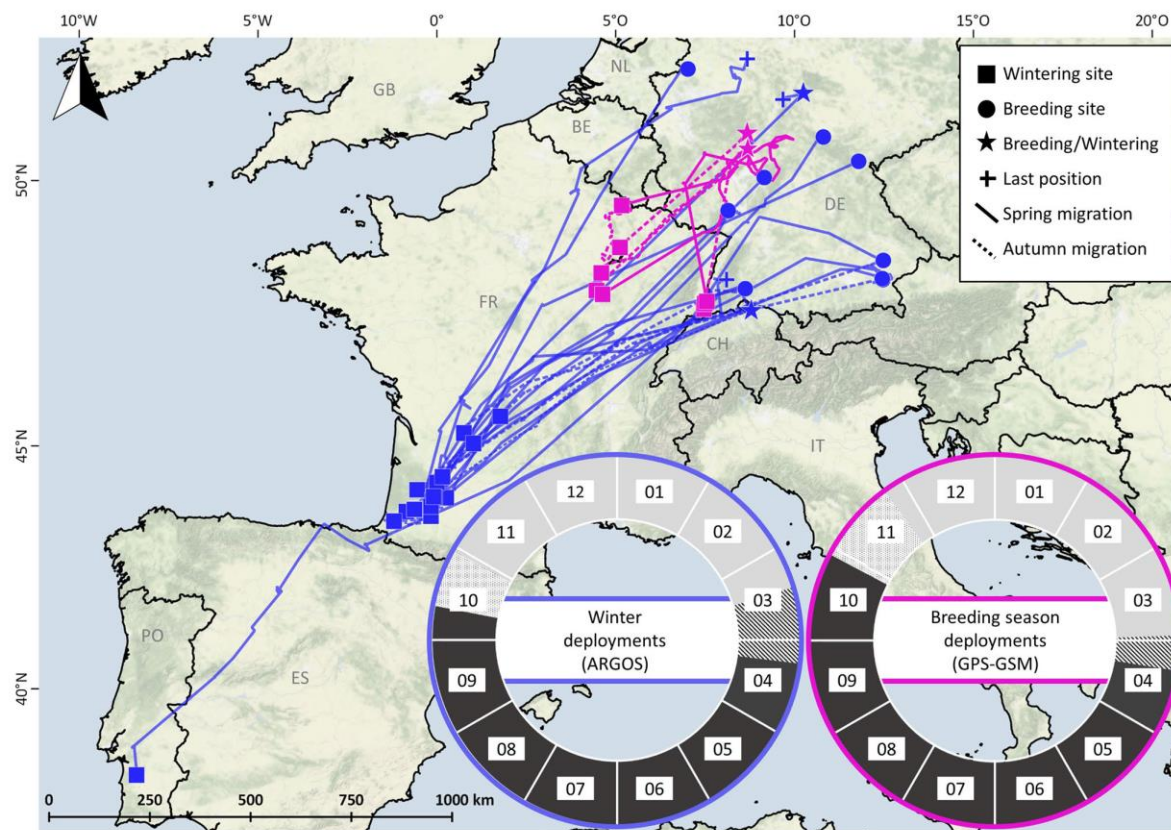
Fig. 2 Migratory movements of Common Woodpigeons *Columba palumbus* equipped with Argos-transmitters (*n* = 12) during the winter season in Portugal and France (dark blue lines and symbols) or during the breeding season with GPS-GSM transmitters (*n* = 4) in Hesse, Germany (pink lines and symbols). The map gives the spatial organization with spring migration (solid line) and autumn migration (dashed line) between the breeding sites (circles) and the winter sites (squares). The star symbol indicates that breeding and winter time in at least 1 year were spent at the same location. Crosses indicate that the last position was transmitted outside the breeding or winter site. The insets show the temporal organization with percentages of time for period spent at the breeding site (dark grey), the non-breeding site (light grey) and on migration (striped = spring migration; dotted = autumn migration) and average arrival and departure for each respective period. Background colours indicate the terrain (Background map: Stamen terrain (map tiles by Stamen Design: maps.stamen.com; data by OpenStreetMap: www.openstreetmap.org))

performed migratory movements, spending the wintering time in France (regions: Grand-Est and Bourgogne-Franche Comté, Figs. 2 and S5). These 4 individuals started autumn migration on average on 28 October (20 October – 16 November) and reached the wintering sites on 24 November (15 November – 2 December) with an average migration time of 27.5 ± 8.9 days, including an average number of 3.0 stopovers (range 1 - 6) and total stopover duration of 20.5 ± 9.0 days. After wintering for 123.7 ± 6.8 days at 1 to 3 wintering sites, Woodpigeons started spring migration on 29 March (24 March – 9 April) and arrived after 11.0 ± 8.9 days of spring migration, including 0 to 1 stopover up to 15 days, on 9 April (1–24 April) at their breeding sites in Hesse.

Three further individuals (#190763, #190764 and #191391; Table 2) spent the wintertime in different sites than during the breeding season, though without leaving Germany. These wintering sites were approx. 10 km, 20 km and 40 km away from the breeding area. Woodpigeons reached them in less than one day (Fig. S6). One individual (#190759; Table 2) changed several times (*n* = 11) between two sites, however, not only during the winter period (Fig. S6), and this was not considered as migration. Migratory movements of Woodpigeons tagged in Hesse could be observed in the wintering seasons 2019/20 (5 of 12 individuals, 41.7%) and 2020/2021 (2 of 15 individuals, 13.3%), whereas no migratory movements were observed during the winter of 2018/19 (2 individuals). For migrating individuals from Hesse of which data were available for two consecutive years (*n* = 5, Table 3), repeated site utilization suggested a high breeding site fidelity (Longitude: *r* = 0.909, Anova: $F_{4,5}$ = 42.9,

$p < 0.001$), whereas wintering site fidelity was weak (Longitude: $r = -0.381$, Anova: $F_{3,4} = 0.4$, $p = 0.732$).

None of the Woodpigeons tagged in Portugal ($n = 10$) exhibited migratory movements. However, two individuals (#191389 and #180783; Table 2) showed movements between sites approx. 20 km apart from each other throughout the annual cycle similar to one of the German birds (#190769; Table 2).

Habitat use and foraging movements of Woodpigeons from two European cities

According to GPS positions, the average size of Woodpigeons core area of use (50% KUD) was 0.7 ± 0.3 km^2 or 1.0 ± 0.3 km^2 and home range (95% KUD) was 4.2 ± 1.5 km^2 or 7.5 ± 1.3 km^2, for individuals from Lisbon and Giessen, respectively. Whereby monthly core areas and home ranges were mostly < 1.0 km^2 (87% and 62%, respectively; Table S2, Figs. S7 and S8). In general, core areas as well as home ranges were larger for Woodpigeons in Giessen compared to individuals in Lisbon (Mann–Whitney: 50% KUD: $W = 22072$, $p < 0.001$; 95% KUD: $W = 21645$, $p < 0.001$).

Whereas the sizes of core area and home range varied across the annual cycle, i.e. was significantly different for the different months, for individuals in Giessen, it was not the case for Woodpigeons in Lisbon (Kruskal–Wallis: Giessen: 50% KUD: $\chi^2 = 56.45$, d.f. $= 11$, $p < 0.001$; 95% KUD: $\chi^2 = 67.68$, d.f. $= 11$, $p < 0.001$; Lisbon: 50% KUD: $\chi^2 = 6.10$, d.f. $= 11$, $p = 0.866$; 95% KUD: $\chi^2 = 7.36$, d.f. $= 11$, $p = 0.770$).

Woodpigeons in Lisbon mainly stayed within the '*Parque Florestal de Monsanto*', an approximately 800 ha wooded park categorized as '*green urban area*' by the CLC land cover data (Fig. 3), in which they were caught and tagged, leaving the park area only occasionally (Table S3). When they left the park area, their monthly home range was significantly larger (Mean 95% KUD: visits outside park: 26.3 km^2, only inside park: 0.4 km^2; Wilcoxon rank sum test: $W = 16$, $p < 0.001$).

Woodpigeons tagged in Giessen regularly left the city area to fly to agricultural areas/farmland located mainly south-westerly of the city, particularly between July and September (Figs. 3 and 4; Table S3), resulting in an enlarged home range size (mean 95% KUD: flights to farmland: 13.0 km^2, only within city area: 0.7 km^2; Wilcoxon rank sum test: $W = 518$, $p < 0.001$). The average distance travelled to the agricultural sites was 5.7 ± 0.2 km (maximum: 19.7 km).

Discussion

Foraging movements and habitat use

While forests were the original breeding habitat, breeding Woodpigeons are increasingly recorded in many European towns since the 1970s (Tomiałojć 1976; Sruoga et al. 2005;

Bea et al. 2011). Typically, urban areas contain novel food items, such as non-native species and intentionally provisioned food. This can cause a diet shift (Murray et al. 2018), which in turn may also alter foraging behaviour. GPS data revealed differences in foraging movements and habitat use between individuals from Lisbon and Giessen and seasons. Birds in Lisbon rarely and seasonally independently left the '*green urban area*', whilst individuals from Giessen regularly visited surrounding farmland (Figs. 3 and 4). This difference is also reflected in the habitat use: Whilst for individuals in Lisbon the proportion of '*artificial surfaces*' barely varies throughout the year, it clearly decreases and is replaced by '*agricultural areas*' in summer and early autumn for Woodpigeons in Giessen, resulting in an enlarged home range size.

Woodpigeons are granivorous-frugivorous with an opportunistic nature adapting their dietary choices according to (seasonal) food availability, resulting in significant variations of consumed items between seasons (Ó hUallachain and Dunne 2013; Gutiérrez-Galán et al. 2017; Negrier et al. 2020). Particularly during summer and beginning of autumn, previous studies pointed out grains of cereal crops as major part of the diet (Murton et al. 1964; Gutiérrez-Galán et al. 2017; Negrier et al. 2020). This is in line with our result showing that Woodpigeons from an urban population of Giessen undertook foraging trips to surrounding agricultural areas mainly from July to September (Fig. 4). Anthropogenic plant species provided at bird feeders were found in faecal samples of columbiform birds in the UK (Dunn et al. 2018) and Woodpigeons are nowadays regularly recorded at bird feeders (Reynolds et al. 2017; Darryl 2018). It is thus evident that Woodpigeons breeding in urban areas find part of their food, and in the case of individuals wintering in Giessen, the majority of their food in their urban areas (but see Tomiałojć 1999). However, comparing the two study sites, it is obvious that different foraging strategies exist: Woodpigeons in Lisbon appear to find their food almost exclusively within the urban park area throughout the year, whereas individuals in Giessen left the urban area to forage on farmland. The covered distance to reach the farmland feeding sites observed in this study (5.7 km averagely) is similar to previously observed distances in other locations (5–15 km: Wrocław, Tomiałojć 1999; min. 6 km: Liverpool, Slater 2001; > 10 km: Bejaia, Moali et al. 2003). The observed main foraging flight direction (south-westerly, Fig. 4) might be influenced by the regional distribution of farmland. However, farmland is surrounding the city of Giessen in various cardinal directions. The spatial directed foraging behaviour might be also caused by the gregarious feeding behaviour of Woodpigeons (Murton et al. 1966, 1971), as previous tracking data support a memory-based model with a flocking behaviour rather than an optimal foraging model as their foraging strategy (Kułakowska et al. 2014). Our results point to a distinct plasticity in foraging habits

Table 3 Details of the annual schedule of migrating Common Woodpigeons *Columba palumbus* equipped with Argos satellite tags (n = 12) or GPS-GSM/GPRS transmitters (n = 7, only individuals showing migratory movements are displayed). Given are the dates [dd.mm] for the first and last transmitted location from each phase of the annual cycle

Transmitter ID	Data type	Year	Wintering		Spring Migration		Breeding		Autumn migration	
			Area	First–Last signal	Yes/No	First–Last signal	Area	First–Last signal	Yes/No	First–Last signal
#39369	Argos	2003	FR: Nouvelle-Aquitaine	ST[a]–08.03	Yes	11.03–20.03	DE: Bavaria	17.08[b]*		
#90094	Argos	2009	FR: Nouvelle-Aquitaine	ST–11.03	Yes	18.03–03.04	DE: Lower Saxony	15.04–NA[c]	No	
		2009/10	DE: Lower Saxony	NA–07.01*						
#90097	Argos	2009	FR: Nouvelle-Aquitaine	ST–13.03	Yes	14.03–03.04	DE: Bavaria	07.04–15.09*	No	
#90099	Argos	2009	PO: Lisbon	ST–12.03	Yes	13.03–13.05	CH	14.05–NA	Yes	11.10–23.10
		2010	CH/DE: Baden Wurtemberg	NA–NA	No		CH	NA–27.09	No	
		2010/11	FR: Nouvelle-Aquitaine	30.10–19.03	Yes	20.03–23.03	CH	25.03–NA	No	
		2011/12	CH	NA–11.10*						
#104633	Argos	2011	FR: Nouvelle-Aquitaine	ST–16.03	Yes	18.03–11.04	DE: Baden Wurtemberg[d]	13.04–18.04*		
#104638	Argos	2011	FR: Nouvelle-Aquitaine	ST–21.03	Yes	22.03–20.04	DE: North Rhine-Westphalia	21.04–20.07*		
#104639	Argos	2011	FR: Nouvelle-Aquitaine	ST–17.03	Yes	20.03–26.03	DE: Rhineland-Palatinate	31.03–04.09*		
#104640	Argos	2011	FR: Nouvelle-Aquitaine	ST–21.03	Yes	23.03–28.05*			Yes	19.10–05.11
#113891	Argos	2012	FR: Nouvelle-Aquitaine; Occitanie	ST–21.02	Yes	25.02–28.03	DE: Bavaria	30.03–17.10	Yes	25.10
		2012/13	FR: Nouvelle-Aquitaine	10.11–09.03	Yes	09.03–03.04	DE: Bavaria	05.04–11.07*		
#123147	Argos	2013	FR: Nouvelle-Aquitaine	ST–07.03	Yes	08.03–13.04	DE: Bavaria	14.04–18.10	Yes	
		2013/14	FR: Nouvelle-Aquitaine	02.12–02.03*						
#133559	Argos	2013/14	FR: Nouvelle-Aquitaine	ST–06.03	Yes	08.03–18.03	DE: Thuringia	20.03–07.05*	Yes	
#141869	Argos	2014/15	FR: Nouvelle-Aquitaine; Occitanie	ST–17.03	Yes	19.03–07.04	DE: Baden Wurtemberg	09.04–26.09	Yes	29.09–20.10
		2015/16	FR: Nouvelle-Aquitaine; Occitanie	22.10–28.01*						
#180777	GPS	2018	DE: Hesse		No		DE: Hesse	ST–NA	No	
		2018/19	DE: Hesse				DE: Hesse	NA–21.10	Yes	21.10–21.11
		2019/20	FR: Grand-Est	21.11–12.02*						
#180784	GPS	2019	FR: Grand-Est; Bourgogne-Franche Comté	02.12–27.03	Yes	28.03–01.04	DE: Hesse	ST–16.11	Yes	16.11–02.12
		2019/20					DE: Hesse	01.04–NA	No	
		2020/21	DE: Hesse	NA–NA	No		DE: Hesse	NA–21.05*		

Table 3 (continued)

Transmitter ID	Data type	Year	Wintering		Spring Migration		Breeding		Autumn migration	
			Area	First–Last signal	Yes/No	First–Last signal	Area	First–Last signal	Yes/No	First–Last signal
#191391	GPS	2019					DE: Hesse	ST–14.11	Yes	14.11–14.11
		2019/20	DE: Hesse	14.11–28.03	Yes	28.03–28.03	DE: Hesse	28.03–NA	No	
		2020/21	DE: Hesse	NA–NA	No		DE: Hesse	NA–21.05*		
#190758	GPS	2019					DE: Hesse	ST–23.10	Yes	23.10–29.11
		2019/20	FR: Grand-Est	29.11–03.04	Yes	03.04–24.04	DE: Hesse	24.04–31.10*		
#190213	GPS	2019					DE: Hesse	ST–20.10	Yes	20.10–15.11
		2019/20	FR: Grand-Est	15.11–23.03	Yes	24.03–01.04	DE: Hesse	01.04–NA	No	
		2020/21	DE: Hesse	NA–NA	No		DE: Hesse	NA–31.05*		
#190763	GPS	2020					DE: Hesse	ST–17.11	Yes	17.11–17.11
		2020/21	DE: Hesse	17.11–09.03*						
#190764	GPS	2020					DE: Hesse	ST–06.11	Yes	06.11–07.11
		2020/21	DE: Hesse	07.11–17.02	Yes	17.02–18.02	DE: Hesse	18.02–31.05*		

* Marks the date on which the last location was transmitted, i.e. end of data transmission

a ST = Start of transmission, i.e. tagging during this period (see Tables 1 and 2)

b Only a single location from the breeding area was received

c NA is given if the period could not be clearly defined as no migratory movements occurred, i.e. resident individual

d Location transmission stopped shortly after arrival. Further movements cannot be ruled out
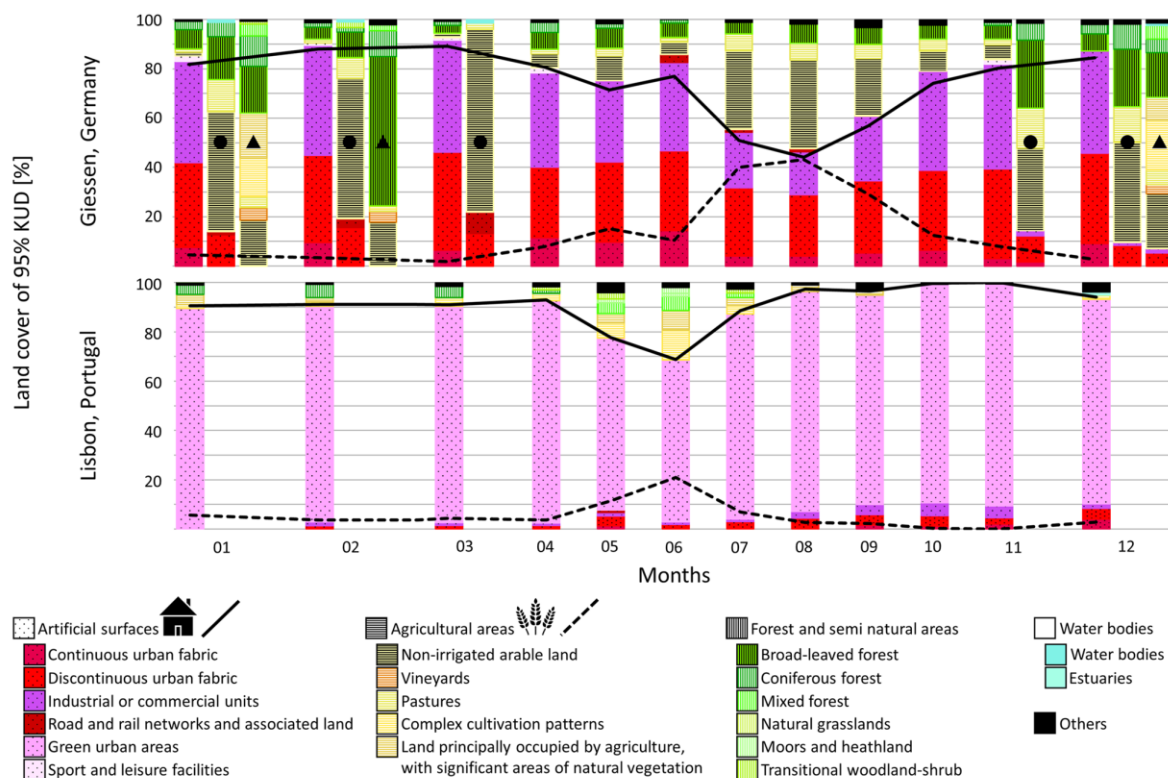
Fig. 3 Average proportions of land cover categories in monthly home ranges used by tagged Common Woodpigeons *Columba palumbus*. Shown are Woodpigeons from two regions (Lisbon, Portugal, $n = 10$ and Giessen, Germany, $n = 19$). The different wintering strategies of individuals from Giessen are shown separately for the wintering period: residents (no symbol), individuals using another distinct site during the winter than during the breeding season, but migratory movements occurred within Germany (black circle symbol), and

Woodpigeons migrating to France (black triangle symbol). Categories occurring with < 1% were combined into '*Others*'. Land cover categories and associated colours were chosen according CORINE land cover (CLC) nomenclature. Black lines represent the average proportion of the land cover main categories '*Artificial surfaces*' (continuous line) and '*Agricultural areas*' (dashed line). Sample sizes and detailed proportions can be found in Table S3

for 'urban' Woodpigeons, most likely adapted to different uses of foraging habitats as the productivity, i.e. available food, of these habitats changes over time (Bendjoudi et al. 2015), e.g. cereal ripening in July, and variable food supply in different cities (Rose et al. 2006), such as the proportion of green urban areas or distance to closest surrounding fields.

Resident or migrant species?

While the Woodpigeons tracked in Lisbon were definitely residents (as expected, see Sruoga et al. 2005), all three methods demonstrated that some Woodpigeons with breeding ground in Germany winter in Germany, whilst other individuals migrate along the East Atlantic flyway to mainly France and less frequently to Spain, Portugal, Belgium, Netherlands and Denmark (Figs. 1 and 2, Table 3). Tracking-based methods provide information on migratory behaviour of individuals for consecutive years, clearly

showing that the individual based migratory decision can vary from year to year. Therefore, Woodpigeons with breeding sites in Germany can be classified as facultative partial migrants (Nilsson et al. 2016; Chambon et al. 2018), performing a non-breeding partial migration, i.e. sympatric breeding and allopatric wintering (Chapman et al. 2011a), with individuals switching migratory strategies (resident vs migrant) between years. This results in annually fluctuating numbers of migrating and resident individuals (e.g. our study: winter 2019/20: 42% vs. winter 2020/21: 13% migrating Woodpigeons).

Fluctuating numbers of Woodpigeons were also recorded at French, Spanish and Portuguese wintering sites (Beitia et al. 2001; Bea et al. 2003; Cohou et al. 2006, 2007; Lanusse et al. 2006; Lormée and Aubry 2018). It was hypothesized that the inter-annual fluctuations of migrants might occur due to a shift of their migratory route and/or wintering sites (Bea et al. 2003; Cohou et al. 2007). Such a shift to wintering sites to
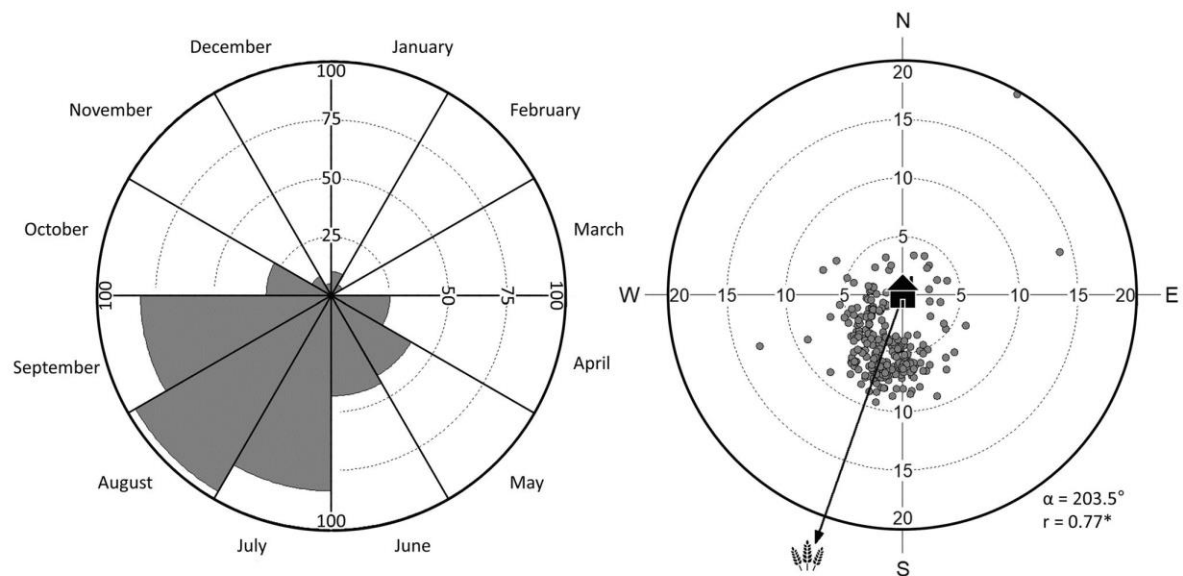
Fig. 4 Foraging behaviour of Common Woodpigeons *Columba palumbus* (*n* = 19) with breeding sites in the city of Giessen, Hesse (*n* = 19). Left: Circular diagram showing the proportion of individuals (0–100%) leaving the city area, i.e. artificial surfaces, to forage outside the city on agricultural used areas. Foraging outside was assumed if parts of the home range (95% Kernel Utilization Distributions KUD) were located on agricultural areas outside the city area. Right: The circular diagram represents the direction (geographic North N corresponds to 0°) and distance (0–20 km) Woodpigeons flew from the city of Giessen to foraging sites outside the city area. Each data point indicates the orientation of one individual bird for its monthly 95% KUD. Arrow indicates the mean direction (α) and vector length (*r*; * *p* < 0.001, Rayleigh test). Symbols used as in Fig. 3

South-Western France was associated with the intensification of maize monoculture there (Lanusse et al. 2006). So far, there was no unambiguous data on the Woodpigeon site fidelity to certain wintering sites (Sruoga et al. 2005). Based on Argos and GPS tracking data, we provided evidence for a low wintering site fidelity, in contrast to being faithful to their breeding sites. Furthermore, the tracking data revealed the use of multiple wintering sites (Figs. S2 and S5), presumably following the availability and accessibility of food resources (Díaz and Martín 1998; Lanusse et al. 2006; Cohou 2013). Annually varying winter sites due to a low wintering site fidelity and exploitation of multiple wintering sites might explain fluctuating counts of Woodpigeons at wintering sites partly. Alternative hypotheses for the observed fluctuations in wintering Woodpigeons might be that the numbers are influenced by fluctuations in breeding success (cf. Robillard et al. 2016) or that due to warmer winters previous migrants may now winter at their breeding sites or only perform shorter-distance migration movements (Hobson et al. 2009; Butkauskas et al. 2019). Migratory movements of only around 10 km to 40 km, leaving the city area to winter in close wood- and farmland, were observed for three individuals in our study (Fig. S6). Interestingly, independent of migration distance (> 100 km outside of Germany vs. < 50 km within Germany), the onset of autumn migration was quite similar (average 11 November and 12

November, respectively), whereas the individuals wintering outside Germany arrived almost 1 month later (08 April) at their breeding sites compared to the migrants wintering closer to their breeding site (09 March; Fig. S4). Wintering closer to the breeding sites might constitute an intermediate tactic between more distant migration and residence, minimizing the disadvantage of migrants in competition over high-quality territories due to later arrival at the breeding sites (see 'arrival time' hypothesis in Chapman et al. 2011a).

Most individuals with breeding sites in the city of Giessen spent the winter season mainly within the urban area without exhibiting any migratory movements (Table 2, Figs. 3 and S4). Generally, urbanization may affect individual migration strategies, favouring resident behaviour, because urban areas are characterized by large and predictable anthropogenic food resources and due to the urban heat island effect are warmer than rural areas (Evans et al. 2012; Jokimäki and Kaisanlahti-Jokimäki 2012; Jokimäki et al. 2016; Bonnet-Lebrun et al. 2020).

Observed plasticity in individual migratory decisions of Woodpigeons suggests that migratory strategy is unlikely to be strictly and solely genetically fixed (see also Ogonowski and Conway 2009; Lundblad and Conway 2020). However, for the data examined here, the question why some individuals migrate and others do not within the same population and even same city still remains. A multi-taxa meta-analysis found consistently

higher fitness of residents over migrants in birds (Buchan et al. 2020, but see Zúñiga et al. 2017). However, further exploration of the effects on fitness in terms of survival and reproductive outcome dependent on the chosen wintering tactic and vice versa would be helpful to evaluate the differences between the migratory strategies (Chambon et al. 2018; Buchan et al. 2020).

Conclusion and outlook

Our study provides the first tracking data of Woodpigeons in Europe for consecutive years, revealing pronounced plasticity in intra-species and intra-individual migration and foraging behaviour. In this way, our results add to the body of evidence that migratory movements in partial migratory birds are not solely a genetically fixed behaviour as they can change from year to year. The observed individual and within-species variation in migratory decision might be influenced by numerous factors and their interactions (reviewed in Chapman et al. 2011a and Hegemann et al. 2019) such as varying (local) food supply, e.g. mast seeding of oaks or beeches (Nilsson et al. 2006; Selås 2017), climatic conditions like winter temperature or snow cover (Mulsow 1979; Resano-Mayor et al. 2020) or individual traits (Chapman et al. 2011b; Fudickar et al. 2013). However, the small sample size available for migrating individuals precluded rigorous statistical comparison and modelling. In general, studying species with a plastic migratory behaviour can lend insight into the intrinsic and extrinsic factors that mediate this decision and how animals respond to environmental dynamics in terms of migration and, hence, gain insight into the evolution of which mechanisms underlie migratory behaviour more generally (Berthold 1999; Bowlin et al. 2010; Bonnet-Lebrun et al. 2020; Lundblad and Conway 2020).

Future studies tracking individuals year-round and over several years, tying migratory decisions to measures of individual fitness and environmental parameters but also to sex and age class and examining effects that carry-over across different stages of the annual cycle will help to understand the proximate and ultimate drivers and consequences of migratory decisions (cf. Lundblad and Conway 2020). In particular, in the framework of ongoing climate change, predicted to have profound effects on migrants (Berthold 1999; Bonnet-Lebrun et al. 2020), and increasing urbanization, which both may interact in their effects on the birds (Greig et al. 2017), studying species with pronounced variation in migratory behaviour, such as Woodpigeons, might be particularly valuable.

Declarations

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CHAPTER 4

DIET COMPOSITION OF WILD COLUMBIFORM BIRDS: NEXT-GENERATION SEQUENCING OF PLANT AND METAZOAN DNA IN FAECAL SAMPLES

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Diet composition of wild columbiform birds: next-generation sequencing of plant and metazoan DNA in faecal samples

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Abstract

Next-generation sequencing technology has become a powerful and non-invasive tool for diet reconstruction through DNA metabarcoding. Accurate knowledge of species' diets is fundamental to understand their ecological requirements. Here we applied next-generation sequencing and DNA metabarcoding on faecal samples of European Turtle Doves *Streptopelia turtur* (n = 19), Stock Doves *Columba oenas* (n = 71) and Common Woodpigeons *C. palumbus* (n = 49) to investigate their dietary composition. By applying primer pairs targeting both the ITS2 region of plant nuclear DNA and the mitochondrial COI region of metazoan DNA, we provide a complete picture of the food ingested and estimate the dietary overlap between the columbiform species. Animal DNA was present very rarely, and a diverse range of plants from the class Spermatopsida dominated the diet, with Asteraceae, Brassicaceae, Cucurbitaceae, Fabaceae and Poaceae as the most frequently represented families. Generally, we detected a variability between species but also among individual samples. Plant species already known from previous studies, mainly visual analyses, could be confirmed for our individuals sampled in Germany and the Netherlands. However, the molecular approach also revealed new plant taxa. Although most of the plant species were categorised as 'wild', the majority of cultivated plants were present with higher frequencies of occurrence, suggesting that cultivated food items likely constitute an important part of the Columbiformes' diet. For Turtle Doves a comparison with previous studies suggested regional differences and that food items (historically) considered as important part of their diet, were missing in our samples. This indicates that regional variations as well as historic and current data on diet should be considered to plan tailored seed mixtures, which are currently proposed as an important conservation measure for rapidly declining Turtle Doves.

Keywords

Introduction

Analyses of diet are important to understand the feeding ecology and habitat requirements of animals as well as to manage and protect species (Oehm et al., 2011; Gong et al., 2019). Conventional methods of dietary studies rely on visually identifying diet components during foraging (behavioural observations) or within stomachs, guts or faeces (morphological classification). These techniques often suffer from misidentification of similar-looking prey items, underrepresentation of soft-bodied or small components, and low taxonomic resolution due to observation distance or digestion stage. Furthermore, analyses of the stomach and crop

content are invasive, as dead individuals are needed or regurgitation needs to be forced (Jordan, 2005; Oehm et al., 2011; Bowser et al., 2013; Gong et al., 2019).

Biochemical and molecular methods like fatty acids, protein electrophoretic, stable isotope analyses and DNA metabarcoding have been adapted to be used in diet studies (Pompanon et al., 2012). The application of DNA metabarcoding in diet studies has increased considerably with the advent of next-generation sequencing (NGS) technology (Pompanon et al., 2012). With NGS it is now possible to identify (rare) prey items of multiple samples up to species level in a single sequencing run while maintaining the ability to trace back each prey to the sample of origin (Bowser et al., 2013; Gong et al., 2019). NGS technology is increasingly used for dietary analyses across a variety of animal taxa (arthropods: Krey et al., 2020; fish: Chow et al., 2019; mammals: Buglione et al., 2020; birds: Dunn et al., 2018, Kleinschmidt et al., 2019) with faeces being the most popular sample type (Alberdi et al., 2018).

Up to now, detailed information about the range and composition of the diets of many free-ranging wild animals is still extremely limited, and often, only a generalized approximation of food items consumed is known. Accurate and comprehensive knowledge of a species' diet is fundamental to understand its ecological requirements (Wood, 1954; Newton, 1998; Jordan, 2005). This is, for example, essential for appreciating resource partitioning between species and competition for food. Furthermore, knowledge of the feeding habits and ecology of a species is important to evaluate how food availability can affect its population status and to identify key resources for designing conservation strategies (Valentini, 2008; Gutiérrez-Galán et al., 2017).

In general, the Turtle Dove is considered an obligate granivore, whereas the other two columbiform species have more generalist diets, including green plant material, fruits and invertebrates, especially if seed availability is low (Murton et al., 1964; Möckel, 1988; Gutiérrez-Galán et al., 2017; Negrier et al., 2021).

The present study aimed to update and improve our knowledge on the diet composition of three species of Columbiformes, sampled in Germany and the Netherlands, representing regions where their feeding ecology was little studied in recent years. NGS technology was used to generate a diet reconstruction through DNA metabarcoding based on faecal samples, which in turn was used to compare the diet of the three species to assess their dietary overlap.

Material & Methods

Faecal sample collection and DNA isolation

Faecal samples (n = 139) were collected from three species Common Woodpigeon (n = 49), European Turtle Dove (n = 19), and Stock Dove (n = 71) at different sampling sites in Germany and the Netherlands (Fig. S1, Table S1). Birds were caught using mist nets, trapping cages, clap nets or in the case of some Stock Doves traps installed to their artificial nest boxes. Faecal samples were collected either opportunistically from the bird during handling or from the inside

of clean bird bags within which the birds were temporarily held. Some faecal samples of Woodpigeons were collected as fresh droppings of active nests or roosting sites (n = 26) or from transport containers of individuals brought to the clinic for birds by the public ('Vetmed', n = 19). Some individuals were caught at temporarily baited sites with seeds used to lure individuals (Table S1). Thus, we expected a small amount of baited seeds (Table S2) to be present in faecal if individuals were using baited sites (cf. Dunn et al. 2018). Sampled nestlings were at least one week or older to ensure they did not receive crop milk only (Glutz von Blotzheim & Bauer, 1987). All faecal samples were stored dark and frozen at −20°C.

Prior to DNA isolation, 180-200 µg of each sample were weighed. If less material was available, the entire sample was used (minimum: 21 µg). DNA was extracted using the QIAamp ® Fast DNA Stool Kit Mini (QIAGEN GmbH, Germany) with the following modifications to the manufacturer's instructions: 2-3 bashing beads (ZR Bashing Bead™ 2.0 mm, Zymo Research, USA) were added to ensure proper homogenization using the Disruptor Genie™ (Scientific Industries SI™). Incubation with Buffer AL and proteinase K was increased from 10 to 30 min.

Two negative extraction controls, i.e. empty Eppendorf tubes, were run along with the faecal samples during isolation and through the entire process. DNA concentration was determined with a NanoDrop2000c UV-Vis Spectrophotometer (NanoDrop Technologies, USA) and samples were diluted to 20 ng/µl if the DNA concentration was higher than 100 ng/µl.

Construction of sequencing library

A sequencing library (NGS library) was constructed with two consecutive PCR reactions, first, an amplicon PCR followed by an indexing PCR. Initial tests (Supplementary material A1) resulted in the following amplicon PCRs: We used primers UniPlantF and UniPlantR amplifying a 187 to 380 bp region encompassing the second internal transcribed spacer of nuclear ribosomal

DNA (ITS2) of plant nuclear DNA (Moorhouse-Gann et al., 2018). The primer pair mICOIintF/dgHCO-2198 (Meyer, 2003, Leray et al., 2013) was used to amplify a fragment of approx. 300 bp of the highly variable mitochondrial cytochrome *c* oxidase subunit 1 (COI) region of metazoan DNA (Supplementary material A2). All used primers had Illumina overhang adapters attached (P5 for forward primers: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3' and P7 for reverse primers: 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3'). PCR runs included PCR grade water as negative control and negative extraction controls, as well as positive controls (DNA isolated directly from plants or Gastropoda). PCR amplicons were visualised using QIAxcel Advanced (QIAGEN) high-resolution capillary gel electrophoresis.

A 5 µl aliquot of the amplicon PCR products was purified using an Illustra™ ExoproStar 1-Step Kit for enzymatic PCR clean-up (GE Healthcare, UK) according to the manufacturer's protocol. After this purification, an index PCR was performed in order to individually mark each

PCR product with specific Illumina indices added to the P5 and P7 sequencing adapters (Supplementary material A2).

Index PCR products were purified and normalised with a SequalPrep™ Normalization Plate Kit (Thermo Fisher Scientific, USA) and 2 µl of each normalised and individually tagged sample were pooled to finalise the NGS library. In total, 136 and 104 samples were successfully amplified with the metazoan and plant primers, respectively, and sent for sequencing (Table S1). The library was sequenced using 250-bp paired-end reads on a MiSeq desktop sequencer (Illumina) at SEQ-IT GmbH & Co. KG, Kaiserslautern, Germany.

Bioinformatics analyses of sequences from faecal samples

To transform the raw Illumina sequence data into a list of MOTUs (molecular operational taxonomic units) with assigned taxonomy, a custom workflow (Masello et al., 2021; for detailed steps see Supplementary material A3) in GALAXY (https://www.computational.bio.uni-giessen.de/galaxy; Afgan et al., 2018) was used.

Subsequently, MOTUs that corresponded to regular fieldwork contaminants in faecal samples (bacteria, soil fungi, bird DNA) were manually discarded (Kleinschmidt et al., 2019). As short fragments are less likely to contain reliable taxonomic information (Deagle et al., 2009), sequences with a length of less than 100 bp and a BLASTn assignment match of less than 98% were discarded. MOTUs were assigned to the lowest shared taxonomic level (Kleinschmidt et al., 2019; Table S3). Those that could not be determined at least at family level were excluded.

Further filter steps were performed to obtain reliable data, i.e. avoid contamination and false positives (Crisol-Martínez et al., 2016): MOTUs were accepted only if they contained a minimum of five sequences or accounted for > 1% of the maximum total of hits per columbiform species. For each MOTU, we identified the highest read number within the negative samples and removed this MOTU from any sample where the read number was below this threshold.

Statistical analysis

All statistical analyses were carried out in R v.4.0.4 (R Core Team, 2021). For dietary overlap analyses and statistical analyses, we used the presence or absence data of each MOTU or respective genus or family. The frequency of occurrence 'FOO' per single species was calculated as FOO% = $(n/t)*100$, where 'n' is the number of samples, in which the MOTU was detected, and t is the total number of samples (Table 1).

Since the data are qualitative data, χ^2 tests (pairwise for species) were performed to compare frequencies of plant families and genera between columbiform species (Table S4, S5). Furthermore, we tested for differences in diet species composition at family and genus level with permutation tests in the R package 'VEGAN' (Oksanen et al., 2009). Non-metric Multidimensional Scaling (NMDS, function *metaMDS*) was used to visualize species differences in diet compositions. NMDS uses rank orders to collapse information from multiple

dimensions into usually two dimensions to facilitate visualization as well as interpretation and is generally considered the most robust unconstrained ordination method in community ecology (Faith et al., 1987; Minchin, 1987). The function *metaMDS* allowed us to investigate the agreement between the two-dimension configuration and the original configuration through a stress parameter (if the stress value < 0.1 the agreement is very good, < 0.2 is a good representation). For this analysis at family level samples containing only a single plant family (n = 8) were discarded. All stress values in the present tests were < 0.25 at family and < 0.19 at genus level. We performed Permutational Multivariate Analysis of Variance Using Distance Matrices (PERMANOVA) with the function *adonis* and checked for the multivariate homogeneity of group dispersions (variances) with the function *betadisper*.

To assess the dietary overlap of each species pair according to the presence/absence data at family and genus level of valid MOTUs, we calculated Pianka's measure of overlap O_{jk} (Pianka, 1986) in the R package 'SPAA' (Zhang, 2016) using the *niche.overlap* function.

To evaluate differences between the species in the proportion of wild to cultivated plant species we categorised the MOTUs in five broad categories according to their likely source (Table S3; Dunn et al., 2018): 'fed' (seeds likely to be offered at bird feeders), 'cultivated' (crop plants as well as those widely cultivated as components of seed mixes sown to provide seed for wild birds), 'natural' (wild plant species), 'tree', and 'brassica' (all MOTUs of the family Brassicaceae). 'Brassica' was considered as a separate category as the family of Brassicaceae includes plants used to provision birds, as well as cultivated and several naturally occurring wild species (Dunn et al., 2018). If a species and respective genus occurred both as separate MOTUs in one species, e.g. *Achillea* sp. and *Achillea millefolium*, they were combined for categorisation.

Results

Diet composition – metazoan DNA

Apart from the consumption of a few insects (9 samples, Table S3), only one valid metazoan prey MOTU was present in the faecal sample of one Stock Dove. This was DNA of a common earthworm *Lumbricus terrestris* (Table S3). Due to the low presence of animal prey in our samples, the further statistical evaluation refers only to the plant components found in the faecal samples.

Table 1 Presence of valid MOTUs (molecular operational taxonomic units) of Spermatopsida in the diet of Common Woodpigeons *Columba palumbus* (WP), European Turtle Doves *Streptopelia turtur* (TD), and Stock Doves *C. oenas* (SD) with the respective frequency of occurrence (FOO%) of each MOTU.

Order	Family	Genus	Species	MOTU	Common name	FOO %		
						WP	TD	SD
Apiales	Apiaceae	*Heracleum*	-	*Heracleum* sp	hogweeds	-	5.6	-
	Araliaceae	*Hedera*	-	*Hedera* sp	ivies	6.3	-	1.9
Asterales	Asteraceae	*Achillea*	-	*Achillea* sp	yarrows	15.6	-	-
			A. millefolium	*A. millefolium*	common yarrow	15.6	-	3.7
		Artemisia	*A. vulgaris*	*A. vulgaris*	common mugwort	6.3	-	5.6
		Bellis	*B. perennis*	*B. perennis*	common daisy	3.1	-	-
		Carthamus	*C. tinctorius*	*C. tinctorius*	safflower	-	-	3.7
		Cichorium	-	*Cichorium* sp	chicories	-	-	3.7
		Cirsum	-	*Cirsum* sp	thistles	-	11.1	-
		Crepis	*C. capillaris*	*C. capillaris*	smooth hawksbeard	3.1	5.6	-
		Dittrichia	*D. graveolens*	*D. graveolens*	stinkwort	-	-	3.7
		Guizotia	*G. abyssinica*	*G. abyssinica*	niger seed	6.3	-	-
		Helianthus	*H. annuus*	*H. annuus*	annual sunflower	21.9	11.1	7.4
		Hypochaeris	*H. radicata*	*H. radicata*	catsear	3.1	-	-
		Lactuca		*Lactuca* sp	lettuce	6.3	-	-
		Scorzoneroides	*S. autumnalis*	*S. autumnalis*	autumn hawkbit	6.3	-	-
		Senecio	*S. inaequidens*	*S. inaequidens*	narrow-leaved ragwort	3.1	-	-
		Sonchus	-	*Sonchus* sp	sow thistles	3.1	-	-
		Taraxacum	-	*Taraxacum* sp	dandelions	21.9	11.1	-
		Tripleurospermum	-	*Tripleurospermum* sp	mayweeds	-	-	5.6
Boraginales	Boraginaceae	*Echium*	*E. vulgare*	*E. vulgare*	blueweed	-	5.6	-
Brassicales	Brassicaceae	*Raphanus*	-	*Raphanus* sp	radishes	-	-	9.3
		Sinapis	*S. alba*	*S. alba*	white mustard	-	5.6	3.7
		Brassica	-	*Brassica* sp	cole crops	28.1	50.0	68.5
			B. juncea	*B. juncea*	brown mustard	-	22.2	33.3
			B. napus	*B. napus*	rapeseed	6.3	22.2	38.9
			B. oleracea	*B. oleracea*	cabbage	-	-	13.0
			B. rapa	*B. rapa*	bird rape	6.3	16.7	38.9
		Cardamine	*C. hirsuta*	*C. hirsuta*	hairy bittercress	-	5.6	-
Caryophyllales	Amaranthaceae	*Chenopodium*	-	*Chenopodium* sp	goosefoots	12.5	16.7	5.6
	Caryophyllaceae	*Cerastium*	-	*Cerastium* sp	mouse-ear chickweeds	3.1	-	-
		Sagina	*S. apetala*	*S. apetala*	annual pearlwort	3.1	-	-
		Silene	-	*Silene* sp	campions	3.1	-	-
			S. latifolia	*S. latifolia*	white campion	3.1	-	-
			S. vulgaris	*S. vulgaris*	bladder campion	-	-	1.9
		Stellaria	*S. media*	*S.media*	chickweed	-	-	7.4
Cucurbitales	Cucurbitaceae	*Cucumis*	-	*Cucumis* sp		40.6	-	25.9
		Cucurbita	-	*Cucurbita* sp	gourd	65.6	27.8	51.9
			C. pepo	*C. pepo*	field pumpkin	43.8	16.7	35.2
Dipsacales	Adoxaceae	*Sambucus*	*S. nigra*	*S. nigra*	elder	6.3	-	-
Ericales	Balsaminaceae	*Impatiens*	-	*Impatiens* sp	snapweeds	-	-	5.6
			I. parviflora	*I. parviflora*	small balsam	-	-	5.6
Fabales	Fabaceae	*Glycine*	*G. max*	*G. max*	soya bean	6.3	11.1	-

- Table 1 continued -

Order	Family	Genus	Species	MOTU	Common name	FOO %		
						WP	TD	SD
		Lotus	-	*Lotus* sp	bird's-foot trefoils	3.1	-	-
		Pisum	*P. sativum*	*P. sativum*	pea	-	-	20.4
		Robinia	-	*Robinia* sp	locusts	-	11.1	-
		Trifolium	*T. pratense*	*T. pratense*	red clover	3.1	-	-
			T. repens	*T. repens*	white clover	6.3	-	-
		Vicia	-	*Vicia* sp	vetches	-	-	55.6
			V. hirsuta	*V. hirsuta*	hairy vetch	-	-	38.9
			V. lathyroides	*V. lathyroides*	spring vetch	-	-	11.1
			V. sativa	*V. sativa*	common vetch	-	-	31.5
			V. sepium	*V. sepium*	bush vetch	-	-	7.4
			V. tetrasperma	*V. tetrasperma*	smooth vetch	-	-	9.3
Fagales	Betulaceae	*Betula*	-	*Betula* sp	birches	9.4	11.1	-
		Carpinus	-	*Carpinus* sp	hornbeams	6.3	-	-
	Fagaceae	*Fagus*	-	*Fagus* sp	beeches	9.4	-	11.1
	Juglandaceae	*Juglans*	*J. regia*	*J. regia*	walnut	3.1	-	1.9
Gentianales	Rubiaceae	*Galium*	-	*Galium* sp	bedstraws	3.1	-	-
Lamiales	Plantaginaceae	*Hippuris*	-	*Hippuris* sp	mare's tails	-	-	5.6
		Plantago	*P. lanceolata*	*P. lanceolata*	buckhorn plantain	18.8	-	5.6
		Veronica	*V. chamaedrys*	*V. chamaedrys*	cat's eyes	-	-	3.7
Liliales	Liliaceae	*Lilium*	-	*Lilium* sp	lilies	3.1	-	-
Malpighiales	Euphorbiaceae	*Euphorbia*	*E. helioscopia*	*E. helioscopia*	sun spurge	-	11.1	3.7
		Mercurialis	*M. annua*	*M. annua*	annual mercury	-	-	5.6
	Linaceae	*Linum*	-	*Linum* sp	flax plants	-	5.6	-
Malvales	Malvaceae	*Tilia*	-	*Tilia* sp	linden	9.4	-	-
			T. platyphyllos	*T. platyphyllos*	large-leaved linden	6.3	-	-
Myrtales	Lythraceae	*Lythrum*	*L. salicaria*	*L. salicaria*	purple loosestrife	-	5.6	-
	Onagraceae	*Epilobium*	-	*Epilobium* sp	willowherbs	-	5.6	1.9
		Oenothera	-	*Oenothera* sp	evening primroses	-	-	5.6
Pinales	Pinaceae	*Picea*	-	*Picea* sp	spruces	9.4	-	22.2
		Pinus	-	*Pinus* sp	pines	-	5.6	-
			P. sylvestris	*P. sylvestris*	European red pine	9.4	5.6	9.3
Poales	Cyperaceae	*Carex*	-	*Carex* sp	sedges	31.3	16.7	22.2
	Poaceae	-	-	Poaceae	grasses	96.9	94.4	88.9
		Agrostis	-	*Agrostis* sp	bentgrasses	12.5	-	1.9
		Alopecurus	*A. myosuroides*	*A. myosuroides*	black-grass	-	-	1.9
			A. pratensis	*A. pratensis*	meadow foxtail	15.6	5.6	-
		Arrhenatherum	-	*Arrhenatherum* sp	oatgrasses	12.5	11.1	-
			A. elatius	*A. elatius*	false oat-grass	12.5	11.1	-
		Avena	-	*Avena* sp	oats	15.6	5.6	11.1
		Dactylis	*D. glomerata*	*D. glomerata*	cock's-foot	6.3	-	11.1
		Elymus	-	*Elymus* sp	couch grasses	-	-	3.7
		Festuca	-	*Festuca* sp	fescues	9.38	-	-
		Holcus	-	*Holcus* sp	soft-grasses	-	11.11	-
		Hordeum	*H. vulgare*	*H. vulgare*	barley	21.9	33.3	27.8
		Lolium	-	*Lolium* sp	ryegrasses	15.6	-	3.7
			L. perenne	*L. perenne*	perennial ryegrass	15.6	-	3.7
		Molinia	*M. caerulea*	*M. caerulea*	purple moorgrass	28.1	-	14.8
		Panicum	*P. miliaceum*	*P. miliaceum*	proso millet	21.9	50.0	18.5
		Phalaris	-	*Phalaris* sp		9.4	-	-

- Table 1 continued -

Order	Family	Genus	Species	MOTU	Common name	WP	TD	SD
		Poa	-	*Poa* sp	meadow-grasses	15.6	11.1	5.6
			P. trivialis	*P. trivialis*	rough bluegrass	12.5	-	-
		Secale	*S. cereale*	*S. cereale*	rye	9.4	16.7	18.5
		Setaria		*Setaria* sp	bristle grasses	6.3	11.1	5.6
		Trisetum	*T. flavescens*	*T. flavescens*	yellow oatgrass	3.1	-	-
		Triticum	-	*Triticum* sp	wheat	68.8	66.7	77.8
			T. aestivum	*T. aestivum*	common wheat	25.0	16.7	35.2
			T. dicoccon	*T. dicoccon*	emmer wheat	9.4	-	-
			T. spelta	*T. spelta*	dinkel wheat	-	-	11.1
		Zea	*Z. mays*	*Z. mays*	maize	34.4	16.7	16.7
Ranunculales	Ranunculaceae	*Ranunculus*	-	*Ranunculus* sp	buttercups	-	27.8	-
Rosales	Cannabaceae	*Cannabis*	*C. sativa*	*C. sativa*	hemp	25.0	27.8	27.8
	Elaeagnaceae	*Hippophae*	*H. rhamnoides*	*H. rhamnoides*	sea-buckthorn	-	-	1.9
	Rosaceae	*Amelanchier*	-	*Amelanchier* sp	shadbushes	6.3	-	-
		Potentilla	-	*Potentilla* sp	cinquefoils	9.4	-	-
		Prunus	-	*Prunus* sp		15.6	-	3.7
			P. avium	*P. avium*	bird cherry	3.1	-	-
		Rosa	-	*Rosa* sp	roses	3.1	11.1	-
		Rubus	-	*Rubus* sp		6.3	22.2	1.9
	Urticaceae	*Urtica*	*U. dioica*	*U. dioica*	common nettle	18.8	16.7	11.1
Sapindales	Sapindaceae	*Acer*	-	*Acer* sp	maples	18.8	-	9.3
			A. platanoides	*A. platanoides*	norway maple	6.3	-	-
			A. pseudoplatanus	*A. pseudoplatanus*	sycamore	6.3	-	-
Saxifragales	Crassulaceae	*Sedum*	-	*Sedum* sp	stonecrops	3.1	-	-
Solanales	Convolvulaceae	*Convolvulus*	*C. arvensis*	*C. arvensis*	field bindweed	3.1	-	-
	Solanaceae	*Solanum*	*S. lycopersicum*	*S. lycopersicum*	tomato	6.3	-	14.8

Diet composition – plants

Of all faecal samples successfully amplified with the plant primers (n = 104), at least one valid MOTU was found in every sample with an average of 9.3 ± 5.8 MOTUs per sample (Stock Dove = 10.0 ± 5.0, Woodpigeon = 9.8 ± 7.5, Turtle Dove = 6.4 ± 4.1). A total of 118 MOTUs were found, with 54.2% of MOTUs determined at species level, 44.9% at genus level and 0.8% at family level (Table 1). Most MOTUs were found in samples of Woodpigeons (79 MOTUs), followed by Stock Doves (67) and Turtle Doves (44). All MOTUs belonged to the class Spermatopsida, distributed among 23 orders and 34 families (Table 1; Fig. 1).

Asteraceae, Brassicaceae, Cucurbitaceae, Fabaceae and Poaceae have been the most frequently represented plant families, occurring with a FOO of over 50% in at least one of the tested species (Fig. 1). These five families contained varying numbers of genera (17, 4, 2, 6 and 19, respectively), that occurred with varying FOO% (Fig. S2; Table S5).
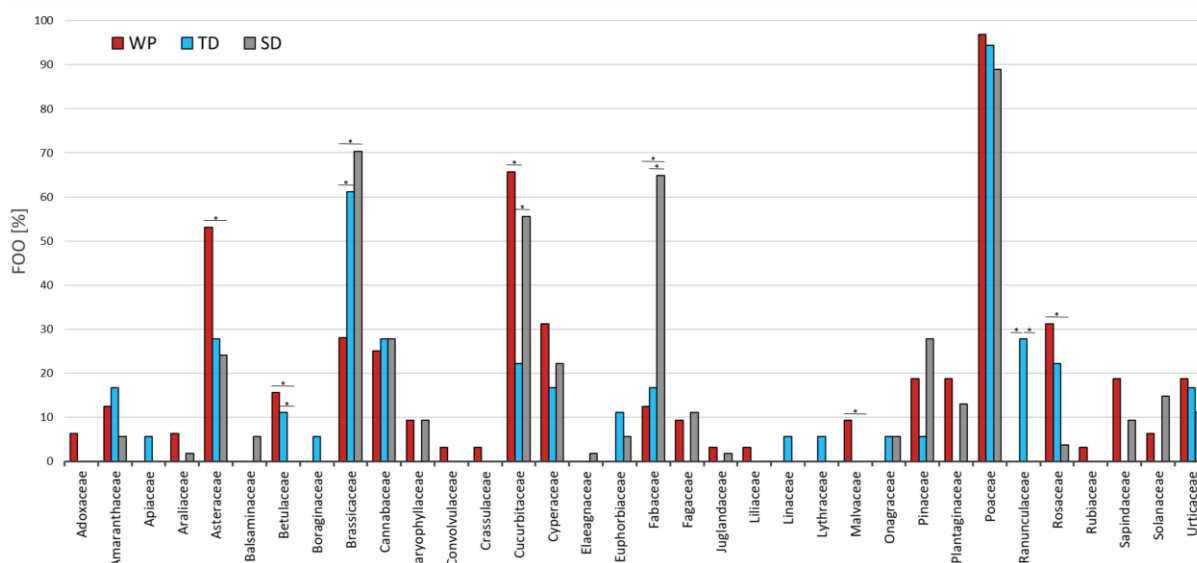
Figure 1 Diet composition of Common Woodpigeons *Columba palumbus* (WP), European Turtle Doves *Streptopelia turtur* (TD) and Stock Doves *C. oenas* (SD). Summary of plant families found in faecal samples represented as frequency of occurrence (FOO). '*' indicates a significant difference ($p < 0.05$) in the occurrence (presence/absence data) of respective families between two species (Table A4).

Diet differences among species of Columbiformes

The frequency of most plant families (77.1%) did not differ significantly between the columbiform species. However, significant differences were present in eight of the families (Fig. 1; Table S4).

Overall, the community analyses showed that the diet composition differed between the columbiform species at plant family level (Fig. 2) as indicated by permutation tests (Permutation test for differences: $F_{93,2} = 7.1$, $p < 0.001$). However, the difference in species explained only 13.3% of the overall variation ($R^2 = 0.133$; Fig. 2). Likewise, the result at genus level (Fig. S3) pointed out differences between the species' diet composition (Permutation test for differences: $F_{101,2} = 4.8$, $p < 0.001$), though this difference also explained a rather small proportion (8.7%) of the overall variation ($R^2 = 0.087$).

According to Pianka's measure of overlap at family level Woodpigeons and Stock Doves showed the highest dietary overlap ($O_{jk} = 0.768$), followed by Stock Doves and Turtle Doves ($O_{jk} = 0.636$). Turtle Doves and Woodpigeons had the least plant families in common ($O_{jk} = 0.551$). Also at genus level, Woodpigeons and Stock Doves showed the highest dietary overlap ($O_{jk} = 0.605$). The overlap between Turtle Doves and Woodpigeons ($O_{jk} = 0.504$) as well as Turtle Doves and Stock Doves ($O_{jk} = 0.504$) was equal.

Most MOTUs were assigned to the category 'natural' (55.1%), followed by 'cultivated' (15.3%) and 'tree' (14.4%). The remaining MOTUs were categorised as 'brassica' (5.9%) or 'fed' (5.1%). Some MOTUs (4.2%) could not be clearly assigned ('NA') to one of the categories (Table S3). In all three species, most MOTUs were assigned to the category 'natural' (Woodpigeon = 52.9%, Turtle Dove = 48.7%, Stock Dove = 46.8%), followed by 'cultivated' for Turtle Doves (17.9%) and Stock Doves (19.4%) and 'tree' for Woodpigeons (17.6%; Fig. 3).

None of the proportion of categories varied significantly between the columbiform species (pairwise t-test all $\chi^2 < 2.6$, df = 1, p > 0.110).
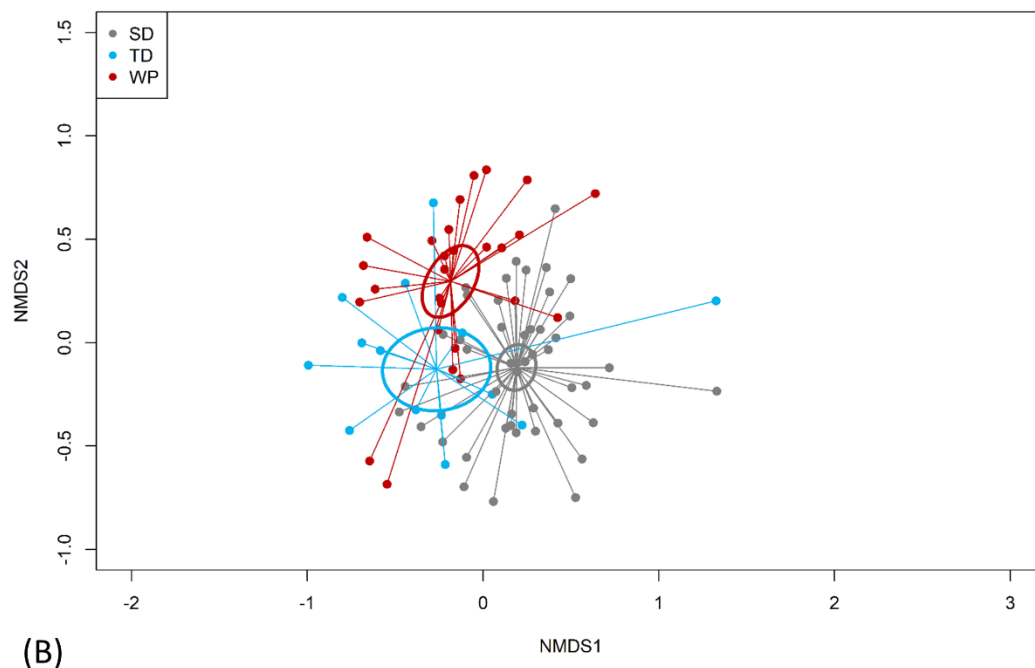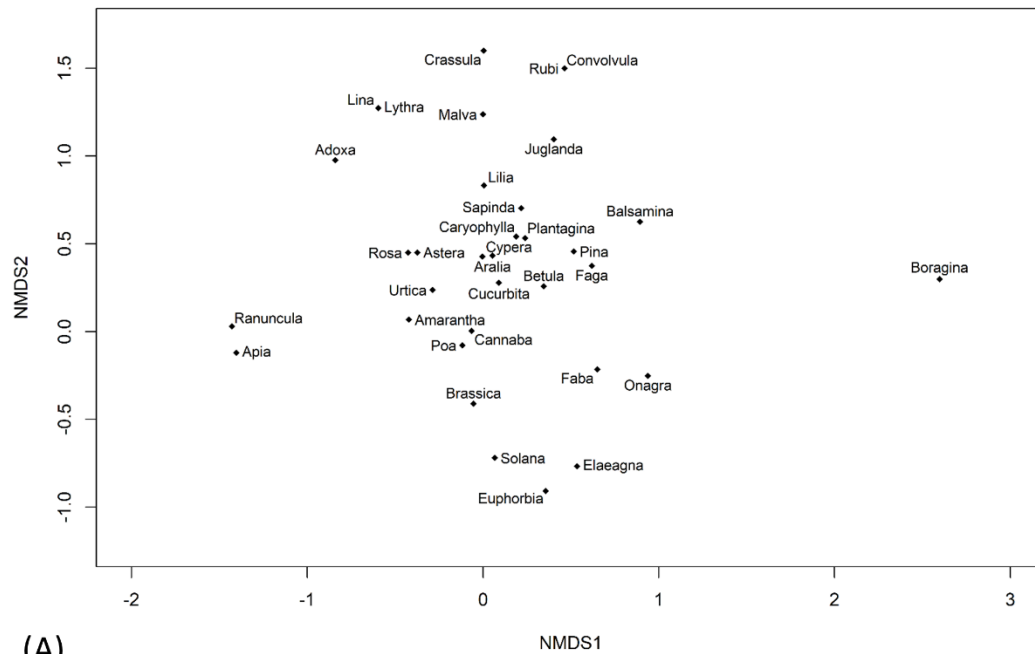

(A)

(B)

Figure 2 Differences in the diet composition at plant family level in three columbiform species (Common Woodpigeon *Columba palumbus* (WP); European Turtle Dove *Streptopelia turtur* (TD); Stock Dove *C. oenas* (SD)), using Non-metric Multidimensional Scaling (NMDS, function metaMDS in the R package 'VEGAN'). Depicted are (A) the distribution of the plant families (the word ending '-ceae' was removed to avoid overlapping) and (B) the distribution of samples and 95% confidence ellipses.
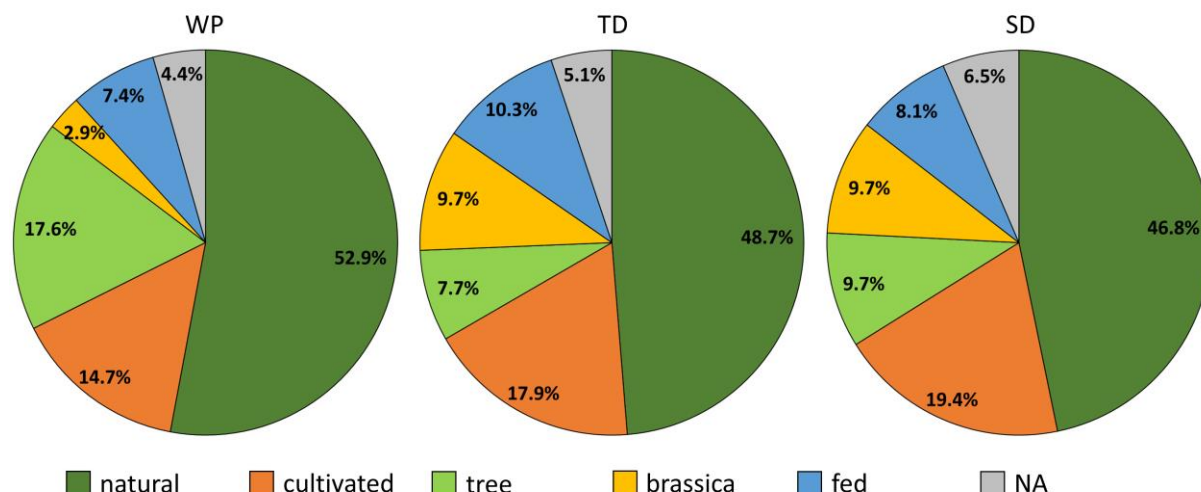
Figure 3 Proportion of dietary component categories of three columbiform species (Common Woodpigeon *Columba palumbus* (WP), European Turtle Dove *Streptopelia turtur* (TD), Stock Dove *C. oenas* (SD)). The categories reflect the likely source of the dietary item (MOTU, Table S3). Proportion is given as percent [%] based on the presence data of MOTUs per species. 'NA' indicates that the MOTU could not be assigned clearly to a category.

Discussion

The main aim of the present study was to analyse the diet composition of Common Woodpigeons, European Turtle Doves and Stock Doves, using NGS technology and metabarcoding, and to compare the diet composition found between the species and to the results of previous studies.

Diet reconstruction based on NGS technology and comparison with previous studies

Animal constituents

The DNA data from faecal samples of the columbiform species show a diverse range of taxa, dominated mainly by plant constituents, while animal prey was present very rarely. In line with our study, most other studies found no or only little numbers of animal material. In a review, the proportion of invertebrate components in the diet of all three species of Columbiformes was below 5% (Holland et al., 2006).

Murton et al. (1964) reported the intake of cocoons of earthworms for Stock Doves. We found earthworm DNA in the faeces of one Stock Dove individual. However, with the applied method we cannot determine the development stage, e.g. cocoon, larvae or imago, of consumed prey.

Woodpigeons sampled in Algeria and Spain were exclusively herbivorous (Jimenez et al., 1994; Kaouachi et al., 2021). In other studies, animal constituents were observed with small volumes and low frequency in Woodpigeon diet (Ó hUallachain & Dunne, 2013; Gutiérrez-Galán et al., 2017; Negrier et al., 2021).

Animal prey was present in only 2.7% of Turtle Doves in Spain or completely absent in other years (Gutiérrez-Galán & Alonso, 2016). Another study on Turtle Dove diet from Spain (Jimenez et al., 1992), finding three gastropods (*Helicella* sp.) in 64 samples, states the animal fraction in the diet as insignificant compared to seeds.

Animal material, particularly calcareous shells, might be mainly consumed to cover the need for calcium (Möckel, 1988; Glutz von Blotzheim & Bauer, 1987), but shells would likely not result in detectable DNA in the faeces. Moreover, the birds might also meet their mineral requirements through the consumption of small clods of earth (Glutz von Blotzheim & Bauer, 1987; Downs et al., 2019) and together, this might explain the absence of animal DNA in the individuals examined here.

A few insect species were detected in the faecal samples (Table S3). Insects and Crustacea have occasionally been found in previous studies, e.g. Cecidomyiidae larvae or Coleoptera in Stock Doves (Möckel, 1988), Coccoidea, larvae and cocoons of Lepidoptera in Woodpigeons (Murton et al., 1964; Glutz von Blotzheim & Bauer; 1987, Ó hUallachain & Dunne, 2013). However, these animals were likely not consumed on purpose but taken 'accidentally' while eating plant components or during plumage care.

Plant constituents

Results obtained in the diet of Columbiformes showed a wide diversity in consumed plants. The applied molecular DNA metabarcoding approach detected a larger number of plant families than former analyses based on visual or observational identification of food items (Table S6-8).

For Stock Doves, we detected 22 plant families (Table S6). Previously, seeds and fruits of plants from the families Poaceae, Fabaceae, Brassicaceae, Polygonaceae and Caryophyllaceae were described as the most important food items for Stock Doves (Glutz von Blotzheim & Bauer, 1987), whereby especially seeds of wild and cultivated vetches *Vicia* sp. (Fabaceae) comprise a major part (Murton et al., 1965; Möckel, 1988). In line with this, four of the five aforementioned plant families were present in Stock Doves sampled in our study (FOO%: Poaceae = 88.9%, Fabaceae = 64.8%, Brassicaceae = 70.4%, Caryophyllaceae = 9.3%, Fig. 1). The important proportion of vetches for Stock Dove diet is also supported by our data. *Vicia* DNA could be traced in 55.6% of all Stock Dove samples with the hairy vetch *Vicia hirsuta* being the most frequent (Table 1), whereas *Vicia* DNA was not found in Woodpigeon or Turtle Dove samples. Nine plant families have not been mentioned as being part of Stock Dove diet in previous studies (Table S6). While seven of these families had a FOO lower than 15%, Pinaceae (27.8%) and Cucurbitaceae (55.6%) were present in more individuals. Overall, our results support the previous assumptions that especially Poaceae, Brassicaceae and Fabaceae, particularly *Vicia* sp., are characteristic for Stock Dove diet. Generally, the diet of Stock Doves seems less intensely studied compared to Woodpigeons and Turtle Doves.

The diet of Woodpigeons has been studied more extensively, likely because they were appraised as a pest of growing crops (Überkermann, 1985). The main food of Woodpigeons, considered a granivorous-frugivorous species, consists of different kinds of seeds and green plant parts. Acorns, beechnuts, maple and other tree seeds are consumed. Cereals, different berries, e.g. elder or ivy and drupes are also eaten, as well as buds, young leaves and young shoots of deciduous trees and conifers. In some areas and times of the year, leaves of rape, cabbage and clover can constitute the main part of the food (Überkermann, 1985; Gutiérrez-Galán et al., 2017).

Woodpigeons are regarded as generalist/opportunistic feeders, feeding on various food items and switching to alternative species when preferred ones are unavailable, leading to a pronounced seasonal variation (Gutiérrez-Galán, et al. 2017; Kaouachi et al., 2021). This generalist feeding is also reflected in our results, as Woodpigeon samples contained the highest number of plant families (n = 25; Fig. 1). However, Woodpigeons were the only species of which we also had samples from the non-breeding season and this may lead to the result of a more variable diet due to the seasonal variation in available and preferred food items. Most of the plant families detected in our samples were already described as part of Woodpigeon diet. Only Crassulaceae, Juglandaceae, Liliaceae, Malvaceae and Sapindaceae were not mentioned in previous studies (Table S7). Some MOTUs found for Woodpigeons in our study might differ from previous studies, as many studies concentrated on sampling in rural and agricultural areas, whereas most of our Woodpigeon samples originated from (sub)urban habitats. Once a typical and exclusive woodland species, Woodpigeons colonised cities of Western and Central Europe since the early 19th century (Fey et al., 2015). Urban areas typically contain novel food items, such as non-native species, intentionally (e.g. bird feeders) or unintentionally provisioned food (e.g. garbage in landfills). Therefore, many wildlife species shift their diets to use these 'novel' food resources (Murray et al., 2018). Some examples for the food items that were most likely found in urban areas solely are the MOTUs *Amelanchier* sp. (ornamental shrub), *Sedum* sp. (ornamental garden plant; roof covering in green roofs) and *Lilium* sp.(ornamental plant). Other MOTUs likely originate from food provided in bird feeders (Table S3, category 'fed'): Relatively frequently found in Woodpigeon faecal samples were e.g. sunflower *Helianthus annuus*, niger seed *Guizotia abyssinica* and proso millet *Panicum miliaceum* (Table 1). Provided seeds in urban areas, e.g. wheat, maize or millet, have probably also contributed to the high FOO% of Poaceae (96.9%) in Woodpigeons, but it also is known that individuals from (sub-)urban areas move out to agricultural areas to feed upon farmland there (Slater et al., 2001). Overall, the diet of the Woodpigeon fits into the known pattern with some peculiarities in the diet of individuals from urban areas.

Turtle Doves are considered obligate granivorous birds (Fisher et al., 2018). Glutz von Blotzheim and Bauer (1987) name seeds of Polygonaceae, Papaveraceae, Brassicaceae, Asteraceae, Poaceae, Pinaceae, Faboideae and *Chenopodium* sp. to constitute the main diet on breeding grounds. While Poaceae (FOO = 94.4%), Brassicaceae (61.1%), Asteraceae (27.8%), Faboideae (16.7%), *Chenopodium* sp. (16.7%) and Pinaceae (5.6%) occurred in our Turtle Dove samples, Polygonaceae and Papaveraceae were not detected.

The feeding ecology of Turtle Doves changed significantly from non-cultivated, natural arable plants, primarily weed seeds, to mainly cultivated plants, such as rape and wheat, from the 1960s to the late 1990s in the UK (Browne & Aebischer, 2003). Also, seeds provided at bird feeders were recently found in Turtle Dove diet (Dunn et al., 2018). Fumitory *Fumaria* sp. historically formed the mainstay of Turtle Dove diet. Individuals sampled in the UK also commonly ate other natural plants, e.g. *S. media*, scarlet pimpernel *Anagallis arvensis*, cock's-foot *Dactylis glomerata*, *Poa* sp., Geraniaceae and Amaranthaceae (Murton et al., 1964; Fisher et al., 2018; Dunn et al., 2018). After the 1990s cultivated seeds, mainly *Triticum* sp. and rape *Brassica napus*, were the main food items in the UK (Browne & Aebischer, 2003).

Our results also reflect the dietary shift from wild plants to cultivated ones On the one hand MOTUs categorized as 'natural', except for *Ranunculus* sp. (27.8%) and *Rubus* sp. (22.2%), occurred with FOO lower than 20%, while cultivated ones reached higher FOO (*Triticum* sp.= 66.7%; *Brassica* sp. = 50.0%, including *B. napus* with 22.2%). On the other hand, we did not find some historically important food items, particularly *Fumaria* sp. and *S. media*, even though they grow in Germany and the Netherlands (Sparrius et al., 2014; Metzing et al., 2018). Similar to our results, these natural plant species, classified as important in Turtle Dove diet, in particular in the UK, were also absent in other regions (Romania and Slovakia: Glutz von Blotzheim & Bauer, 1987; Russia: Murton et al., 1965; Spain: Gutiérrez-Galán & Alonso, 2016). The comparison with previous studies shows that only the plant family Poaceae was present in Turtle Dove diet in all the represented European countries (Table S8). To our knowledge, the families Betulaceae, including the MOTU *Betula* sp., Cyperaceae (MOTU: *Carex* sp.) and Lythraceae (MOTU: *Lythrum salicaria*) were so far not mentioned as part of Turtle Dove diet (Table S8). The observed regional dietary differences may be due to climatic and biogeographical differences as well as variation in habitat, e.g. agricultural landscape vs forest, and occurrence and availability of certain plant species (Gutiérrez-Galán & Alonso, 2016; Mansouri et al., 2019).

Dietary composition differences between species

The degree of dietary overlap between the studied columbiform species pairs was slightly lower than observed by Dunn et al. (2018) with Pianka's measure, ranging from 0.7 to 0.9 compared to 0.5 to 0.8 in our study. Dietary overlap between the species suggests that some resources are shared and the species might compete for food. However, it has been suggested that the related columbiform species select different feeding sites, occupy different ecological

niches or utilise superabundant supplies if taking the same food items, indicating rather little or no competition between them (Murton et al., 1964; Jimenez et al., 1994). For some plant families and genera, we found significant differences in their occurrence for the tested species (Table S4, S5). In addition, the permutation tests indicated significant variance in diet composition among the species (Fig. 2, S3). However, both at plant family and genus level, the differences among species explained only a rather small proportion of the overall variation (13.3 and 8.7%, respectively). This implies a rather pronounced variability within species, which is also supported by the rather strongly varying number of MOTUs detected per sample (1 to 33). Furthermore, with the use of DNA metabarcoding, we cannot distinguish which part of the plant was eaten and the different species may feed on different parts of the same plant species, e.g. Woodpigeons eat the young leaves of Brassicaceae, whereas Turtle Doves feed on Brassica seeds. This can result in the degree of dietary overlap being overestimated.

Another limitation of the method is that only presence/absence data of food items are obtained and thus, quantitative assessment of the proportion of consumed plants is not possible. Even though most MOTUs were assigned to the category 'natural' for all three species (Fig. 3), it cannot be assumed that these proportionally form the main part of the diet. Based on previous studies, seeds and plant material of 'cultivated' species are expected to constitute the main fraction of the diet nowadays. In Woodpigeons sampled in Spain, 97.6% in volume corresponded to cultivated plants (Jimenez et al., 1994). Wheat and rape seeds averaged 61% of the seeds eaten by Turtle Doves in the UK (Browne & Aebischer, 2003). In Stock Doves, wheat and barley made up 80-90% of the diet in April (Möckel, 1988).

Application of results for conservation measures

While the population trends of Woodpigeons and Stock Doves are moderately increasing, Turtle Doves are declining across their entire European breeding range (-80% since the 1980s, PECBMS, 2021). The population decline occurred concurrently with decreases in the abundance of many noncultivated, natural arable plants and along with a decrease in reproductive output (Calladine et al., 1997; Browne & Aebischer, 2004; Dunn et al., 2018). Changes in farming practice and agricultural intensification have caused a serious decrease in weed seed abundance in European farmland areas and the availability of seeds from natural arable plants has declined. Therefore, it has been suggested that the dietary change is associated with a reduction in food availability during important periods of the breeding season, when seeding natural arable plants have become scarce, and this may constitute a stressor for Turtle Dove populations (Browne & Aebischer, 2004; Dunn et al., 2015, 2018; Gutiérrez-Galán & Alonso, 2016). For instance, the condition of Turtle Dove nestlings fed with cultivated seeds was poorer than that of those fed with wild seeds (Dunn et al., 2018) and the availability of wild plant seeds is considered one of the main breeding habitat requirements (Dunn & Morris, 2012).

Thus, the development of an extensive, seed-provisioning option is considered vital for the conservation of Turtle Doves and options to enhance food availability should favour the provision of wild seeds rather than cultivated seeds (Dunn et al., 2015). However, most existing options, e.g. agri-environment schemes (AES) or agri-environmental policies (AEP), are suboptimal in providing accessible food for Turtle Doves (Dunn et al., 2015). A tailored sown mix, based on plant species known to be present in Turtle Dove diet historically, has been devised by an RSPB/Natural England project aiming to provide optimal foraging conditions. However, even though the sown plots provided more seeds compared to control plots, sown plots developed a too dense vegetation structure to attract foraging Turtle Doves. Therefore, modifications for the tailored sown mix were recommended (Dunn et al., 2015).

Updated and improved knowledge of the seeds included in Turtle Dove diet will help to plan and carry out tailored management options as well as optimise feeding during rehabilitation and possible *ex situ* conservation projects, in particular as data on the Turtle Dove diet is fragmentary and limited (Mansouri et al., 2019). Our results and their comparison with previous studies highlight the presence of regional differences in Turtle Dove diet composition and that some plant species, (historically) considered important food items in some regions, might not be the major part of Turtle Dove diet in other regions despite their existence in the studied areas. Further studies should focus on identifying regional dietary differences as they might play an important role in planning tailored seed mixes. It is probably advisable to plan the composition of seed mixtures according to locally preferred and known wild plant species in order to achieve the best possible acceptance of the managed foraging and feeding areas by the Turtle Doves.

Statements

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Information on the 'Supplementary Material' can be found in the appendix
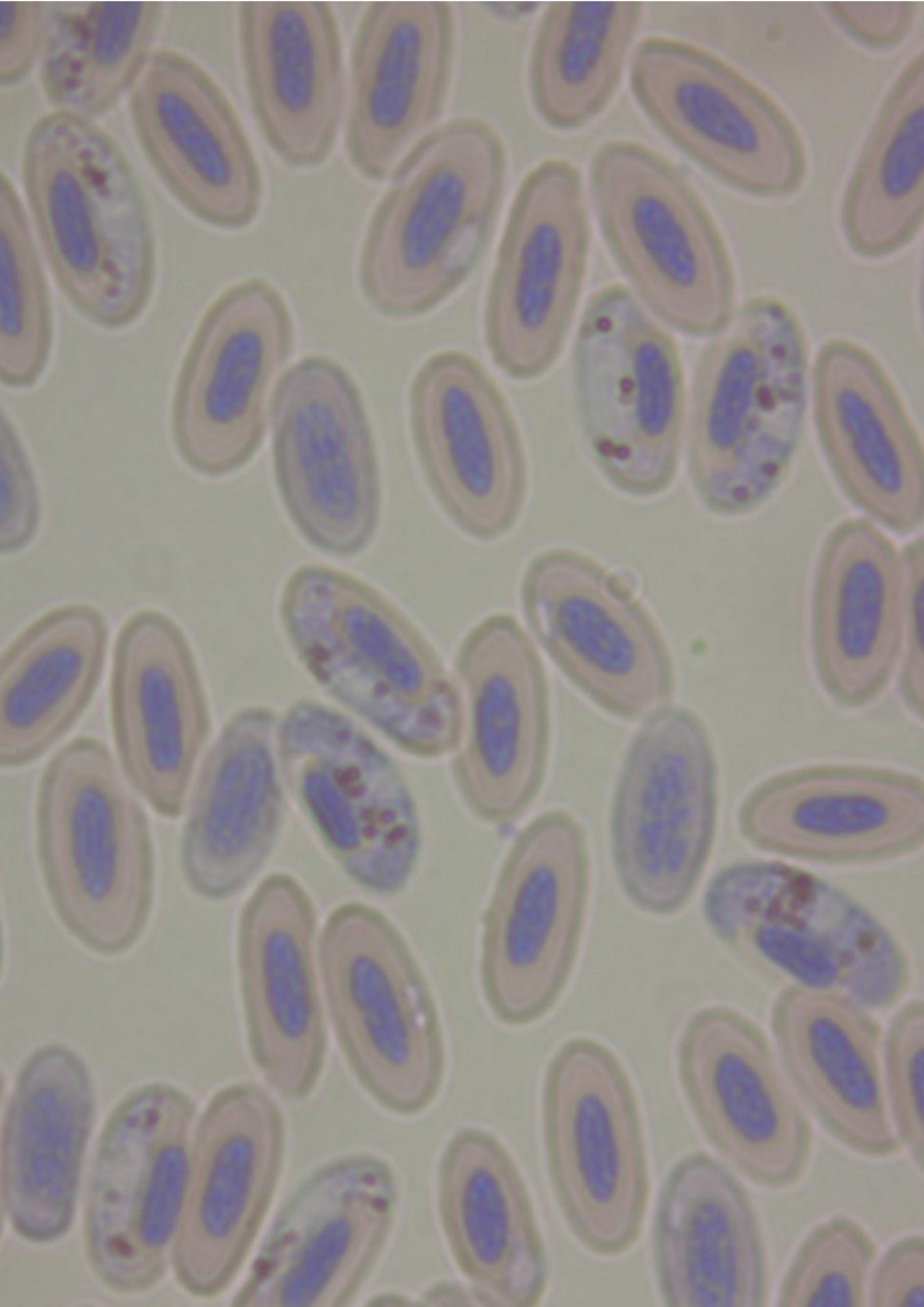
CHAPTER 5

PREVALENCE AND GENETIC DIVERSITY OF AVIAN HAEMOSPORIDIAN PARASITES IN WILD BIRD SPECIES OF THE ORDER COLUMBIFORMES

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IMMUNOLOGY AND HOST-PARASITE INTERACTIONS - ORIGINAL PAPER

Prevalence and genetic diversity of avian haemosporidian parasites in wild bird species of the order Columbiformes

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Abstract

Diseases can play a role in species decline. Among them, haemosporidian parasites, vector-transmitted protozoan parasites, are known to constitute a risk for different avian species. However, the magnitude of haemosporidian infection in wild columbiform birds, including strongly decreasing European turtle doves, is largely unknown. We examined the prevalence and diversity of haemosporidian parasites *Plasmodium*, *Leucocytozoon* and subgenera *Haemoproteus* and *Parahaemoproteus* in six species of the order Columbiformes during breeding season and migration by applying nested PCR, one-step multiplex PCR assay and microscopy. We detected infections in 109 of the 259 screened individuals (42%), including 15 distinct haemosporidian mitochondrial cytochrome *b* lineages, representing five *H.* (*Haemoproteus*), two *H.* (*Parahaemoproteus*), five *Leucocytozoon* and three *Plasmodium* lineages. Five of these lineages have never been described before. We discriminated between single and mixed infections and determined host species-specific prevalence for each parasite genus. Observed differences among sampled host species are discussed with reference to behavioural characteristics, including nesting and migration strategy. Our results support previous suggestions that migratory birds have a higher prevalence and diversity of blood parasites than resident or short-distance migratory species. A phylogenetic reconstruction provided evidence for *H.* (*Haemoproteus*) as well as *H.* (*Parahaemoproteus*) infections in columbiform birds. Based on microscopic examination, we quantified parasitemia, indicating the probability of negative effects on the host. This study provides a large-scale baseline description of haemosporidian infections of wild birds belonging to the order Columbiformes sampled in the northern hemisphere. The results enable the monitoring of future changes in parasite transmission areas, distribution and diversity associated with global change, posing a potential risk for declining avian species as the European turtle dove.

Keywords Avian malaria · Woodpigeon · Turtle dove · Stock dove · Parasite ecology

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Introduction

There is increasing evidence that pathogens can play a significant role in species decline (Bunbury et al. 2007). Haemosporidian parasites, including *Plasmodium*, known as avian malaria, and related malaria-like pathogens *Leucocytozoon* and subgenera *Haemoproteus* and *Parahaemoproteus* have been associated to negatively affect bird population dynamics (Yanga et al. 2011; Yoshimura et al. 2014). Several studies demonstrated different costs on life-history traits associated with haemosporidian infections, such as impairment on the body condition (Valkiūnas et al. 2006), reduced reproductive success (Merino et al. 2000; Marzal et al. 2005; Knowles et al. 2010) and lower chance of survival (Earle et al. 1993; Sol et al. 2003; Bunbury et al. 2007; Lachish et al. 2011).

Haemosporidian parasites are widespread and infect a great variety of avian host species (Valkiūnas 2005; Boundenga et al. 2017). Nevertheless, most studies have specifically addressed avian haemosporidians of passerine birds, while research on non-passerine host species is underrepresented (Santiago-Alarcon et al. 2010; Clark et al. 2014). There is only a small number of recent publications dealing with haemosporidian parasites in wild columbiform birds, particularly in Europe, apart from feral pigeon *Columba livia domestica* (e.g. Sol et al. 2003; Foronda et al. 2004; Scaglione et al. 2015).

In general, given their common evolutionary background, closely related host species (i.e. species belonging to the same family) are expected to be similar in their susceptibility to parasitic infestations and exposure to vectoring dipterans and their parasite community (Ricklefs and Fallon 2002; Dubiec et al. 2016; Ciloglu et al. 2020a; Ellis et al. 2020). However, only few studies have presented data on the prevalence and diversity of haemosporidian parasites in closely related bird species. Differences in prevalence between species are associated with several factors and the interactions between those, including life-history traits and ecology of the hosts and vectors, parasite characteristics and environmental conditions, that may affect the activity of vectors and the development of parasites (Sol et al. 2000; Gupta et al. 2011; Quillfeldt et al. 2011; Hellard et al. 2016; Chakarov et al. 2020; Ciloglu et al. 2020b; Ellis et al. 2020). Also different behavioural characteristics (e.g. cavity-nesting vs. open-nesting or migrant vs. resident species) may influence haemosporidian prevalence and community (Dunn et al. 2017; Emmenegger et al. 2018), whereas no evidence that closely related host species share parasites due to overlapping geographic ranges was found (Ciloglu et al. 2020a). Cavity-nesting species may be shielded from vector exposure due to their enclosed surroundings, while open-nesting birds should be more susceptible to flying dipteran vectors. Migratory species, particularly long-distance migrants, are expected to host a higher diversity of parasites

(Walther et al. 2016; Emmenegger et al. 2018; Ciloglu et al. 2020b) as they encounter parasites and their vectors in multiple ecosystems each year, whereas residents only encounter parasites in one ecosystem (Møller and Erriyzøe 1998). The European turtle dove *Streptopelia turtur* (henceforth turtle dove) is the only long-distance migrant among the columbiform birds we tested. The European population follows three main migration flyways (western, central and eastern) between Europe and sub-Saharan Africa (Marx et al. 2016). The population trend of turtle doves across Europe declined by almost 80% since the 1970s, whereas population trends of other columbiform species, like Common woodpigeon *C. palumbus* (henceforth woodpigeon) and stock dove *C. oenas*, are increasing (PECBMS 2020). Stock doves and woodpigeons from Central Europe are partial migrants. Migratory individuals are mainly wintering in France and Iberia (Cramp 1985; von Blotzheim and Bauer 1994). The main reasons for the turtle dove population decline are the loss of good-quality habitats as well as illegal and unsustainable legal hunting. Additional threats were identified, but these are either considered to have a small or unknown impact or need further research (Fisher et al. 2018); among these are diseases like haemosporidian infections.

We used molecular and microscopic techniques to screen the columbiform species for haemosporidian infections and to identify genetic lineages in order to test the following hypotheses: (i) the prevalence of haemosporidian parasites is higher in long-distance compared to short-distance migratory or resident species, (ii) the diversity of lineages differs among related species and (iii) the prevalence and lineage occurrence in turtle doves varies across their flyways due to possible differing parasite-vector-communities at different breeding, stopover and wintering areas.

Material and methods

Origin and preparation of the samples

Blood samples from 259 individuals belonging to six species of the order Columbiformes were collected from 2013 to 2019 over a broad geographical extent (Table 1; Fig. S1) by venipuncture of the brachial or metatarsal vein and stored on Whatman FTA cards (Whatman®, UK). A blood smear was prepared in the field for 251 of the sampled birds. The blood smears were fixed with methanol (100%) for 30 s and stained with Giemsa in a work solution prepared with buffer pH 7.0 (ratio 1:5) for 30 min. For DNA isolation, a 3 × 3 mm piece of each sample was cut out of the FTA card. Subsequently, the DNA was extracted according to the ammonium-acetate protocol by Martínez et al. (2009) and purified with Zymo-SpinTM IIC columns (Zymo Research, USA). DNA

Table 1 Number of blood samples analysed, split by species year and site

Country	Location	Species[a]	Sampling year	Sampling period[b]	Sample size FTA (adult/juvenile)[c]	Sample size blood smear	Flyway
Egypt	Lake Nasser	TD[d]	2019	BS	9 (5/4)	9	-
		LD	2019	-	4 (4/0)	4	-
		CD	2019	-	1 (0/1)	1	-
		ND	2019	-	1 (1/0)	1	-
France	Chizé	TD	2014	BS	5 (X/X)[e]	5	West
	Île d'Oléron	TD	2014	BS	34 (X/X)	34	West
Germany	Brandenburg	TD	2018/19	BS	4 (4/0)	4	Central/east
	Saxony	SD	2013	BS	2 (2/0)	2	-
	Hesse	TD	2014/18/19	BS	7 (7/0)	7	West
		SD	2013/14/18/19	BS	50 (50/0)	49	-
		WP	2018/19	Year-round	15 (14/1)	14	-
		WP VetMed[f]	2019	Year-round	45 (9/36)	44	-
Greece	Soufli	TD	2015	BS	3 (0/3)	3	Central/east
	Antikythira Island	TD	2018/19	AM/SM	3/46 (48/1)	47	Central/east
Italy	Ventotene Island	TD	2014	SM	27 (27/0)	24	Central/east
Malta	Comino Island	TD	2014	SM	2 (2/0)	2	Central/east
Spain	National Park Monfragüe	TD	2013	BS	1 (X/X)	1	West

[a] TD = European turtle dove *Streptopelia turtur*, LD = laughing dove *S. senegalensis*, CD = collared dove *S. decaocto*, ND = Namaqua dove *Oena capensis*, SD = stock dove *Columba oenas*, WP = common woodpigeon *C. palumbus*

[b] BS = breeding season (sampled June to August), SM = spring migration (sampled April to May) and AM = autumn migration (sampled in September)

[c] Juvenile = hatched during the current calendar year. No nestlings were included

[d] Subspecies *S. t. rufescens* (Brehm 1845). All other sampled turtle doves belong to the nominate subspecies *S. t. turtur* (Linnaeus 1758)

[e] X' is given when no information about the age was available

[f] Woodpigeons were brought to the Clinic for Birds, Reptiles, Amphibians and Fish in Giessen by the public

concentration and purity were quantified by using NanoDrop2000c UV-Vis spectrophotometer (NanoDrop Technologies, USA).

Parasite detection

Nested PCR assay and Sanger sequencing

The presence or absence of avian haemosporidians was determined through nested polymerase chain reaction (PCR) targeting a 479 base pair (bp) region of the cytochrome *b* gene (cyt *b*; Hellgren et al. 2004). For the initial PCR reaction, the primer pair HaemNFI/HaemNR3 was applied. A 4 μl aliquot of this PCR product was subsequently used as template DNA for the second PCR reactions with specific primer pairs HaemF/HaemR2 for *Haemoproteus* (henceforth *Haemoproteus* refers to both subgenera H. (*Haemoproteus*) and H. (*Parahaemoproteus*) infections, unless explicitly defined) and *Plasmodium* and HaemFL/HaemR2L for *Leucocytozoon* amplification. All PCR reactions were carried out in a 25 μL reaction volume containing 12.5 μl 2x DreamTaq Master-Mix (Thermo Fisher Scientific, USA),

1.65 μl of each primer (10 μM), 4 μl template DNA (20–80 ng/μl) and 5.2 μl deionized water. DNA from passerine birds with known infection and deionized water were included in each PCR run as positive and negative controls, respectively. PCR protocols (see Hellgren et al. 2004 for cycling conditions) were carried out on a Biometra TOne Cycler (Analytik Jena, Germany).

As multiple PCR runs can produce additional positives (Dunn et al. 2017), each sample resulting in a negative PCR reaction was conducted a second time to confirm the absence of parasites, whereas a single positive PCR result was interpreted as an infected bird. PCR products of samples rendering a clear band during gel electrophoresis (QIAxcel Advanced, Qiagen, Switzerland) were Sanger sequenced bi-directional by Microsynth-Seqlab (Sequence Laboratories Goettingen GmbH, Germany). Forward and reverse sequences were assembled and trimmed in CLC Main Workbench 7.6.4 (CLC Bio, Qiagen, Denmark) and checked for mixed infections (Ferreira Junior et al. 2017).

To identify lineages, the sequences were aligned with reference sequences deposited in MalAvi database (Bensch et al. 2009) using BLASTN 2.3.0+ (Zhang et al. 2000). Sequences

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are considered as distinct lineages if they differ by one or more nucleotides in the cyt *b* fragment (Hellgren et al. 2004; Bensch et al. 2009). Lineages with no database records in MalAvi were considered novel. For novel lineages, PCR and sequencing were performed twice to verify the results. Novel sequences and sequences found in a host species for the first time are deposited in GenBank under accession numbers MT888848-60.

One-step multiplex PCR assay

The aforementioned widely used nested PCR assay is sufficient for genus and lineage identification. However, it is ineffective at detecting mixed infections of *Haemoproteus* and *Plasmodium* because it favours the amplification of the most abundant parasite in the sample or the parasite for which the primers are a better match (Ciloglu et al. 2019). But since mixed infections are very common and have been shown to be particularly virulent (Valkiūnas et al. 2006; Bernotienė et al. 2016), a PCR assay for simultaneous detection of *Plasmodium*, *Haemoproteus* and *Leucocytozoon* was additionally applied for samples tested positive for either *Haemoproteus* or *Plasmodium* by the nested PCR assay.

The PCR was performed according to Ciloglu et al. (2019) by using equimolar concentrations of three primer sets PMF/PMR, HMF/HMR and LMF/LMR in a single reaction tube, targeting different sized fragments (approx. 380 bp fragment of non-coding region of *Plasmodium* mtDNA, approx. 530 bp fragment between the 5′ end of cyt *b* and a non-coding region of mtDNA of *Haemoproteus*, and approx. 220 bp fragment of the cytochrome *c* oxidase subunit 1 (COX1) gene of *Leucocytozoon*, respectively).

The reactions were set up in total volumes of 20 μl containing 10 μl of 2x Multiplex PCR Master-Mix (Qiagen, Hilden, Germany), 0.4 μl of each primer (10 μM), 3.6 μl of deionized water and 4 μl of DNA template. PCR protocols (see Ciloglu et al. 2019 for cycling conditions) were carried out on a Biometra TOne Cycler. Every PCR run contained positive and negative samples (cf. nested PCR assay). PCR amplicons were visualized using QIAxcel Advanced (Qiagen, Switzerland) high-resolution capillary gel electrophoresis.

Examination of blood films

To confirm the presence or absence of intracellular parasite gametocytes, blood smears (*n* = 251) were examined at × 1000 magnification for at least 10,000 monolayered erythrocytes using a light microscope (PrimoStar Zeiss, Germany). The intensity of parasitemia was determined by counting the number of infected blood cells per 10,000 erythrocytes (Godfrey et al. 1987). Identification of haemosporidian parasites, limited to genus level, followed the criteria of Clark et al. (2009).

Phylogenetic and statistical analyses

Constructions of lineage networks, using the medium joining network method, were performed with PopART 1.7 (Leigh and Bryant 2015).

For phylogenetic tree reconstruction, in addition to newly found lineages, we downloaded one sequence from NCBI GenBank for each haemosporidian lineage (*n* = 148) shown to infect species of the order Columbiformes and deposited in MalAvi (MalAvi 2020). Some sequences (*n* = 45) were excluded due to insufficient sequence length or only partial coverage of the chosen 477 bp cyt *b* fragment. However, all lineages detected in the present study are represented in the phylogenetic analysis.

The best-fit model of DNA sequence evolution was selected using jModeltest 2.1.7 (Darriba et al. 2012). According to the Akaike information criterion, we used the General Time Reversible model including invariable sites and variation among sites (GTR+I+G; Gu et al. 1995). Phylogenetic reconstruction was performed with BEAST 1.8.4. (Drummond et al. 2012). Tree priors were selected using the interface BEAUTi 1.8.4. with strict clock and a Yule speciation process (Yule 1925; Gernhard 2008). Markov chain Monte Carlo (MCMC) simulations were run with 50,000,000 generations and one tree was recorded every 1000 generations. In all, 10% of the trees were discarded as burn-in in TreeAnnotator (BEAST package). We validated the results of the Bayesian analyses in Tracer 1.6. (Drummond and Rambaut 2007). The phylogenetic tree was constructed with FigTree 1.4.3 (Rambaut 2007).

Statistical analyses were performed with R 3.6.3 (R Core Team 2016). Due to a sufficient sample size (cf. Jovani and Tella 2006), we selected turtle doves (*n* = 141), woodpigeons (*n* = 60) and stock doves (*n* = 52) to assess whether overall prevalence and prevalence per genus varied across species. To compare the equality of proportions (e.g. to assess the difference in prevalence between species), the frequency distribution test 'Pearson's chi-squared test' was applied. To determine whether prevalence (infection status as determined by nested PCR expressed as binominal contrast: presence/absence of infection) of turtle doves was associated with timing of sampling (breeding season vs. spring migration) or flyway, we constructed a general linear model (GLM). A significance level of $p < 0.05$ was used.

Results

Prevalence of haemosporidian parasites

Of the 259 individuals screened for haemosporidian parasites using specific nested PCR assay, 109 were infected (42.1%). We successfully obtained a sequence from all positive PCR

results ($n = 109$). Most individuals ($n = 40$) were infected with a single *Leucocytozoon* lineage (15.4%), 40 with a single *Haemoproteus* lineage (15.4%; divided in *H.* (*Haemoproteus*) 13.1%, *H.* (*Parahaemoproteus*) 2.3%) and nine with a single *Plasmodium* lineage (3.5%). Two individuals (0.8%) had mixed homogeneric infections, 17 columbiform birds (6.6%) showed different types of heterogeneric infections and one turtle dove had both (*Haemoproteus/Haemoproteus/Leucocytozoon*) (Table 2).

Woodpigeons had the highest overall prevalence (61.7%), followed by turtle doves (48.9%), while stock doves had the lowest prevalence (3.8%) (Fig. 1). Overall prevalence differed significantly between the species ($\chi^2 = 43.2$, df = 2, $p < 0.001$). Comparing the species pairwise, there was a significant difference between stock doves and the other two species (stock dove/woodpigeon $\chi^2 = 41.0$, df = 1, $p < 0.001$; stock dove/turtle dove $\chi^2 = 33.2$, df = 1, $p < 0.001$), while there was no significant difference between turtle doves and woodpigeons ($\chi^2 = 2.7$, df = 1, $p = 0.098$). In turtle doves, a single infection with *Haemoproteus* was the most prevalent (25.5%), whereas in woodpigeons, it was a single infection with *Leucocytozoon* (48.3%). Turtle doves were significantly more often infected with *Haemoproteus* compared to woodpigeons ($\chi^2 = 10.6$, df = 1, $p = 0.001$) and stock doves ($\chi^2 = 20.7$, df = 1, $p < 0.001$). Conversely, woodpigeons significantly showed more infections with *Leucocytozoon* than turtle doves ($\chi^2 = 33.1$, df = 1, $p < 0.001$) and stock

doves ($\chi^2 = 44.1$, df = 1, $p < 0.001$). An infection with *Plasmodium* was present at a rather low frequency in turtle doves (5.6%) and stock doves (1.9%) and absent in woodpigeons. *Haemoproteus* and *Plasmodium* infections were found in only one stock dove individual each (1.9%). We did not detect *Leucocytozoon* infections in any sampled stock doves. Mixed infection occurred in turtle doves and woodpigeons with a similar prevalence (9.9% and 10%, respectively) (Fig. 1).

There was no age-related significant difference (juveniles vs. adults) in woodpigeons for neither *Haemoproteus* ($\chi^2 = 0.9$, df = 1, $p = 0.334$) nor *Leucocytozoon* infections ($\chi^2 = 1.3$, df = 1, $p = 0.256$).

In turtle doves, an infection with *Haemoproteus* and *Leucocytozoon* depended on the sampling season, while there was no effect of the migration flyway (*Haemoproteus*: season: $\chi^2 = 4.0$, df = 1, $p = 0.045$; flyway: $\chi^2 = 0.1$, df = 1, $p = 0.781$; *Leucocytozoon*: season: $\chi^2 = 5.1$, df = 1, $p = 0.024$; flyway: $\chi^2 < 0.001$, df = 1, $p = 1.0$). Independent of sampling location in Europe, *Haemoproteus* was most prevalent in turtle doves sampled during spring migration ($n = 75$; 52.0%) compared to breeding season ($n = 54$; 11.1%) and autumn migration ($n = 3$; 0.0%). *Leucocytozoon* infections (32.0%) could be detected for individuals sampled during spring migration only. *Plasmodium* infections were neither dependent on flyway nor sampling season (season: $\chi^2 = 0.7$, df = 1, $p = 0.392$; flyway: $\chi^2 = 1.5$, df = 1, $p = 0.227$).

Table 2 Number of avian haemosporidian infections per sampled species and sites. The composition of occurring mixed infections is shown in the right-most column. We refer 'infected' birds to individuals tested positive through nested PCR assay

Host species[a]	Sampling location	Total	Prevalence [%][b]	INF	H[c]	P	L	M	Mixed
TD	Egypt	9	33.3	3	3	0	0	0	
	France	39	15.4	6	2	4	0	0	
	Germany	11	45.5	5	4	1	0	0	
	Greece	52	75.0	39	22	2	3	12	10HL, 1PL, 1HHL
	Italy	27	55.6	15	4	1	8	2	1HL, 1LL
	Malta	2		1	1	0	0	0	
	Spain	1		0	0	0	0	0	
	Total TD	141	48.9	69	36	8	11	14	11HL, 1PL, 1HHL, 1LL
WP	Germany	60	61.7	37	2	0	29	6	5HL, 1LL
SD	Germany	52	3.8	2	1	1	0	0	
ND	Egypt	1		1	1	0	0	0	
LD	Egypt	4		0	0	0	0	0	
CD	Egypt	1		0	0	0	0	0	
Overall total		259	42.1	109	40	9	40	20	

[a] TD = European turtle dove *Streptopelia turtur*, LD = laughing dove *S. senegalensis*, CD = collared dove *S. decaocto*, ND = Namaqua dove *Oena capensis*, SD = stock dove *Columba oenas*, WP = common woodpigeon *C. palumbus*

[b] Prevalence is only given if the sample size was ≥ 5

[c] H = *Haemoproteus* (subgenera *H.* (*Haemoproteus*) and *H.* (*Parahaemoproteus*) combined), P = *Plasmodium*, L = *Leucocytozoon*, M = mixed infections

Fig. 1 Prevalence of infection [%] for haemosporidian genera (*Haemoproteus* refers to the subgenera *H.* (*Haemoproteus*) and *H.* (*Parahaemoproteus*) combined) and mixed infections in the three species: European turtle doves *Streptopelia turtur* (*n* = 141), common woodpigeons *Columba palumbus* (*n* = 60) and stock doves *C. oenas* (*n* = 52). We refer 'infected' birds to individuals tested positive through nested PCR assay
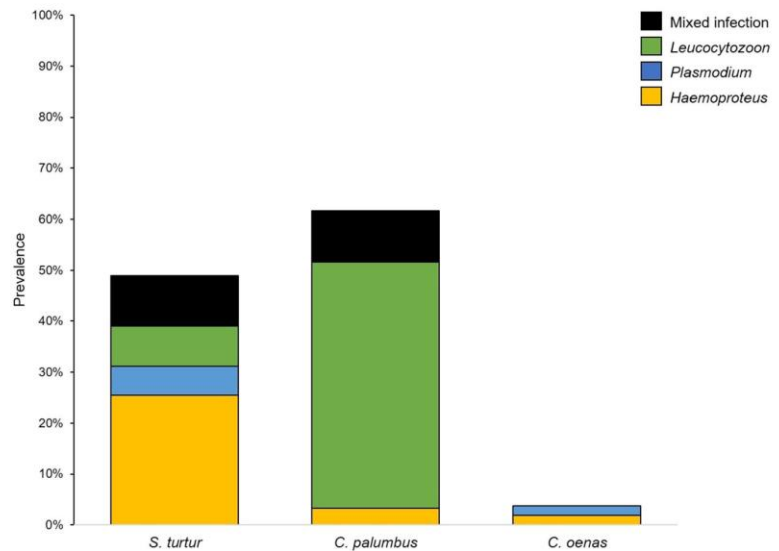
Lineage diversity and phylogenetic analyses

The 109 positive samples represented 15 distinct lineages, including five *H.* (*Haemoproteus*), two *H.* (*Parahaemoproteus*), three *Plasmodium* and five *Leucocytozoon* lineages (Fig. 2, Table 3). The highest lineage diversity was found in turtle doves (*n* = 11), followed by woodpigeons (*n* = 5) and stock doves (*n* = 2). The most frequently occurring lineage was AEMO02 (*Leucocytozoon*), followed by COLIV04 (*Leucocytozoon*) and the *H.* (*Haemoproteus*) lineages STRTUR02 and STRTUR03. All other lineages had a prevalence of less than 10% (Table 3). Eleven lineages were present in a single host species, whereas the remaining four infected two host species (Fig. 2).

We identified five novel lineages. Novel lineages were found in turtle doves (STRTUR04 *H.* (*Haemoproteus*), MT888848 and STRTUR05 *H.* (*Parahaemoproteus*), MT888849) and in the Namaqua dove sample (OENCAP01 *H.* (*Haemoproteus*), MT888850). Two novel *Leucocytozoon* lineages infected woodpigeons (COLPAL03 MT888851 and COLPAL04 MT888852). For some already known lineages, we provide first records of them infecting species of the order Columbiformes (Table 3).

Turtle doves following the central/eastern flyway showed a higher lineage diversity for all haemosporidian genera than individuals using the western flyway (*Haemoproteus*: five vs. three, *Plasmodium*: three vs. one, and *Leucocytozoon*: three vs. zero lineages, respectively). All lineages infecting turtle doves sampled along the western flyway were also found in individuals following the central/eastern flyway (Fig. 3).

The Bayesian-based phylogeny of mitochondrial cyt *b* gene fragment revealed three well-supported major clades, representing *Leucocytozoon*, *Plasmodium* and *Haemoproteus*. Whereby *Haemoproteus* showed two monophyletic subclades, indicating the subgenera *H.* (*Parahaemoproteus*) and *H.* (*Haemoproteus*) (Fig. 4). From the 109 lineages included in the phylogenetic tree reconstruction, 22 belong to *Leucocytozoon*, 15 to *Plasmodium* and 72 to *Haemoproteus*, divided in 54 lineages clustering to *H.* (*Haemoproteus*) and 18 to *H.* (*Parahaemoproteus*).

Blood slide screening and one-step multiplex PCR assay

The overall prevalence according to counts of infected erythrocytes in blood smears was 28.3% (*Haemoproteus* 12.7%, *Plasmodium* 1.2%, *Leucocytozoon* 4.8%; 27 samples could not be assigned reliably to one of the genera). This overall prevalence is lower than the prevalence according to nested PCR results (42.1%) (χ^2 = 10.6, df = 1, *p* = 0.001). With the exception of COLPAL03, MILANS06 and RTSR1, we found in at least one sample per lineage infected erythrocytes (Table 3).

The average parasitemia estimated from blood smears was 17.6, ranging from 0.8 to 912.3 parasites per 10,000 erythrocytes. However, 90.1% of the samples had a parasitemia lower than 10 infected blood cells per 10,000 erythrocytes. One turtle dove sampled during spring migration in 2019 in Greece showed an extremely high parasitemia with 912.3 parasites per 10,000 erythrocytes (Fig. S2) compared to the remaining samples (maximum of 43.6 parasites per 10,000 erythrocytes).

From the 259 samples, 67 were tested positive for *Haemoproteus* or *Plasmodium* with the nested PCR assay. These samples were included in the one-step multiplex PCR

Table 3 Haemosporidian lineages found in bird species of the order Columbiformes with the respective GenBank accession number and lineage prevalence. If mixed homogeneric infection occurred, the lineage combination of the two lineages is presented

Parasite genus[a]	Lineage (MalAvi)	Accession number (GenBank)	Host species[b] (no. infected individuals)	First time record for[c]	Lineage prevalence (%)[d]	Homogeneric mixed with
H	AFR119	KM056425	WP (7)[#]		5.4	-
H	OENCAP01*	MT888850	ND (1)[#]		0.8	-
ParaH	STRTUR01	KJ488784	TD (6)[#]	SD	5.4	-
			SD (1)[#]			
H	STRTUR02	KJ488786	TD (14)[#]		10.8	STRTUR04
H	STRTUR03	KJ488826	TD (22)[#]		16.9	-
H	STRTUR04*	MT888848	TD (6)[#]		4.6	STRTUR02
ParaH	STRTUR05*	MT888849	TD (1)[#]		0.8	-
L	AEMO02	KJ488804	TD (4)[#]	TD	21.5	COLIV04
			WP (24)[#]			STRORI02
L	COLIV04	AB741510	TD (12)[#]	TD	16.9	AEMO02
			WP (10)[#]	WP		
L	COLPAL03*	MT888851	WP (1)		0.8	-
L	COLPAL04*	MT888852	WP (1)[#]		0.8	-
L	STRORI02	AB741508	TD (10)[#]	TD	7.7	AEMO02
P	GRW02	AF254962	TD (7)[#]	TD	6.2	-
			SD (1)[#]	SD		
P	MILANS06	JN164715	TD (1)	TD	0.8	-
P	RTSR1	KJ488785	TD (1)		0.8	-

[a] H = *H. (Haemoproteus)*, ParaH = *H. (Parahaemoproteus)*, L = *Leucocytozoon*, P = *Plasmodium*

[b] TD = European turtle dove *Streptopelia turtur*, LD = laughing dove *S. senegalensis*, CD = collared dove *S. decaocto*, ND = Namaqua dove *Oena capensis*, SD = stock dove *Columba oenas*, WP = Common woodpigeon *C. palumbus*

[c] According to MalAvi (2020)

[d] Percentage of each lineage among all lineage sequences ($n = 130$)

*Novel lineages found in the present study

[#] Lineages present during microscopic examination (infected erythrocytes)

runs. The one-step multiplex PCR assay showed a positive PCR result for 64 (95.5%) samples. All *Plasmodium* lineages GRW02, MILSAN06 and RTSR1 were displayed at the expected band height. While *Haemoproteus* lineages STRTUR01 and STRTUR05 were displayed at the expected band height, AFR119, STRTUR02, STRTUR03 and STRTUR04 were displayed at a height expected for *Plasmodium* lineages. All *Haemoproteus* lineages with 'wrong' band heights cluster in the phylogenetic tree into the *H. (Haemoproteus)* clade (Fig. 4) and group together in the networks (Figs. 2 and 3). An apparent mixed infection of *Haemoproteus* and *Plasmodium* was present in three turtle dove samples according to the results of the one-step multiplex PCR assay. No mixed *Haemoproteus*/*Plasmodium* infection could be proved by the nested PCR assay (Table 2) and for one turtle dove only by microscopic examination. However, for the mixed infections according to the one-step multiplex PCR assay, we cannot distinguish between *Plasmodium*/*Haemoproteus* mixed infections and mixed infections of the two subgenera *H. (Haemoproteus)* and *H.*

(*Parahaemoproteus*) due to the 'wrong' band heights displayed for *H. (Haemoproteus)* lineages. Given these inconsistencies, we classified individuals tested positive through nested PCR-based assay (and verified by Sanger sequencing) as 'infected'.

Discussion

Interspecific differences and lineage diversity

Even though common lineages were detected in the sampled columbiform birds, the overall and genus-specific prevalence as well as the lineage diversity differed among the species. Similar differences in parasite prevalence were reported from other closely related species (e.g. tree sparrow *Passer montanus* and house sparrow *P. domesticus*, Lee et al. 2006; great tit *Parus major* and blue tit *Cyanistes caeruleus*, Dubiec et al. 2016).

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Fig. 2 Median-joining network of mitochondrial cytochrome *b* gene lineages (*n* = 130, 496 bp fragment) of haemosporidian parasites *Haemoproteus* (refers to the subgenera *H.* (*Haemoproteus*) and *H.* (*Parahaemoproteus*) combined), *Leucocytozoon* and *Plasmodium* infecting columbiform birds. Circles represent lineages, and the circle sizes are proportional to the lineage frequencies in the sample set. Lineage names are noted at the associated circles. One hatch mark represents one mutation. Sampled host species are represented by different colours
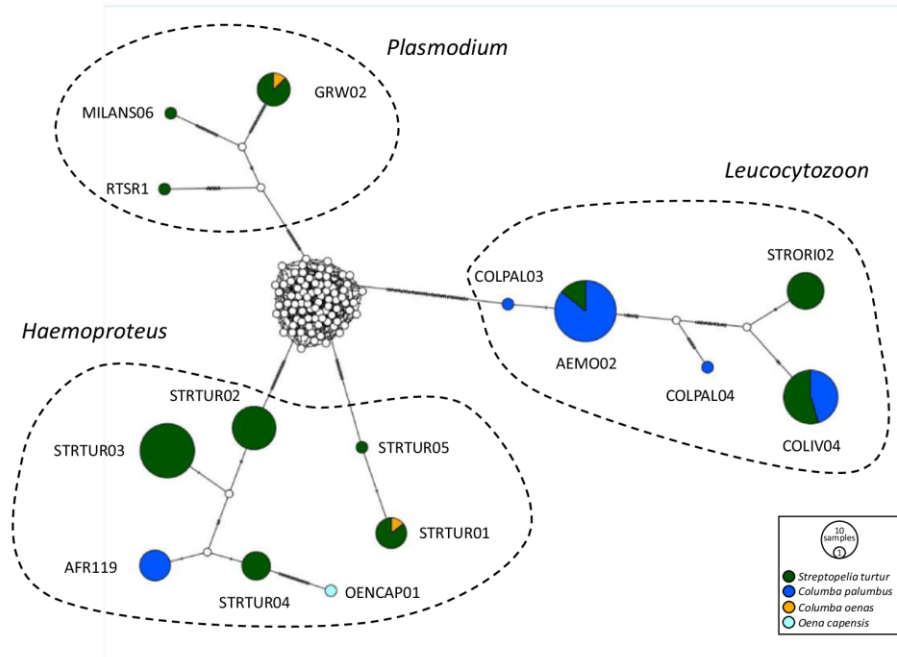
Our results could have been affected by changes in prevalence over time, as the samples were collected from various years. However, there are studies that found no significant changes in the parasite or lineage prevalence between years (Bensch et al. 2007; Shurulinkov and Ilieva 2009, but see Dunn et al. 2017) and the majority of studies investigating prevalence are based on non-uniform distributed data (e.g. birds of different age or variable sample sizes among sample locations; see Dubiec et al. 2016). Therefore, we deem at least our observed main patterns of prevalence and lineage diversity as reliable and evaluable.

The general pattern with woodpigeons showing the highest overall prevalence, followed by turtle doves and stock doves being the least infected ones, is quite similar with the results of Dunn et al. (2017), who tested nestlings of these species.

Fig. 3 Median-joining network of mitochondrial cytochrome *b* gene lineages (*n* = 81, 496 bp fragment) of haemosporidian parasites *Haemoproteus* (refers to the subgenera *H.* (*Haemoproteus*) and *H.* (*Parahaemoproteus*) combined), *Leucocytozoon* and *Plasmodium* infecting European turtle doves *Streptopelia turtur*. Circles represent lineages, and the circle sizes are proportional to the lineage frequencies in the sample set. Lineage names are noted at the associated circles. One hatch mark represents one mutation. Positive samples of individual birds from the western (*n* = 10 sequences) and the central/eastern flyway (*n* = 71 sequences) are represented by different colours
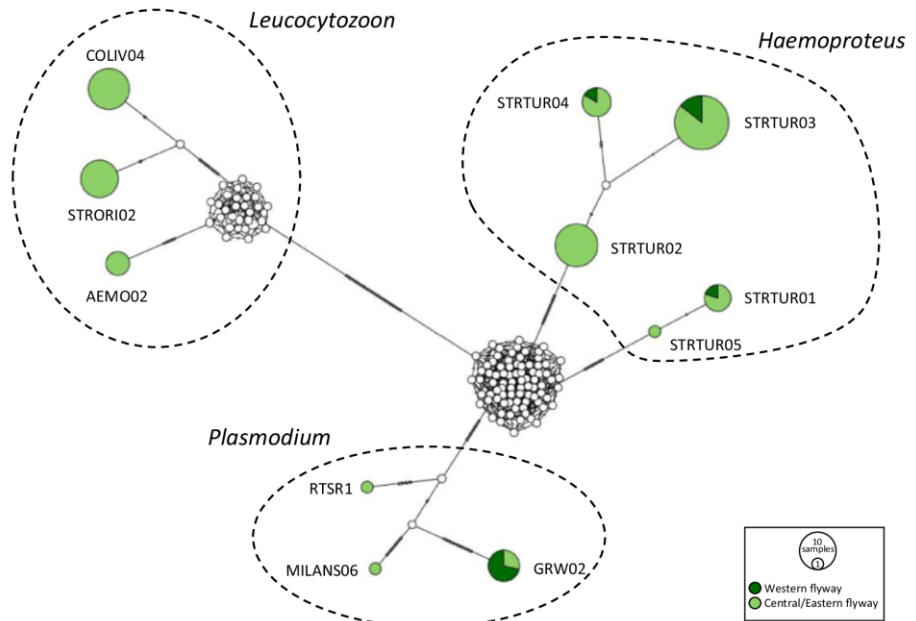
However, in contrast to Dunn et al. (2017), we found evidence for *Plasmodium* infections in turtle doves and stock doves. The fact that woodpigeons and turtle doves showed a significantly higher infection rate than stock doves might be due to different nesting behaviours. Open-nesting species are expected to have higher rates of infection than cavity-nesters (Hellard et al. 2016; Dunn et al. 2017, but see Quillfeldt et al. 2011). While turtle doves and woodpigeons built open nests in shrubs or trees, stock doves breed in tree cavities or artificial nest boxes (von Blotzheim and Bauer 1994). We found the overall prevalence was markedly higher in open-nesting vs. cavity-nesting species. Since only one stock dove individual each was infected with *Haemoproteus* or *Plasmodium*, it is possible that also *Leucocytozoon* infections occur in stock doves at a similar low rate, even if we could not prove this in our study. Dunn et al. (2017) could prove an infection with *Leucocytozoon* for a single stock dove nestling. The nesting behaviour together with other behavioural traits (e.g. being less gregarious or having a low habitat overlap with other columbiform birds, particularly feral pigeons) may explain the low prevalence in stock doves. However, also other factors such as differences in the host immune system, resistance to parasites and other idiosyncrasies of vectors and parasites (Reinoso-Pérez et al. 2016) may influence the low infection rates in stock doves.

Haemoproteus is the most frequently reported blood parasite in birds, followed by *Leucocytozoon* and *Plasmodium* (Carlson et al. 2013; Heym et al. 2019). In this study, this pattern was observed for turtle doves, but not for woodpigeons, for which *Leucocytozoon* was the most prevalent. Although the *Leucocytozoon* prevalence was rather high (58.3%), the genetic diversity was rather low. The two lineages AEMO02 and COLIV04 were present in 94.4% of *Leucocytozoon*-infected woodpigeons. Both lineages were detected in feral pigeons previously (MalAvi 2020). Feral pigeons are widely distributed and their number is increasing, especially in urban areas (Haag-Wackernagel and Moch 2004). In this study, the majority of sampled woodpigeons (82%) were from urban areas. Hence, it is likely that feral pigeons could have acted as a reservoir and blackflies transmitted the lineages from feral pigeons to woodpigeons and vice versa. While Scaglione et al. (2015) states that *Leucocytozoon* parasites are not routinely found in pigeons, a study sampling blackflies in Central Europe showed pigeons to be one of the main targets of ornithophilic blackflies (Chakarov et al. 2020). High rates of *Leucocytozoon* in the sampled woodpigeons may have been favoured by the opportunities for transmission that the woodpigeons offered (e.g. flocking behaviour, increased host densities in urban areas, proximity to feral pigeons; cf. Sol et al. 2000).

High infection rate with *Leucocytozoon* and lower rates of *Plasmodium* and *Haemoproteus* in residents or short-distance migrants (woodpigeons) compared to higher Haemoproteidae prevalence in long-distance migrants (turtle doves) were observed. This is in line with previous research, as Haemoproteid transmission to the birds breeding in the northern hemisphere takes place mostly at wintering areas and along the migration route of the long-distance migrants in contrast to *Leucocytozoon* that is transmitted mainly at the breeding grounds (Valkiūnas 2005; Shurulinkov and Ilieva 2009).

Turtle doves hosted the highest parasite diversity, being the only species infected with all tested haemosporidian genera and harbouring the highest number of lineages. This is in line with previous studies, which have shown that migratory birds have a higher prevalence and diversity of blood parasites than resident or short-distance migratory species (Figuerola and Green 2000; Jenkins et al. 2012; Walther et al. 2016; Ciloglu et al. 2020b). This could be explained by the fact that residents and short-distance migrants travel between areas that are likely to be within a single transmission area, e.g. within a continent, and so are confronted with a single parasite fauna (Hellgren et al. 2007; Jenkins et al. 2012). In contrast, long-distance migrants move between vastly separated areas and thereby encounter different faunas of parasites (Waldenström et al. 2002). Being a long-distance migrant also increases the time being exposed to parasites compared to residents or short-distance migrants in temperate regions, which lack parasite transmission during autumn and winter when vector activity wanes (Cosgrove et al. 2008). Furthermore, environmental challenges, inducing stress and increased day length at the distant wintering grounds may induce infections (Valkiūnas et al. 2004). Since migration is an energetically costly, strenuous physical activity, resources may be traded-off from immune defence, making it likely that migrant species are more susceptible than residents (Waldenström et al. 2002; Jenkins et al. 2012, but see Hegemann et al. 2012).

Migratory birds can transport parasite lineages to novel environments (Waldenström et al. 2002; Adamik et al. 2016). It has been suggested that some lineages are transmitted in Africa only, while others are transmitted in Europe only and a few in both continents (Ferraguti et al. 2019). The presence of the seven lineages found in woodpigeons and stock doves (Table 3) indicates that these lineages are transmitted in Europe. For turtle doves of the subspecies *S. t. turtur* as Palearctic-African migrants, transmission can take place both on the African and European continents, whereas for the subspecies *S. t. rufescens*, infected with the lineages STRTUR01, STRTUR02 and STRTUR03, transmission can occur in Africa only. As for some lineages (COLPAL03, MILSAN06 and RTSR1), no infected erythrocytes could be found during blood smear screening and possible mixed infections of lineages belonging to the same genus were not considered in the microscopic examinations, assigned transmission areas are likely but not definitely proven.
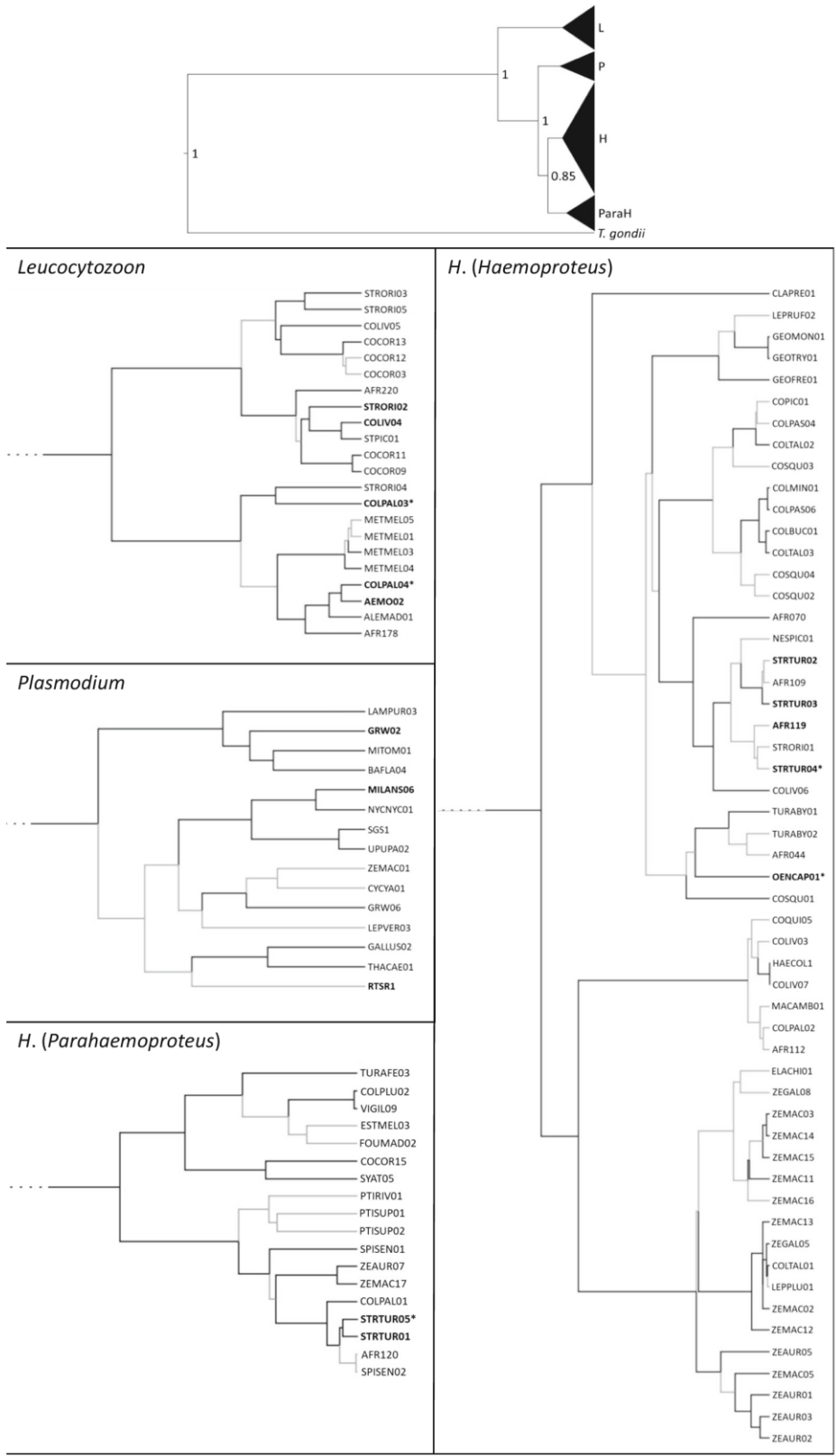
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◀ **Fig. 4** Phylogeny of mitochondrial cytochrome *b* gene lineages (477 bp fragment) of avian haemosporidian parasites (*n* = 109) isolated from blood samples of columbiform birds based on a Bayesian analysis. The four different clades *Leucocytozoon* (L), *Plasmodium* (P), *H.* (*Haemoproteus*) (H) and *H.* (*Parahaemoproteus*) (ParaH) are shown in the overview tree and in one individual zoomed-in tree each together with lineage names. Lineages found in the present study are shown in bold. Newly discovered lineages are marked with '*'. *Toxoplasma gondii* (KM657812) was included as outgroup. GenBank accession numbers are given in Table S1. Nodal support values indicate posterior clade probabilities. If the node support is not shown by digits, nodes with posterior probabilities < 90% are coloured in grey

Despite a rather high overall prevalence, the infection intensity (parasitemia) was in general rather low. Studies on parasitemia are still limited despite their importance (Huang et al. 2020). The damage produced on the host species greatly depends on the infection intensity with the harmful effects being most pronounced when parasitemia is very high (Sol et al. 2003). However, medication experiments have shown that also chronic infections at lower intensities can influence host reproductive success and conditions (Merino et al. 2000; Knowles et al. 2010). To our knowledge, there is no study examining the parasitemia of the species sampled here. However, the average parasitemia (approx. 10 parasites per 10,000 erythrocytes) was lower than in wild columbiform birds sampled in Nigeria (100 per 10,000; Akinpelu 2008), on the Canary Islands (148 per 10,000; Foronda et al. 2004) and in India (*Haemoproteus*: 350, and *Plasmodium* 150 per 10,000; Gupta et al. 2011). Only one of our sampled birds, a turtle dove, showed a severe *Haemoproteus* infection with parasitemia of approx. 900 parasites per 10,000 erythrocytes. However, the adverse effects of haemosporidian infections to the avian host depend on many factors (e.g. host immunity, food availability, or infection intensity) (Chagas et al. 2016) and are therefore difficult to assess for our sampled birds.

Intraspecific differences in turtle doves

Population genetic analyses have shown that turtle doves are not genetically structured across their flyways (Calderon et al. 2016). For haemosporidian parasites infecting turtle doves, we found differences in *Haemoproteus* and *Leucocytozoon* prevalence with the timing of sampling, but no significant differences in prevalence between the flyways. These results are consistent with other studies that have described seasonal variation in the prevalence over the annual cycle (Klei and DeGiusti 1975; Cosgrove et al. 2008; Hellgren et al. 2013; Dubiec et al. 2016; Walther et al. 2016; Pulgarín-R et al. 2018; Soares et al. 2020).

Leucocytozoon prevalence was highest in turtle doves sampled during spring migration. This pattern fits previous work showing that *Leucocytozoon* infections mainly occur in spring and autumn (Atkinson and van Riper 1991). However, other

studies found contrasting *Leucocytozoon* prevalence patterns. Significantly, lower infection rates at spring migration stopover sites compared to breeding areas were detected for redstarts *Phoenicurus phoenicurus* (Rintamäki et al. 1999). In garden warblers *Sylvia borin*, pooled *Leucocytozoon* infections showed no circannual variation in prevalence, but variation could be detected for some lineages when analysed individually (Hellgren et al. 2013). The higher infection rate of *Leucocytozoon* in turtle doves in spring might be due to a seasonal outbreak. The infection peak might be induced by a spring relapse due to physiological cues in the host (Applegate and Beaudoin 1970; Valkiūnas et al. 2004; Cornelius et al. 2014) and/or with the return of the simuliid vectors (adult female blackflies) in spring, when environmental conditions enable increased blackfly emergence and activity (Reidelbach and Christl 2002). Mechanisms for seasonal peaks in infection outbreaks include changes in the behaviour and physiology of the parasite, vector and host, but these are difficult to tease apart as many of these changes occur simultaneously (Cornelius et al. 2014). *Haemoproteus* prevalence in turtle doves was particularly high during migration compared to the breeding season. Klei and DeGiusti (1975) determined a peak of *H. columbae* infection in feral pigeons during autumn; other studies demonstrated *Haemoproteus* peaks not only during autumn migration but also during the breeding season (Hellgren et al. 2013; Pulgarín-R et al. 2018). Besides sampling date, *Haemoproteus* prevalence and diversity might be influenced by differences at the African wintering grounds, where *Haemoproteus* transmission mainly takes place (Valkiūnas 2005; Waldenström et al. 2002; Shurulinkov and Ilieva 2009). Mirroring the different migration flyways of turtle doves, studies indicate different winter regions in the western, central and eastern Sub-Sahara (Zwarts et al. 2009). Pathogen transmission may be an important driver of site selection during the non-breeding period. Some migration strategies are thought to be the result of species actively avoiding parasite-rich habitats by choosing a winter site with low prevalence of haemosporidian parasites (Waldenström et al. 2002; Clark et al. 2016). However, many variables shape migration patterns and non-breeding habitat choice, and the relative importance of active parasite avoidance compared to other factors needs further research (Clark et al. 2016).

Plasmodium infections showed no seasonality in our study. Contrarily, seasonal variation in pooled *Plasmodium* prevalence was found in a population of blue tits with prevalence peaks in spring and autumn. However, this variation was present in pooled *Plasmodium* infections only, whereas *P. relictum* prevalence was more stable through the annual cycle (Cosgrove et al. 2008). In garden warblers, *Plasmodium* infection peak was during the wintering stage. However, this pattern was not consistent for all lineages, and one of the most common *Plasmodium* lineages SGS1 showed no significant circannual variation (Hellgren et al. 2013). To give a more accurate picture

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of genus-specific or even lineage-specific seasonality of hae-mosporidian parasites in turtle doves, we would need samples from the autumn migration and wintering season.

Haemosporidian parasite prevalence was rather high in turtle doves and these parasites can have negative effects on hosts, but as observed parasitemia was rather low, we deem the contribution of haemosporidian infections to the turtle doves' decline to be rather insignificant. *Plasmodium* is known to cause mortality in wild susceptible bird populations, especially when birds are co-infected with other pathogens such as Usutu virus (Rouffaer et al. 2018). However, *Plasmodium* infection in turtle doves was rather rare and the impact of *Haemoproteus* and *Leucocytozoon*, which had a higher prevalence, on avian populations is generally thought to be less severe (Yanga et al. 2011). Nevertheless, the future development of avian haemosporidians in declining turtle doves should be monitored. Under a scenario of global change, a temperature increase and anthropogenic land-use change may provide new opportunities for blood parasite transmission in areas where they were previously absent as well as alter their diversity and composition (Dunn and Outlaw 2019; Ferraguti et al. 2019; Heym et al. 2019).

The identification of current transmission areas and parasite diversity is highly relevant to recognize and understand possible future changes (Ciloglu et al. 2020b). As habitat destruction and land-use intensification are among the main reasons causing the sharp decline of turtle doves (Fisher et al. 2018), they seem especially prone to be affected by these changes, and thus, parasites could gain importance as threats in the future.

Methodological inconsistencies regarding parasite prevalence

To achieve an assessment of prevalence as accurately as possible, we applied two PCR assays as well as microscopic examination. Different methodological approaches led to differing prevalence. A lower prevalence was derived from blood smear counts. This is in line with other studies on columbiform birds, determining a lower prevalence based on microscopic examination compared to molecular techniques (Dunn et al. 2017; Tavassoli et al. 2018). The absence of gametocytes in blood smears of birds PCR positive can be explained by light gametocyte parasitemia, DNA amplification of circulating sporozoites or presence of remnants of parasites that aborted development (Valkiūnas et al. 2009; Chagas et al. 2016). The PCR-based method displays the detection of parasitic genome, but does not reveal whether parasites have or will develop into a successful infection (Valkiūnas 2005). Therefore, microscopic examination is important to distinguish between abortive and successful chronic infections. In general, when finding a 'rare' lineage, we cannot exclude the scenario that the host is a 'dead-end' (Hellgren

et al. 2013). For three 'rare' lineages (COLPAL03, MILANS06, RTSR1), we could not find infected erythrocytes, indicating a potential abortive infection, i.e. parasites initiate development in a 'wrong' host, in which sporozoites initiate exo-erythrocytic development, which is then aborted, resulting in merozoites and gametocytes do not appear (Ciloglu et al. 2020b). Abortive infections are 'dead ends' of transmission, but might still be virulent (Valkiūnas and Iezhova 2017) and therefore are important to determine as such. Furthermore, microscopy can quantify infection intensity. Quantifying the parasitemia as average number of parasites in one affected host is important, as infection intensity appears to be a more reliable predictor of the parasite virulence than prevalence, given as the number of infected animals per total number of animals (Sol et al. 2003). The parasitemia was rather low for the majority of our samples, indicating mainly chronic instead of acute infections. Pathological effects are expected to be higher at the acute infection than at chronic stages (Townsend et al. 2018).

The applied nested PCR method may have underestimated mixed infections of *Haemoproteus* and *Plasmodium*. Therefore, we have used a second PCR assay. Ciloglu et al. (2019) stated that the multiplex PCR assay was designed for amplification of *H. (Parahaemoproteus)* and that it needs to be tested whether the amplification of primers works for *H. (Haemoproteus)*. We could show that the primers amplify *H. (Haemoproteus)* infections, but display them at the same band height as *Plasmodium* infections. Hence, it was not possible to distinguish between *H. (Haemoproteus)* and *Plasmodium* infections according to obtained PCR bands. Therefore, the multiplex PCR assay seems not effective for species that are prone to *H. (Haemoproteus)* infections. Besides the order of Columbiformes, this mainly applies to the orders Pelecaniformes, Charadriiformes and Suliformes (Valkiūnas 2005; Levin et al. 2011, 2012; Merino et al. 2012). Occasionally, *H. (Haemoproteus)* also infects Passeriformes (Lacorte et al. 2013; Ferreira Junior et al. 2017). Our results indicate that the amplified fragment of non-coding mtDNA is more similar between *H. (Haemoproteus)* and *Plasmodium* than between *H. (Haemoproteus)* and *H. (Parahaemoproteus)*. However, the PCR amplified a rather short fragment only. In general, there is no agreement on deep-level phylogenetic of avian haemosporidian parasites (Walther et al. 2016). While some authors refer to *H. (Haemoproteus)* and *H. (Parahaemoproteus)* as subgenera (Iezhova et al. 2011; Valkiūnas et al. 2013), others propose splitting them into two separate genera (Borner et al. 2016; Galen et al. 2018; Soares et al. 2020). Even though our study did not clarify the phylogenetic classification of *H. (Haemoproteus)* and *H. (Parahaemoproteus)*, we could show that both infect columbiform birds. Several studies stated that all *Haemoproteus* parasites found in birds of the order Columbiformes belong to *H. (Haemoproteus)* (Boundenga et al. 2017). In fact, our phylogenetic analyses placed most

lineages infecting columbiform birds into the *H.* (*Haemoproteus*) clade, but some also clustered within the *H.* (*Parahaemoproteus*) clade (STRTUR01 and STRTUR05). These results are in line with a few other studies, which isolated *H.* (*Parahaemoproteus*) from columbiform species (Križanauskienė et al. 2013; Boundenga et al. 2017).

In conclusion, this study contributes to our understanding of the haemosporidian parasite diversity circulating in free-living birds of the order Columbiformes. In addition to reporting novel lineages and novel host species, the data obtained here contribute to improve our knowledge on the taxonomic relationship of avian haemosporidians and offer reference information to monitor likely future changes in parasite ranges and diversity as a consequence of climate change, representing a potential relevant risk for declining turtle doves.

Compliance with ethical standards

Ethics approval and consent to participate   All applicable institutional and/or national guidelines for the care and use of animals were followed. The blood samples at the veterinary clinic were obtained as surplus from those taken initially in course of routine examinations of sick or injured birds upon admission to the veterinary service to perform necessary serological, hematological or blood chemical diagnostics. All authors voluntarily agree to participate in the elaboration and publication of this manuscript.

Consent for publication   All authors declare that they participated in the study and in the development of the manuscript as well as read the final version and give consent for the article to be published in *Parasitol Res*.

Conflict of interest   The authors declare no competing interests.

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APPENDIX

<u>SUPPLEMENTARY MATERIAL</u>

The following supplementary material is saved on a CD (attached to this thesis). The files are stored in folders sorted and named according to the chapters:

CHAPTER 1 | **Electronic supplementary material**

- *Supplementary Figure 1* Assignment to likely wintering origin (moulting areas of winter-grown primary feathers) of European turtle doves *Streptopelia turtur* (N = 181) sampled in seven different European countries (labeled and gray shaded) predicted from a multivariate normal probability distribution function based on tenth primary feather (P10) δ^2H and δ^{13}C isotope assignments of individual birds. Assignment probabilities of individuals (0 to 1) were summed according to the maximum value obtained in a pixel during the assignment process for the single countries (shown in red: a: Spain, b: France, c: Germany, d: Italy, e: Malta, f: Bulgaria, and g: Greece) representing the percent of individuals potentially originating from a cell in the isoscape. The assignment is restricted to a hitherto described turtle dove wintering range (red outline)

- *Supplementary Figure 2* Assignments to likely wintering origin (moulting areas of winter-grown primary feathers) of European turtle doves *Streptopelia turtur* predicted from a normal probability distribution function based on tenth primary feather (P10) δ^2H isotope assignments of individual birds. **(a)**: All individuals (n = 181), sampled in seven different European countries (labeled and shaded gray). Separated for **(b)**: the western flyway (n = 121) and **(c)**: the central/eastern flyway (n = 55). Assignment probabilities of individuals (0 to 1) were summed according to the maximum value obtained in a pixel during the assignment process for the respective sample set representing the percent of individuals potentially originating from a cell in the isoscape. The assignments are restricted to a hitherto described turtle dove wintering range (outline in red)

CHAPTER 2 | **Electronic supplementary material**

- Electronic supplementary material File 1, including:
  - *Supplementary Table 1* Percentage [%] of land cover classes occurring in the 95% Epanechnikov kernels of breeding habitats of satellite-tracked European turtle dove individuals
  - *Supplementary Table 2* Percentage [%] of land cover classes occurring in the 95% Epanechnikov kernels of wintering habitats of satellite-tracked European turtle dove *Streptopelia turtur* individuals

o *Supplementary Table 3* Results of the principal component analysis (PCA) conducted for seven habitat variables obtained through the Environmental Data Automated Track Annotation System (Env-DATA) on Movebank (movebank.org) for positions of satellite-tracked European turtle doves *Streptopelia turtur*

o *Supplementary Table 4* Studies on habitat selection patterns and habitat requirements of European turtle doves *Streptopelia turtur*

o *Supplementary Figure 1* Individual satellite tracks of European turtle doves *Streptopelia turtur* during migration between European breeding (red circles) and African wintering grounds (blue circles). Tracks are given for the individuals (**a-e**) which reached the wintering grounds and for individuals (**f-n**) which did not reach the sub-Saharan wintering grounds. Black circles correspond to stopover sites and '‡' indicates stopover moult sites. Catching and tagging position is displayed as yellow cross and a black cross indicates the end of data transmission. Autumn migration is shown as solid black line and spring migration as dashed black line. Partial tracks are displayed in grey. Duration at the different sites is given in dd.mm (ST = Start of data transmission; ET = End of data transmission). Background colours indicate the terrain and grey lines indicate national borders (Background map: Stamen terrain (map tiles by Stamen Design: http://maps.stamen.com; data by OpenStreetMap: www.openstreetmap.org))

o *Supplementary Figure 2* Breeding and post-breeding sites of European turtle dove #161050. Presented are the three years we received data during the entire breeding period (2017 = green, 2018 = orange, 2019 = blue). Shown are the filtered Argos location fixes as points and calculated Epanechnikov kernels (95% and 50% Kernel Utilization Distributions 'KUD' as dashed and solid lines, respectively). Duration at the different sites is given in dd.mm. Background map: Stamen terrain (map tiles by Stamen Design: http://maps.stamen.com; data by OpenStreetMap: www.openstreetmap.org)

o *Supplementary Figure 3* Overview chart of migration pattern of 13 satellite tracked European turtle doves *Streptopelia turtur*. Shown are the countries used for stopovers during autumn and spring migration and wintering for turtle doves with different breeding areas (colors indicate the country or federal state the breeding site was located). Country codes are given as two-letter code (ISO-3166-1 ALPHA-2). 'X' represents that no stopover was done. Digits in brackets or at the lines indicate the number of individuals using the site. 'Western', 'Central' and 'Eastern' indicate the used flyway. '*' as we have data from individual #161050 for consecutive years every of the years is depicted as 'single' count. If the data transmission has ended, the line was not continued from the last site

- o *Supplementary Figure 4* Habitat niches of satellite-tracked European turtle doves *Streptopelia turtur* (n = 5) for wintering (blue) and breeding (red) grounds. Obtained from Argos-positions and kernel densities of principal component scores of environmental parameters (n = 9) obtained through the Environmental Data Automated Track Annotation System (Env-DATA) on Movebank. **a**: individual #161046; **b**: #161048; **c**: #161050; **d**: #181091; **e**: #181092. Environmental parameters determining respective PC1 and PC2 as well as their eigenvalues can be found in detail in Supplementary Table 3
- Electronic supplementary material File 2, excel file including:
  - o Keys for 'TrackingData' sheets and 'EnvData' sheet
  - o Raw Argos tracking data

CHAPTER 3 | **Electronic supplementary material**

- Electronic supplementary material File 1, including:
  - o *Supplementary Table 1* Circumstances of recovery of ringed Common Woodpigeons *Columba palumbus*. All individuals here were recovered during wintering timea in the different countries.
  - o *Supplementary Table 2* Home range (95% KUD, Epanechnikov kernel) and core area (50% KUD, Epanechnikov kernel) size of Common Woodpigeons *Columba palumbus* equipped with GPS-GSM transmitters. KUDs are given for individuals in Lisbon, Portugal (L; n = 10), Giessen, Germany (G; n = 19) and wintering sites of migrating individuals per month throughout the annual cycle [Mean ± SE]. Given in the brackets for 50% and 95% KUD is the number of months and individuals (no. months / no. individuals). Given in brackets in the column 'Movements' are the number of individuals visiting the farmland outside the city and the total number of individuals (no. visiting farmland / total no. of individuals).
  - o *Supplementary Table 3* Average proportions [%] of land cover categories in monthly home ranges calculated on the basis of GPS locations of tagged Common Woodpigeons *Columba palumbus*. Numbers are given for individuals from two regions and with different migrations strategies (L: Lisbon, Portugal; G: Giessen, Germany, residents and individuals during the non-wintering season; MD: Individuals using another distinct site during the wintering season than during the breeding season, but migratory movements occurred within Germany; MF: Woodpigeons migrating to France). Land cover categories were named according CORINE Land Cover (CLC) nomenclature.

o *Supplementary Figure 1* Overview of validated ring recoveries of Common Woodpigeon *Columba palumbus* with breeding and wintering grounds within Germany. Coordinates where ringed Woodpigeons were spotted or caught during breeding time (pink circles) and during wintering time (blue squares) are connected by a straight dashed line. In case of identical coordinates during breeding and wintering time, the location is displayed by a yellow star symbol. Background colours indicate the terrain, black lines national borders and grey lines borders of the federal states in Germany (Background map: Stamen Design http://maps.stamen.com; data by OpenStreetMap: www.openstreetmap.org))

o *Supplementary Figure 2* Annual cycle of Common Woodpigeons *Columba palumbus* equipped with Argos-transmitters during the non-breeding season in Portugal and France. The map gives the spatial organisation with spring migration (solid line) and autumn migration (dashed line) between the breeding sites (circles) in Germany and the nonbreeding sites (squares). The star symbol indicates that breeding and non-breeding time were spent at the same location. The triangles indicate stopover sites and crosses that the last Argos-position was transmitted outside the breeding or non-breeding site. The inset shows the temporal organisation with percentages of time for period spent at the breeding site (dark grey), the non-breeding site (light grey) and on migration (striped = spring migration; dotted = autumn migration) and average arrival and departure for each respective period. Background colours indicate the terrain and grey lines indicate national borders (Background map: Stamen terrain (map tiles by Stamen Design: http://maps.stamen.com; data by OpenStreetMap: www.openstreetmap.org))

o *Supplementary Figure 3* Alteration of longitudes of Argos locations of tracked Common Woodpigeon *Columba palumbus* within the annual cycle. The average longitude per decade is presented. Grey shaded time periods correspond to spring and autumn migration periods (for all individuals combined)

- o *Supplementary Figure 4* Diagram representing the migratory systems based on satellite tracking data of the studied Common Woodpigeon *Columba palumbus* populations: Resident population of Portugal, Lisbon (n = 12 winter periods from 10 individuals equipped with GPS transmitters) and partial migratory population of Germany, Hesse (GPS: n = 30 winter periods from 19 individuals; Argos: n = 7 winter periods from 5 individuals only data of the second year was included). Annual seasons are separated by month (given as numbers 01-12) and displayed by colour (yellow = summer, orange = autumn, blue = winter, green = spring). During the months (November – March) of the non-breeding season migrants and residents of partial migratory population winter in different habitats, whereas individuals of Lisbon share the same habitat the entire year. Height of boxes is proportional to the sample sizes of the two breeding populations and proportion of wintering strategies
- o *Supplementary Figure 5* Annual cycle of migrating Common Woodpigeons *Columba palumbus* equipped in Hesse (DE) with GPS-GSM transmitters. The map gives the spatial organisation with spring migration (solid line) and autumn migration (dashed line) between the tagging and breeding sites in Germany (star symbol: C = Caldern, G = Giessen) and the non-breeding, i.e. wintering sites (squares). Black dashed lines depict movements between wintering sites. The triangles indicate stopover sites and the cross that the last GPS position was transmitted on the wintering site. The inset shows the temporal organisation with percentages of time for period spent at the breeding site (dark grey), the non-breeding site (light grey) and on migration (striped = spring migration; dotted = autumn migration) and average arrival and departure for each respective period. Background colours indicate the terrain and black lines indicate borders (Background map: Stamen terrain (map tiles by Stamen Design: http://maps.stamen.com; data by OpenStreetMap: www.openstreetmap.org))
- o *Supplementary Figure 6* Winter movements of Common Woodpigeons *Columba palumbus* equipped in Hesse (DE) with GPS-GSM transmitters. Individuals that wintered within Germany in proximity to their breeding ground in Giessen are shown, as well as the movements of individual #190759 between Giessen (G) and Herborn (H). The map shows spring movements (solid line) and autumn movements (dashed line) between the breeding sites (star symbol) and the non-breeding, i.e. wintering sites (squares). Black dashed lines depict movements between wintering sites and the cross that the last GPS position was transmitted on the wintering site. Background colours indicate CLC land cover categories (Corine Land Cover CLC 2018 v.2020_20u1 raster land cover data; Copernicus Land Monitoring Service)

o *Supplementary Figure 7* Core area size (50% KUD) of Common Woodpigeons *Columba palumbus.* Shown are Woodpigeons from two regions (Lisbon, Portugal and Giessen, Germany) and with different migrations strategies (Giessen (DE): residents and individuals during the non-wintering season; Migration (DE): Individuals using another distinct site during the wintering season than during the breeding season, but migratory movements occurred within Germany; Migration (FR): Woodpigeons migrating to France). Boxplots denote the median value, interquartile range (25–75th percentiles) and range of core area size. The star symbol represents the mean value. Outliers are plotted as individual points

o *Supplementary Figure 8* Home range size (95% KUD) of Common Woodpigeons *Columba palumbus.* Shown are Woodpigeons from two regions (Lisbon, Portugal and Giessen, Germany) and with different migrations strategies (Giessen (DE): residents and individuals during the non-wintering season; Migration (DE): Individuals using another distinct site during the wintering season than during the breeding season, but migratory movements occurred within Germany; Migration (FR): Woodpigeons migrating to France). Boxplots denote the median value, interquartile range (25–75th percentiles) and range of home range size. The star symbol represents the mean value. Outliers are plotted as individual points

CHAPTER 4 | **Electronic supplementary material**

- Electronic supplementary material File 1, including:
    o *Supplementary material A1* Initial tests for amplicon PCR
    o *Supplementary material A2* Amplicon and index PCR setups and cycling conditions
    o *Supplementary material A3* GALAXY workflow
    o *Supplementary Table 1* Collected faecal samples from three species of the order Columbiformes (TD = European Turtle Dove *Streptopelia turtur*, SD = Stock Dove *Columba oenas*, WP = Common Woodpigeon *C. palumbus*) at different sampling sites in Germany and the Netherlands from the years 2013 to 2020 and results of amplicon PCR amplifications of plant and metazoan DNA
    o *Supplementary Table 2* Seed mixes used at baited sites in Germany and Netherlands to attract columbiform species
    o *Supplementary Table 3* Best blast results for each of the 118 detected valid MOTUs using the UniPlant primer pair (Plant) and mICOIintF/dgHCO-2198 (Metazoa) corresponding accession number, the identity with the blast reference sequence, the sequence length and the bitscore. If determination to species level was not clearly determinable, MOTUs were assigned to the lowest shared taxonomic level. Order of the plant MOTUs equates to their order in Table 1

- o *Supplementary Table 4* Differences in the occurrence (presence/absence data) of plant families determined in the diet of three columbiform species (WP = Common Woodpigeon *Columba palumbus*, TD = European Turtle Dove *Streptopelia turtur*, SD = Stock Dove *C. oenas*). Differences among species were tested pairwise with chi² tests. Statistically significant results (p < 0.05) and associated families are marked bold

- o *Supplementary Table 5* Differences in the occurrence (presence/absence data) of plant genera determined in the diet of three columbiform species (WP = Common Woodpigeon *Columba palumbus*, TD = European Turtle Dove *Streptopelia turtur*, SD = Stock Dove *C. oenas*). Genera of the five most frequently represented families are shown. Differences among species were tested pairwise with chi² tests. Statistically significant results (p < 0.05) and associated genera are marked bold

- o *Supplementary Table 6* Stock Dove *Columba oenas* diet composition: Compilation of our results and results from previous studies. Given are the plant families and animal prey taxa found in the diet of Stock Doves based on different methods. The plant or animal item is marked with "x" if it was found in the respective study

- o *Supplementary Table 7* Common Woodpigeon *Columba palumbus* diet composition: Compilation of our results and results from previous studies. Given are the plant families and animal prey taxa found in the diet of Woodpigeons based on different methods. The plant or animal item is marked with "x" if it was found in the respective study

- o *Supplementary Table 8* European Turtle Dove *Streptopelia turtur* diet composition: Compilation of our results and results from previous studies. Given are the plant families and animal prey taxa found in the diet of Turtle Doves based on different methods. The plant or animal item is marked with "x" if it was found in the respective study

- o *Supplementary Figure 1* Sampling locations of faecal samples of three species from the order of Columbiformes (Common Woodpigeon *Columba palumbus*, European Turtle Dove *Streptopelia turtur*, Stock Dove *C. oenas*) in Germany and the Netherlands. Black triangles represent temporarily baited sites and white triangles sites without bait. 1: Helgoland, 2: Wilhelmshaven, 3: Zak van Zuid-Beveland, 4: Lieberoser Heide, 5: Lausitz, 6: Caldern, 7: Zeulenroda, 8: Giessen, 9: Hungen-Villingen, 10: Cleeberg, 11: Eichkopf, 12: Weilbacher Kiesgruben, 13: Groß-Umstadt. The cross marks the location of the clinic for birds ('Vetmed', WP). For exact sample numbers per site see Table A1

o *Supplementary Figure 2* Diet composition of Common Woodpigeons *Columba palumbus* (WP), European Turtle Doves *Streptopelia turtur* (TD) and Stock Doves *C. oenas* (SD). Genera of the five most frequently represented plant families (**A**: Asteraceae, **B**: Brassicaceae, **C**: Cucurbitaceae, **D**: Fabaceae and **E**: Poaceae) found in faecal samples represented as the frequency of occurrence (FOO%) per species. '*' indicates significant difference ($p < 0.05$) in the occurrence (presence/absence data) of respective genera between two species (Table A5)

o *Supplementary Figure 3* Differences in the diet composition at plant genus level in three columbiform species (Common Woodpigeon *Columba palumbus* (WP); European Turtle Dove *Streptopelia turtur* (TD); Stock Dove *C. oenas* (SD)), using Non-metric Multidimensional Scaling (NMDS, function *metaMDS* in the R package 'VEGAN'). Depicted are (A) the distribution of the plant genera (the first four or five letters of the genera are given) and (B) the distribution of samples and 95% confidence ellipses

CHAPTER 5 | Electronic supplementary material

- *Supplementary Figure 1* Map of sampling locations for blood samples of columbiform birds
- *Supplementary Figure 2* Giemsa-stained blood smear of an adult, male European turtle dove (*Streptopelia turtur*) sampled 2019 on Antikythira Island, Greece
- *Supplementary Table 1* Lineage names and associated GenBank accession numbers for avian haemosporidian lineages (n = 109) used for phylogenetic tree construction based on a Bayesian analysis

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LIST OF PUBLICATIONS

Peer-reviewed paper

Quillfeldt P, **Schumm YR**, Marek C, Mader V, Fischer D, Marx M (2018) Prevalence and genotyping of *Trichomonas* infections in wild birds in central Germany. PLoS ONE 13: e0200798. DOI: 10.1371/journal.pone.0200798

Schumm YR, Wecker C, Marek C, Wassmuth M, Bentele A, Willems H, Reiner G, Quillfeldt P (2019) Blood parasites in Passeriformes in central Germany: prevalence and lineage diversity of Haemosporida (*Haemoproteus*, *Plasmodium* and *Leucocytozoon*) in six common songbirds. PeerJ 6: e6259. DOI: 10.7717/peerj.6259

Schumm YR, Bakaloudis D, Barboutis C, Cecere JG, Eraud C, Fischer D, Hering J, Hillerich K, Lormée H, Mader V, Masello JF, Metzger B, Rocha G, Spina F, Quillfeldt P (2021) Prevalence and genetic diversity of avian haemosporidian parasites in wild bird species of the order Columbiformes. Parasitology Research 120: 1405-1420. DOI: 10.1007/s00436-021-07053-7

Castaño-Vázquez F, **Schumm YR**, Bentele A, Quillfeldt P, Merino S (2021) Experimental manipulation of cavity temperature produces differential effects on parasite abundances in blue tit nests at two different latitudes. International Journal for Parasitology: Parasites and Wildlife 14: 287-297. DOI: 10.1016/j.ijppaw.2021.03.010

Fecchio A, Clark NJ, Bell JA, Skeen HR, Lutz HL, De La Torre GM, Vaughan JA, Tkach VV, Schunck F, Ferreira FC, Braga EM, Lugarini C, Wamiti W, Dispoto JH, Galen SC, Kirchgatter K, Sagario MC, Cueto VR, González-Acuña D, Inumaru M, Sato Y, **Schumm YR**, Quillfeldt P, Pellegrino I, Dharmarajan G, Gupta P, Robin VV, Ciloglu A, Yildirim A, Huang X, Chapa-Vargas L, Álvarez-Mendizábal P, Santiago-Alarcon D, Drovetski SV, Hellgren O, Voelker G, Ricklefs RE, Hackett SJ, Collins MD, Weckstein JD, Wells K (2021) Global drivers of avian haemosporidian infections vary across zoogeographical regions. Global Ecology and Biogeography 30: 2393-2406. DOI: 10.1111/geb.13390

Schumm YR, Metzger B, Neuling E, Austad M, Galea N, Barbara N, Quillfeldt P (2021) Year-round spatial distribution and migration phenology of a rapidly declining trans-Saharan migrant - evidence of winter movements and breeding site fidelity in European turtle doves. Behavioral Ecology and Sociobiology 75: 152. DOI: 10.1007/s00265-021-03082-5

Marx M, **Schumm YR**, Kardynal KJ, Hobson KA; Rocha G, Zehtindjiev P, Bakaloudis D, Metzger B, Cecere JG, Spina F, Cianchetti-Benedetti M, Frahnert S, Voigt CC, Lormée H, Eraud C, Quillfeldt P (2022) Feather stable isotopes (δ^2H$_f$ and δ^{13}C$_f$) identify the Sub-Saharan wintering grounds of turtle doves from Europe. European Journal of Wildlife Research 68: 21. DOI: 10.1007/s10344-022-01567-w

Conference contributions

Masello JF, Rösner S, **Schumm Y**, Ehmig M, Lindner K, Quillfeldt P (2019) Movement ecology, energy landscapes, and the microhabitat choice of Common Woodpigeons *Columba palumbus*. 152. Annual Conference, Deutschen Ornithologen Gesellschaft, Marburg [*Poster*]

Rösner S, Lindner K, Ehmig M, Strehmann F, **Schumm YR**, Quillfeldt P, Farwig N, Masello JF (2019) Competition, stress and parasite prevalence: A bird community approach in an interior forest ecosystem. 152. Annual Conference, Deutschen Ornithologen Gesellschaft, Marburg [*Poster*]

Schumm Y, Metzger B, Barbara N, Neuling E, Lachmann L, Quillfeldt P (2019) Ist die Turteltaube ein Gewohnheitstier? Ergebnisse satellitentelemetrischer Untersuchungen an europäischen Turteltauben. 152. Annual Conference, Deutschen Ornithologen Gesellschaft, Marburg [*Talk*]

Schumm Y (2020) Wildtauben in Hessen: Vergleich ökologischer Aspekte von Turtel-, Ringel- und Hohltaube. Fachsymposium 2020 – Vielfalt der Natur, NABU Landesverband Hessen, Wetzlar [*Talk*]

Strehmann F, Lindner K, Becker M, Schumm YR, Quillfeldt P; Masello JF, Farwig N, Lindner K, Becker M, **Schumm YR**, Quillfeldt P, Masello JF, Farwig N, Schabo D, Rösner S (2021) Prevalence of blood parasites in a temperate forest bird community. 50th Annual Meeting of the Ecological Society of Germany, Austria and Switzerland. Online Conference [*Electronic Poster*]

Schumm YR, Masello JF, Vreugdenhil-Rowlands J, Fischer D, Hillerich K, Quillfeldt P (2022) Who ate what? Diet composition of Common Woodpigeons, European Turtle Doves and Stock Doves determined by next-generation sequencing of plant and metazoan DNA in faecal samples. 13th European Ornithologists' Union Congress. Online Conference [*Electronic Poster*]

ACKNOWLEDGEMENTS

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ILLUSTRATION CREDITS

Credits for included illustrations from scientific publications are given in the respective figure captions.

Laura Prause generously created the Woodpigeon illustration included on the thesis title page.

Aleksandra Czylok generously created the Turtle Dove included in Figure 2 and the Woodpigeon with nestlings added to the last page.

Photo of the Turtle Dove with Argos transmitter used as the title page for chapter 2 was taken by Mélibée Morel and the photo of the Woodpigeon with GPS-GSM/GPRS transmitter used for chapter 3 by Benjamin Metzger.

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