Population connectivity of European Turtle Doves (*Streptopelia turtur*) –

Threats affecting European populations and modelling of species-habitat relationships at German breeding grounds

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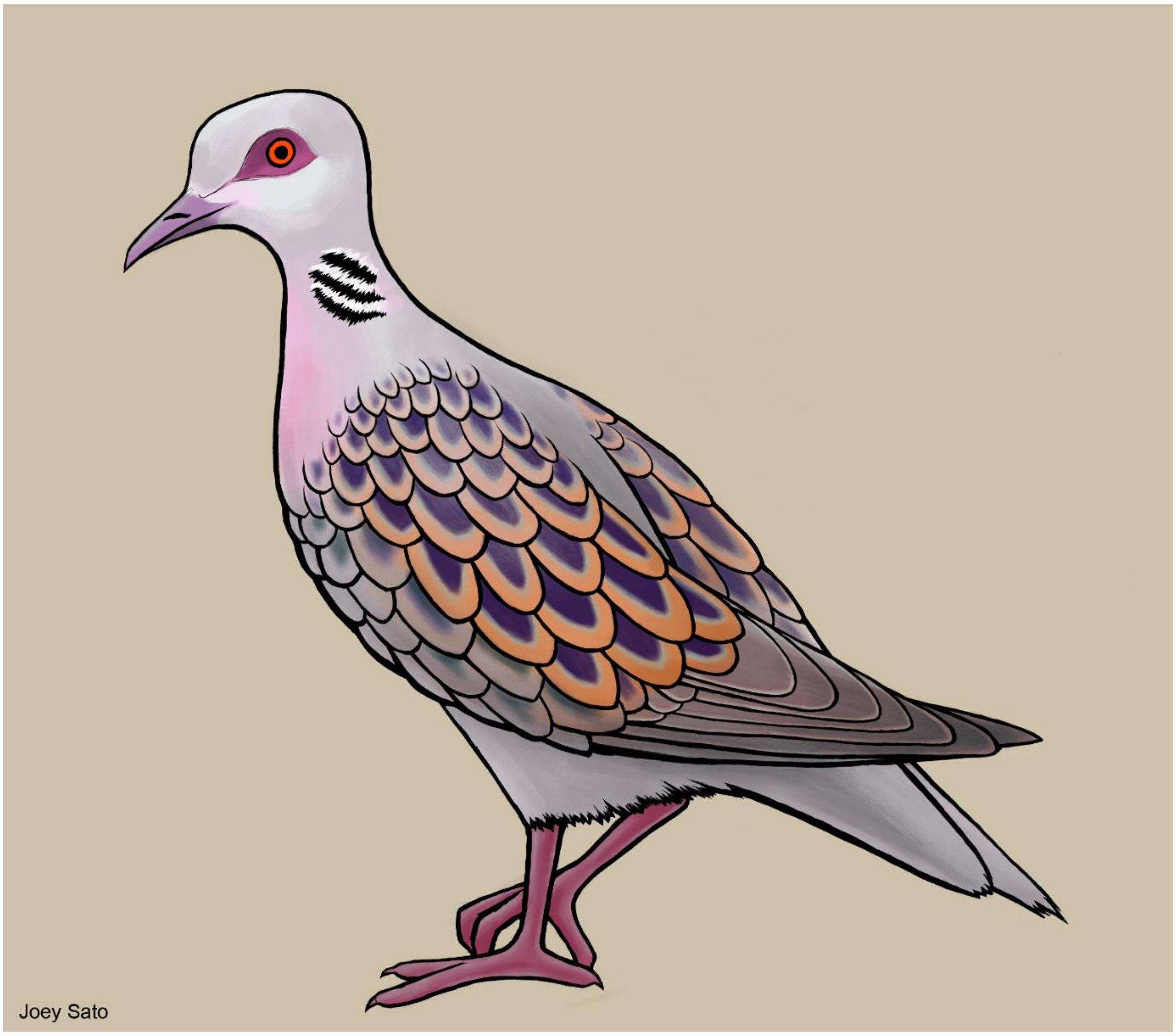
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Abstract

European Turtle Doves (*Streptopelia turtur*), Europe's only long distance migrating columbids, experienced a strong population decline of more than 78% since 1980. Studies about Turtle Dove's population connectivity and threats affecting the population are needed. Therefore, this dissertation aims to infer the population connectivity of Turtle Doves and evaluate the potential impact of hunt, with a) a ring re-encounter analysis (chapter 1) and b) a stable isotope assignment (chapter 2). Furthermore, prevalences and genetic lineages of the parasite *Trichomonas gallinae* were analysed (chapter 3) and species distribution models (SDMs) were applied to determine habitat requirements and potential key breeding areas in Germany (chapter 4).

The first chapter suggests a strong connectivity for Turtle Doves in western Europe, mainly following a western flyway, and a rather weak connectivity for birds from central and eastern Europe, which used both, a central and an eastern flyway. The second chapter demonstrates that stable hydrogen isotopes could be used to distinguish four large areas within the breeding ranges, but also supports the suggestion of a weaker connectivity for some birds. The methods applied in both chapters could not identify spatially explicit breeding areas, but clearly demonstrated the high hunting impact on migrating birds in Europe, particularly on those originating from other countries than from those where hunting occurs. The evaluation for *Trichomonas gallinae* highlights a general high prevalence, but an infection with a highly pathogenic lineage could not be proven for Turtle Doves. However, as pathogenic lineages were already demonstrated in British Turtle Doves and were present in German Stock Doves (*Columba oenas*), present results should be taken seriously, due to potential epidemic outbreaks caused by possible inter- and intra-specific disease transfer at life-cycle stages. Germany wide applied SDMs using presence only (PO) data highlighted key breeding sites and were mainly driven by climatic variables, but the land use parameters available were not suitable to define specific local habitat characteristics. To specify those, other variables such as land management practices or soil type should be introduced in future SDMs.

To conclude, the present dissertation shows the successful application of ring re-encounter analysis and stable isotope assignment to study the population connectivity and estimate a potential hunting impact on migrating Turtle Doves. Furthermore, a high prevalence of *Trichomonas gallinae* was demonstrated, which can become a serious threat due to its potentially epidemic character. Moreover, a possible breeding distribution across Germany was modelled, which highlights PO data as a powerful data source for SDMs.

Zusammenfassung

Europäische Turteltauben (*Streptopelia turtur*), Europas einzige langstreckenziehenden Tauben, erlebten seit 1980 einen starken Populationsrückgang von mehr als 78%. Studien zur Populationskonnektivität von Turteltauben und zu Gefahren, die die Population beeinflussen, werden benötigt. Deshalb beabsichtigt diese Dissertation, die Populationskonnektivität von Turteltauben und potenzielle Gefährdung durch Bejagung mit a) einer Ringwiederfundanalyse (Kapitel 1) und b) einer Zuordnung stabiler Isotope (Kapitel 2) abzuleiten und auszuwerten. Weiterhin wurden auch die Prävalenz und genetischen Linien des Parasiten *Trichomonas gallinae* analysiert (Kapitel 3) und Habitatmodellierungen (SDMs) angewendet, um Habitatansprüche und potenzielle Hauptbrutareale in Deutschland zu bestimmen (Kapitel 4).

Das erste Kapitel weist auf eine starke Konnektivität für Turteltauben aus Westeuropa hin, die vorrangig einer Westzugroute folgten und auf eine eher schwache Konnektivität für Vögel aus Zentral- und Osteuropa, welche eine zentrale und eine östliche Zugroute nutzten. Das zweite Kapitel zeigt, dass stabile Wasserstoffisotope genutzt werden konnten, um vier große Areale innerhalb der Brutgebiete zu ermitteln, die aber auch den Vorschlag einer schwächeren Konnektivität für manche Vögel unterstützen. Die angewandten Methoden konnten keine räumlich genauen Brutareale identifizieren, aber sie konnten deutlich den Jagdeinfluss auf migrierende Turteltauben in Europa darlegen, insbesondere auf solche, die aus anderen Ländern stammen als aus jenen in denen Jagd erfolgt. Die Auswertung zu *Trichomonas gallinae* zeigt eine generell hohe Prävalenz, aber eine Infektion mit einer pathogenen Linie konnte in Turteltauben nicht nachgewiesen werden. Da pathogene Linien aber bereits in britischen Turteltauben und deutschen Hohltauben (*Columba oenas*) nachgewiesen wurden, sollten die hier dargelegten Ergebnisse aufgrund der potenziell epidemischen Ausbrüche durch mögliche inter- und intraspezifischen Krankheitsübertragungen während verschiedener Lebensphasen dennoch ernst genommen werden. Deutschlandweit angewandte SDMs mit Präsenzdaten (PO) verdeutlichen Hauptbrutstandorte und wurden vor allem durch klimatische Variablen modelliert, aber diese konnten keine spezifischen lokalen Habitateigenschaften definieren. Um solche Wissenslücken zu füllen, sollten andere Variablen wie Landbearbeitungsmethoden oder Bodentyp in zukünftige SDMs eingefügt werden.

Die vorliegende Dissertation zeigt die erfolgreiche Anwendung einer Ringwiederfundanalyse sowie die Zuordnung stabiler Isotope um Populationskonnektivität zu untersuchen und den potenziellen Jagdeinfluss auf migrierende Turteltauben einzuschätzen. Weiterhin wurde ein hoher Befall durch *Trichomonas gallinae* verdeutlicht, der eine ernst zu nehmende Gefahr aufgrund der potenziell epidemischen Eigenschaften werden kann. Darüber hinaus wurde

eine potenzielle Brutgebietsverbreitung in Deutschland modelliert, die PO-daten als eine leistungsstarke Datengrundlage für SDMs aufzeigt.

General Introduction

Migration is a common and regular process to move between places of different life-cycle stages, occurring in many birds. Mainly those stages are breeding and non-breeding areas with certain conditions and events, which can affect reproduction and survival. Also the way between them can hold several impacts affecting a species but also a population (Webster et al. 2002).

Population connectivity

Studying the population or migratory connectivity of a species can give insights into its ecology and evolution (Webster et al. 2002). The degree of connectivity is strong, when one breeding population winters together in one area and is weaker when one breeding population winters in more dispersed regions (Webster et al. 2002). Furthermore, knowing the whereabouts and habitat requirements of a species during different life-cycle stages can contribute to the species conservation management at these sites (Williams and Araújo 2000, Webster et al. 2002, Martin et al. 2007, Ocampo-Peñula and Pimm 2014). For one species, habitat requirements can vary geographically depending on local conditions (Jaberg and Guisan 2001, Fisher et al. 2018). Furthermore, events occurring at breeding, stopover or wintering sites can affect reproductive success and / or annual survival and need to be studied, because they can influence the population dynamic (Webster et al. 2002). These impacts on the population level can vary geographically within a species (Fallon et al. 2006) and knowledge about threats affecting the population size is crucial to develop long-term conservation plans.

Methods to study population connectivity

Different methodologies are applied to study population connectivity (Webster et al. 2002). These can include mark-recapture studies (e.g. Reichlin et al. 2009, Korner-Nievergelt et al. 2012, 2014), satellite telemetry (e.g. Trierweiler et al. 2014), genetic analyses of the study species (e.g. Calderón et al. 2016) or its pathogens (e.g Haemoproteus or Plasmodium; Fallon et al. 2006), but also stable isotope approaches (Hobson et al. 2009, Hobson et al. 2012 a, b, Cardador et al. 2015). In the present cumulative dissertation, a ring re-encounter analysis and stable isotope assignments were applied to infer the population connectivity of European Turtle Doves (*Streptopelia turtur*). Additionally, genetic analyses of Turtle Dove tissue samples by Calderón et al. (2016), which were conducted alongside this dissertation, contributed information about connectivity. Studying population connectivity with genetic analyses of tissue samples is an indirect method and is based on the genetic variation among migratory populations (Webster et al. 2002). It can be an extremely powerful tool when there are specific genetic markers to particular populations, which are able to allocate individuals to specific breeding grounds (Webster et al. 2002).

Mark-recapture studies on the basis of ringing data, for instance provided by EURING (http:\\www.euring.org) have a great potential to describe general migration patterns due to a great number of collected data covering a long time period. With those datasets it is possible to describe flyway directions or breeding and non-breeding distribution areas, but also population connectivity (e.g. Reichlin et al. 2009, Korner-Nievergelt et al. 2012, 2014). Also, they can be used to detect changes in arrival and departure date (Both et al. 2005), or to evaluate a possible impact of threats on distribution and survival (McCulloch et al. 1992, Aebischer et al. 1999, Péron et al. 2011). However, a drawback of this approach is the non-random nature of both ringing and recapture. Ringing efforts depend often on individual ringers and their interests and recapture would ideally occur in the winter quarters, but in practice frequently involved hunted birds at stopover migration sites (e.g. McCulloch et al. 1992, Chamberlain CP et al. 2000, Schaub and Pradel 2004, Reichlin et al. 2009).

The stable isotope approach benefits from naturally occurring elements, such as hydrogen, carbon, and nitrogen, which vary across landscapes. For instance, stable isotope ratios of hydrogen (δD measure for ratio $^{2}H:^{1}H$, relative to a standard) are determined by precipitation. In Europe and North America, it was shown that δD differs gradually with latitude, elevation, and from coasts to inland (Rozanski et al. 1993, Hobson and Wassenaar 1997, Hobson et al. 2004, West et al. 2008). Therefore, δD is more positive at lower latitudes, lower elevation and at coasts. Conversely, δD is more negative at higher latitudes, higher elevation and towards the continental interior (Rozanski et al. 1993, Hobson and Wassenaar 1997, Hobson et al. 2004, West et al. 2008). In terrestrial ecosystems, $\delta^{13}C$ (ratio of $^{13}C:^{12}C$, relative to a standard) varies geographically with occurrence and frequency of C3 and C4 plants (Cerling at al. 1997, Wang et al. 2010). $\delta^{13}C$ values were shown to be usually more positive in C4 plant types and more negative in C3 plants (Cerling at al. 1997, Still and Powell 2010). Also $\delta^{15}N$ (ratio of $^{15}N:^{14}N$, relative to standard) varies with climatic conditions, foliar nitrogen concentrations and method of nitrogen fixation (Craine et al. 2009). Generally, the findings of the study by Craine et al. (2009) suggested that dry and warm ecosystems have the highest nitrogen availability, which, on average, are usually occupied by plants enriched with positive stable nitrogen (Craine et al. 2009).

That relationship between environmental factors and occurrence of heavier and lighter isotopes can be used to assign origins of animals, but a discrimination factor from diet to analysed tissues needs to be considered (e.g. Rubenstein and Hobson 2004, Hobson et al. 2009, Wunder 2010). The tissue must be chosen according to the temporal period, when the geographical information of the breeding stage of interest was incorporated by feeding and drinking, thus the tissue reflects food-web conditions at the area visited during this stage (Rubenstein and Hobson 2004). For metabolically active tissues the time period represented

by tissues also depends on metabolic turnover rates in tissues (Bearhop et al. 2002). To track whereabouts of migrants, usage of metabolically inert tissues was applied frequently, because those incorporate elements during growth period and do not change during movements (Hobson 1999, Rubenstein and Hobson 2004). For example, those tissues are feathers and claws in migratory birds (Bearhop et al. 2003), or fur and claws in mammals (Voigt et al. 2003), but also wing membranes of insects (Hobson et al. 1999). For breeding and wintering origin assignment of birds, feathers are used regularly (Rocque et al. 2006, Hobson et al. 2009). Therefore, it is important to know which feathers were moulted at breeding grounds and at wintering sites (Hobson 1999).

In case of an assignment of European origins, assignment models can be based on δD (Hobson et al. 2004, Hobson et al. 2009), because of 1.) the availability of δD precipitation data from a variety of measurement stations across Europe, and 2.) the strong correlation of δD in feathers and δD from precipitation (Hobson et al. 2004).

Possible threats affecting a species

For many species it is not possible to survive in human dominated habitats (Shaffer 1981). They require essentially undisturbed habitats to survive in the wild (Shaffer 1981). Others, like farmland birds, depend on the (extensive) management of habitats (Chamberlain et al. 1999, Chamberlain DE et al. 2000). However, due to large colonised land areas but also enormous use and alterations of ecosystems by humans (Vitousek et al. 1997), major threats to many species' are habitat loss and degradation, but also overexploitation, for instance by hunt or resource usages (Smith et al. 2006, Kirby et al. 2008, IUCN 2018). Also, anthropogenic driven climate change (Vitousek et al. 1997, Mitchell et al. 2001) is contributing to reduce species abundance and distribution (e.g. Warren et al. 2001, Walther et al. 2002), form habitats (Vitousek et al. 1997, Martin 2001), and affect pathogen occurrence (Epstein 2001, Harvell et al. 2002, Pounds et al. 2006, Altizer et al. 2013). Further threats affecting 30 – 50% of migratory species are pollution, natural system modifications (e.g. dam building and wetland drainage), residential and commercial development, and human disturbance (Kirby et al. 2008).

Above mentioned threats affect many species – residents and migrants, but migrants have to face different threats during their different life-cycle stages. Depending on the population connectivity and migration strategies used, threats can affect a population to different degrees. In case of weakly connected breeding populations of a species, theoretically, one breeding population can experience no or little decline while the other is decreasing drastically due to e.g. habitat loss at stopovers (Weber et al. 1999, Runge et al. 2014). Also, occupied winter sites can be of different quality and therefore influence the condition of individuals, which can

affect departure and arrival dates, but also breeding performance (Baillie and Peach 1992, Webster et al. 2002, Norris et al. 2003, Marra et al. 2005). Therefore, carry-over effects from one stage or even season to the other can influence the reproductive success, annual survival or population size (Marra et al. 1998, Webster et al. 2002, Norris 2005, Norris and Taylor 2006, Norris and Marra 2007).

As habitat loss and degradation take place globally (Vitousek et al. 1997), these kinds of threat should apply to all breeding populations of migrants and has been demonstrated for instance in monarch butterflies (*Danaus plexippus*), whose severe population declines are mainly driven by the loss of host plants in all breeding ranges (Flockhart et al. 2015). However, habitat loss at wintering sites and extreme weather events caused by ongoing climate change are also negatively affecting this species (Flockhart et al. 2015). Similar to monarch butterflies, almost 80% of migratory land- and waterbird species are affected by habitat loss and degradation due to changes in agri- and aquaculture (Kirby et al. 2008).

Besides the major threat of habitat loss, the threat of hunt is also a serious factor impacting migrants, especially birds (Smith et al. 2006, Kirby 2008, IUCN 2018). Throughout the Mediterranean millions of birds are shot or trapped legally and illegally when they migrate between Africa and Europe (McCulloch et al. 1992, Brochet et al. 2016) and illegal hunt is recognised as a large pan-European problem affecting several bird species (Hirschfeld and Heyd 2005, Larnaca Declaration 2011). For instance, hunt can be assumed to accelerate the decline of some species e.g. Lapwing (*Vanellus vanellus*), Garganey (*Anas querquedula*), Skylark (*Alauda arvensis*), Quail (*Coturnix c. coturnix*), Jack Snipe (*Lymnocryptes minimus*) or Turtle Dove (Hirschfeld and Heyd 2005, Fisher et al. 2018).

Also, the possibility for infections with parasites or diseases (hereafter called pathogens) might be higher for migrants, especially when pathogens are not host specific and several potential hosts meet at stopovers or wintering grounds (Krauss et al. 2010). Hence, there also might be a higher possibility of cross-species transfer and infection of previously unexposed individuals (Altizer et al. 2011). The chance for pathogen transmission is particularly high at stopovers due to the dense aggregation of possible hosts (Feare 2007, Krauss et al. 2010, Altizer et al. 2011). Besides larger probabilities for pathogen transfer, migrants are also able to tolerate some pathogens or can escape from contaminated areas (Loehle 1995, Altizer et al. 2011). However, it is also suggested that escapes will be more successful when pathogens have short-lived infection stages or do not cause chronic infections (Altizer et al 2011). For instance, monarch butterflies were shown to be able to escape from areas with high pathogen densities, but some populations experienced also migratory culling caused by infections (Altizer et al. 2011). Generally, pathogenic infections are thought to weaken migratory animals

tremendously and thus, those individuals are less likely to migrate long distances. In fact, most of infected animals die before reaching their destination sites or transferring pathogens to other individuals (Feare 2007, Weber and Stiliankis 2007). Furthermore, due to global warming many pathogens are expected to become more lethal or to spread more rapidly (Epstein 2001, Harvell et al. 2002), which might affect migratory species seriously.

In the present cumulative dissertation, the pathogen *Trichomonas gallinae* was investigated, which primarily infects the crop and oropharynx of infected birds and can develop necrotic lesions. Latter named symptoms are sign for the outbreak of the disease trichomonosis, which leads to death by starvation or suffocation (Stabler 1954). However, not all infected birds show clinical signs or die, which is due to the existence of different *Trichomonas gallinae* lineages varying in their pathogenicity (Bunbury et al. 2008, Sansano-Maestre et al. 2009, Robinson et al. 2010).

Habitat requirements of a species

Evaluating threats is substantial to be able to protect a species. However, to establish reserves and develop management strategies for cultivated landscapes, information about habitat requirements at breeding, stopover and wintering sites is crucial to understand the species' needs (Webster et al. 2002), and information should be gathered to conserve favourable conditions. For a given species, requirements can vary geographically depending on its life-cycle stage. For instance, a diet shift from plant dominated food resources to a protein rich diet (young grass or sprouting legumes) was shown for Greyleg Geese (*Anser anser*) in their breeding ranges (Glutz von Blotzheim and Bauer 1994). These protein demands are associated with egg production and gonad development (Glutz von Blotzheim and Bauer 1994). Also, information about favoured habitat characteristics of occupied sites is useful to maintain or improve management practices, species compatible usage of resources and also resource conservation (Finch 1991, Lambeck 1997).

European Turtle Doves

Turtle Doves show a wide breeding distribution range, which covers most of Europe and extends into Asia, with a northern limit reaching central England and Estonia (Glutz von Blotzheim and Bauer 1994). They are the only long-distant migrating columbids in Europe (Cramp 1985, Glutz von Blotzheim and Bauer 1994) and travel annually between European breeding areas and sub-Saharan winter quarters (Cramp 1985, Glutz von Blotzheim and Bauer 1994). Besides being a migratory species, Turtle Doves are mostly associated to (extensively managed) farmland. Both groups, migrants and farmland birds, show the largest population declines among birds (Donald et al. 2001, Sanderson et al. 2006). Turtle Doves experience steep population declines since the 1980s of more than 78% (BirdLife International

2015) and are listed as “Vulnerable” species (BirdLife International 2017). To investigate reasons for the decline, surveys are needed to obtain information about their flyways and key breeding or wintering sites, but also threats appearing at different stages and therefore, affecting the different European populations. Suggested threats affecting the study species are habitat loss at both breeding and winter quarters (Donald et al. 2001, Europäische Umweltagentur 2003, Eraud et al. 2009, Vickery et al. 2014), which is further aggravated by a change of food availability and quality (Browne and Aebischer 2001, 2003, Eraud et al. 2009, Vickery et al. 2014). Also, legal and illegal hunt is stated to have great negative impacts on the species (Lutz 2007, Fisher et al. 2018). Furthermore, Turtle Doves are shown to be vulnerable to infections with *Trichomonas gallinae* (Lennon et al. 2013).

Research objectives and thesis topics

In order to collect information about connectivity, threats and habitat requirements, which is needed to develop and define species conservation strategies (Fisher et al. 2018), the aims of the present cumulative dissertation were to:

- identify the population connectivity of Turtle Doves from different breeding areas across Europe,
- evaluate the effects of threats, in particular legal hunt during migration and possible impact of the parasite *Trichomonas gallinae*,
- study habitat requirements at potential key breeding sites from Turtle Doves in Germany.

Therefore, the present thesis consists of four different papers, each presented as an individual chapter. The first two chapters contribute information about connectivity of European Turtle Doves and evaluate possible impacts on Turtle Doves by legal hunt, performed in several European countries. In the first chapter, ring re-encounter data were analysed, and in the second chapter stable isotope assignment was applied. The third chapter concentrates on the infection with *Trichomonas gallinae* lineages of Turtle Doves and other wild columbid species using genetic approaches. The results were compared for the different species and possible impacts evaluated. Species distribution modelling was applied in the fourth chapter to describe favoured habitat conditions on German breeding grounds as well as the potential distribution across the country.

CHAPTER 1 – Analysis of ring recoveries of European Turtle Doves *Streptopelia turtur* – flyways, migration timing and origin areas of hunted birds

Chapter 2 –	Stable isotope assignment of migrating European Turtle Doves (*Streptopelia turtur*) to breeding ranges in Europe
Chapter 3 –	High prevalence of *Trichomonas gallinae* in wild columbids across western and southern Europe
Chapter 4 –	Species distribution models of European Turtle Doves in Germany are more reliable with presence only rather than presence absence data

Chapter outline

Chapter 1 (published)

Outline – This paper focuses on the analysis of ring re-encounter data from the EURING database of Turtle Doves. I filtered the data according to three different investigation aims: 1.) identify flyways and their proportional use by Turtle Doves from different countries to evaluate the population connectivity (692 data points), 2.) survey the timing of migration along the identified flyways (262 data points), and 3.) assess the timing and origin of birds at major hunting sites (418 data points). By using kernel density analysis I identified three flyways - a western, a central and an eastern route. Multinomial mark-recovery models highlighted that only birds following the western flyway (mainly French, German and British Turtle Doves) demonstrated a strong population connectivity. The central and eastern flyway suggested an overlap of birds crossing between those two flyways, which may highlight a weak migratory connectivity for those populations. Nevertheless, Czech birds mainly followed a central flyway and Hungarian birds mostly used an eastern flyway. The timing along the flyways was visually inspected with boxplots. It was similar along all flyways and according to latitudinal distribution over the year, birds were at their breeding grounds in June and July and in their wintering ranges from September until April. Autumn migration started in August and spring migration lasted until May. Major hunting times fell into migration times of Turtle Doves indicating by the highest numbers of hunted birds in September, April and May, predominantly in the 1960’s and 1970’s. Coinciding with identified flyways and their proportional use, geographic analysis of ringing and hunting sites highlighted that British and French birds were mainly shot along the western flyway in France and Spain. Italian birds were mainly shot in Italy along the central route and Czech birds were predominantly hunted in Greece following the eastern flyway. Although these results were affected by different time- and country-dependent ringing and recovery efforts, they exemplify both lower ringing activities after 1980s and decreasing Turtle Dove numbers.

Contributions – Lead author, flyway analysis (together with Fränzi Korner-Nievergelt), analysis of migration timing, analysis of origin and timing of hunted birds

CHAPTER 2 (SUBMITTED)

Outline – In this study, I used stable hydrogen values of 101 primary feather samples from five stopover sites across Europe to assign them to potential breeding grounds. The assignment was based on a European deuterium isoscape, which was calibrated with stable isotope values of 101 primary feathers with known origins from 13 different countries. I generated assignment maps for 1.) all Turtle Dove samples together as one group, 2.) grouped by country of collection (stopover sites) and 3.) individually. The map for all Turtle Doves together agreed with the known distribution patterns, but the map grouped by country of collection highlighted a range of potential origins in southern and central Europe for samples from Greece, Italy, Malta and Spain. Bulgarian samples were mainly assigned to Russian sites. The individual assignment offered the possibility to show four large distribution ranges following a gradient from cool/humid (Russia and Baltic States) to hot/dry (Mediterranean Sea) climatic zones. Also, using the individual assignment, I was able to define proportions of certain migrants from foreign countries, which varied among the stopover sites. This study has implications, which are important for hunting management and highlights stable isotope analysis as powerful tool, which can be efficiently applied in conservation measures.

Contributions – Lead author, fieldwork and sample collection in Bulgaria, sample collection at the Natural History Museum Berlin (together with Sylke Frahnert), feather sub-sampling from wings or bodies obtained from collaborators, stable isotope assignment with Isorix, statistics

CHAPTER 3 (PUBLISHED)

Outline – The study deals with the infestation by different *Trichomonas gallinae* lineages and the possibly occurring threat of avian trichomonosis as result of an infection with a pathogenic lineage, which can negatively affect wild columbid populations. Particularly, the occurrence and distribution of both potentially pathogenic and non-pathogenic *Trichomonas gallinae* lineages in Turtle Doves were genetically analysed from swab samples and compared to samples from other wild columbid species across Europe (Stock Dove (*Columba oenas*), Wood Pigeons (*Columba palumbus*), Collared Doves (*Streptopelia decaocto*)). A prevalence of 74% was evident among all species, and Turtle Doves had the third highest prevalence (67%), after Wood Pigeons and Stock Doves. I identified three new lineages and in total, I discovered seven lineages occurring in the samples. Only Stock Doves and Collared Doves were infested with pathogenic lineages, but although Turtle Doves were not shown to be infested with such a lineage, results are worrying for this threatened species due to their vulnerability on population level, and especially in the light of high prevalence and possible transfer of pathogens across species at water or drinking places.

Contributions – Lead author, fieldwork and sample collection in Malta, molecular work, phylogenetical and statistical analyses

CHAPTER 4 (PUBLISHED)

Outline – In this paper, I analyse habitat requirements from Turtle Doves on German breeding grounds. Therefore, I run different habitat suitability models with presence absence (PA) and presence only (PO) datasets in Biomod 2. PA data came from standardised field observations and PO data were records obtained by a citizen-science online platform. Environmental information was included in the form of climate and land coverage variables. Results obtained with PO data seemed more reliable than those obtained with PA data. However, algorithms with both datasets (PA and PO) mainly revealed that climatic variables shape Turtle Dove occurrence. Thus, the minimum temperature in January and the precipitation of the warmest quarter were of major importance. Although probability maps for both datasets differed substantially, both excluded mountainous regions as potential presence areas. Nonetheless, only probability maps created with PO data were more discriminatory, demonstrating the utility of PO data in SDMs and highlighting potential presence areas near Saarbrucken, west of Dusseldorf, in the Black Forest and in Lusatia. Although the study did not reveal land coverage variables to be of major importance for Turtle Dove occurrence, future habitat suitability models should include variables such as soil type or agricultural management strategies. This may contribute specified information about occupied habitats.

Contributions – Lead author, data preparation and processing in DIVA-GIS and ArcGIS (with assistance of Patrick Lückel), modelling with Biomod 2

General Conclusions and future outlook

The present thesis studied the population connectivity of Turtle Doves from across Europe and further highlights threats occurring at different spatiotemporal life-cycle stages. Furthermore, it identified suitable breeding areas in Germany with climatic conditions favourable for the occurrence of breeding Turtle Doves.

Population connectivity

The first chapter identified three main flyways all indicating a similar timing for departure from and arrival at breeding sites. The flyways followed a western, central and eastern route with crossings between the last two, which was described for Turtle Doves for the first time. These findings suggested a strong population connectivity for Turtle Doves in western Europe and a rather weak connectivity for birds from central and eastern Europe. The second chapter contributed information suggesting a rather weak population connectivity, highlighted by large individual assignments along four different latitudinal bands, which was mainly driven by the gradual distribution of δD across Europe. Furthermore, the results were supported by unstructured genetics in Turtle Dove samples from different European countries likely highlighting a panmictic behaviour (Calderón et al. 2016). According to the breeding ranges assigned along latitudes, the most northern band, covering Russia and the Baltic States, was especially interesting, due to scarcity of data from these breeding areas of Turtle Doves. Migration from the other breeding ranges is better known, both from ring re-encounters (chapter 1) and increasingly from tracking data (e.g. Eraud et al. 2013, https://blogs.nabu.de/zugvoegel/, http://turtledoveresearch.com/fr/).

Although I was able to identify main flyways and delimit possible breeding ranges along latitudinal bands, it was not possible to highlight spatially exact breeding or wintering areas. Considering the ring re-encounter analysis, aggregations of ringed Turtle Doves did not mirror key breeding sites, but places of high ringing activity, which varied spatiotemporally. Furthermore, there were only a few data points collected on the African continent, which were insufficient to identify further stopovers or wintering areas. Those would add information about connectivity of Turtle Dove populations.

In case of the stable isotope assignment, model results showed broad geographic bands of possible breeding ranges, which usually occur in species with a general wide breeding distribution, as in Turtle Doves, and is further driven by the gradual variation of δD in Europe. The latter offers the possibility to coarsely assign a breeding range along gradients, but to get some insight into specific breeding sites of Turtle Doves, studies using satellite tracking devices would be recommendable (e.g. Webster et al. 2002). Therefore, birds could be equipped while attending stopover sites. Information gathered from tracking data would not only reveal breeding sites, but also exact movements, stopover and wintering sites in Africa. Furthermore, to discover wintering sites in Africa, a spatially-explicit multi-isotope likelihood assignment method (Royle and Rubenstein 2004, Hobson et al. 2009, Wunder 2010) is in preparation to be applied. Such a model considers the isotopes δD, $\delta^{13}C$ and $\delta^{15}N$ of feathers grown in Africa compared to an isoscape based on African environmental conditions.

Threats

Although the datasets used in the first two chapters were of minor utility to identify spatially accurate key habitats, they were powerful tools to evaluate the impact of hunt at stopover sites in southern Europe. Both chapters reliably reflected high hunting effects on migrating Turtle Doves originating from other countries than those of collection or re-encounter. But also the possible impact of hunting on likely national breeding birds were shown, especially for Turtle Doves originating from Italy and shot in Italy.

In addition to the threat of hunt, *Trichomonas gallinae* infections were shown to be numerous in Turtle Doves, although no infection with a pathogenic lineage was shown in this study. However, compared to the other wild columbid species studied in the third chapter, Turtle Doves are the only species showing such a severe population decrease. This is why results should be taken seriously, due to the occasional epidemic character of trichomonosis, as well as the already demonstrated infections with pathogenic lineages in the UK (Lennon et al. 2013) which can be transferred at different whereabouts of Turtle Doves during their life-cycle. Therefore, individuals are not only exposed to potential vectors at breeding or wintering sites, but also along flyways at stopovers. Thus, the potential impact of this pathogen might become a major issue for the currently decreasing and weakened Turtle Dove population.

To add information about further pathogens affecting Turtle Doves, it should be considered to analyse blood samples for prevalence of Haemosporidia, for example.

Habitat requirements

The fourth chapter highlighted the usefulness of PO data from citizen-science programs. These reliably modelled certain climatic conditions being essential for Turtle Dove occurrence and, more importantly, indicate potentially suitable areas as breeding sites across Germany. Therefore, habitat modelling was shown to be a useful instrument in conservation planning, because of its ability to mark areas, which likely are of high conservation interest. Although climatic variables were demonstrated to contribute to Turtle Dove occurrence in Germany, they could not be used to describe specific habitat characteristics. This is why more precise variables should be applied in future habitat models. Incorporating information about land management practices, soil type, existence of field margins and its vegetation coverage might provide appropriate background information to describe suitable Turtle Dove habitats. A similar approach could be applied for African wintering grounds. Therefore, a multi-isotope assignment could delimit a spatial range in Africa. Tracking data can reveal first insight into occupied habitat types, which could be evaluated via aerial images in first place. Theoretically, a modelling approach similar to the habitat modelling with PO data could be applied to unveil important key variables contributing to Turtle Dove occurrence in certain areas.

Besides modelling habitat requirements at certain life-cycle stages, an analysis of diet composition would also help to ascertain essential food resources. Preferably, molecular analyses (e.g. Dunn et al. 2018) should be used for that, because observation data from different areas are difficult to obtain, time consuming and cost-intensive.

To sum up, regarding the unstructured genetics (Calderón et al. 2016) and the current phase of effective population size contraction in Turtle Doves, this species is suggested to be particularly vulnerable to current anthropogenic threats. Thus, the results shown in this cumulative thesis are of importance for the conservation management planning of Turtle Doves and its propagation in term of environmental education programs at breeding, stopover and wintering sites.

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CHAPTER 1

ANALYSIS OF RING RECOVERIES OF EUROPEAN TURTLE DOVES *STREPTOPELIA TURTUR* – FLYWAYS, MIGRATION TIMING AND ORIGIN AREAS OF HUNTED BIRDS

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Analysis of ring recoveries of European Turtle Doves *Streptopelia turtur* — flyways, migration timing and origin areas of hunted birds

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Abstract. Knowledge about flyways, breeding and overwintering sites is important for conservation efforts, but little is known about migration patterns and population connectivity of declining European Turtle Doves *Streptopelia turtur*. EURING ring-recovery data were used to estimate directions and proportional usage of flyways. The timing of migration was compared along these routes and breeding origins of shot individuals were determined. Ring recoveries of Czech, Hungarian, British, German and French birds suggested three main flyways with westerly, central and easterly directions. The proportional usage was estimated by multinomial mark-recovery models. Major parts of French (62%), German (92%) and British (94%) Turtle Doves followed a western flyway. Czech birds used the central route (56%) and 55% of Hungarian birds followed the eastern flyway. Thus, a migratory divide between the Czech Republic and Germany could be suggested. The timing of migration showed a similar latitudinal pattern of migration along all flyways. Birds were at the breeding grounds in June and July and from September to April in their southernmost distribution ranges. Outward migration started in August. Return migration was still evident in May. The majority of reported hunted doves were from the 1960s and 1970s. High hunting numbers were present in September, April and May. France and Spain mainly shot birds from the UK and France. In Italy predominantly Italian birds were shot. Doves shot in Greece mostly came from the Czech Republic. Given the decreasing population numbers, large ringing numbers seem unlikely in the future. Thus, low recovery numbers in recent decades parallel both, the population decrease and a lower ringing activity.

Key words: Turtle Dove, *Streptopelia turtur*, EURING, multinomial model, flyways, timing of migration, hunting

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INTRODUCTION

More than 2 billion European birds migrate twice a year to and from Africa (Hahn et al. 2009). To arrive at their destination points, they follow different migration strategies. Many of these birds follow broad front migration patterns (Zink 1973–1995, Berthold 2000). Further studies describe two main flyways in westerly and easterly directions over the Iberian Peninsula or along Middle East European land, which can be induced by a migratory divide (Zink 1973–1995, Berthold 2000, Reichlin et al. 2009).

When a breeding population winters together in one area, the migratory connectivity for this population is described as high (Webster et al. 2002). The degree of migratory connectivity sinks when large parts of one breeding population disperse to different wintering grounds (Webster et al. 2002).

The phenology of different species and bird populations is believed to be controlled by endogenous mechanisms. Thus, the start of migration is primarily regulated by genetic factors, but also triggered by circumannual rhythms, particularly changes in day length (Berthold 2000). Nonetheless, some studies showed an adjustment of migration patterns. Therefore, bird migration can also be flexible to a certain degree in some species (Marra et al. 2005).

European Turtle Doves *Streptopelia turtur* are the only long-distance migrants among European columbids that participate in trans-Saharan migration. They have a wide breeding distribution range across most of Southern, Central and Eastern Europe and into Asia, with a northern limit reaching Central England and Estonia (Glutz von Blotzheim & Bauer 1987). There are two widespread races of European Turtle Doves — *S. t. turtur* and *S. t. arenicola* and three endemic races on the African continent — *S. t. hoggara*, *S. t. isabellina* and *S. t. rufescens*. *S. t. turtur* is the nominate form and the study species in this paper. It inhabits most of the west and central Palearctic up to Afghanistan and North-West China (Glutz von Blotzheim & Bauer 1987).

Although the general distribution ranges are well described, knowledge of Turtle Dove migration flyways is still insufficient. Some authors suggested a broad front migration pattern (del Hoyo et al. 1997), while others described three main migration routes, crossing the Sahara incoastal NW-Africa, the central Sahara with its oases, or the Nile region (Glutz von Blotzheim & Bauer 1987). Studies from France and the UK traced the main passage through France and Iberia, along Morocco to sub-Saharan Africa during spring and autumn migration (Aebischer et al. 2001, Eraud et al. 2013). Furthermore, a central migration flyway over Italy and Malta and an eastern flyway over Greece, Turkey and Bulgaria have been described (Cramp 1985, Dimaki & Alivizatos 2014). Geolocation data of Turtle Doves from France suggested a loop migration with more eastern migration patterns back to their breeding sites compared to used flyways in autumn (Eraud et al. 2013). Nevertheless, especially for Turtle Doves from Central and East European countries, the used flyways are still insufficiently known.

Across Europe, Turtle Doves are listed as vulnerable in the European Red List, because the population trend has declined by more than 70%, particularly since the 1970s (BirdLife International 2015). Such strong population declines of wild birds in Europe are particularly known in long-distance migrants and farmland birds (Berthold et al. 1998, Schifferli 2000, Newton 2004, Sanderson et al. 2006). This coincides with agricultural intensification, which caused habitat loss and changes in temporal and spatial food availability as well as quality in both breeding and overwintering habitats (Browne & Aebischer 2001, 2003a, Eraud et al. 2009, Vickery et al. 2014). European Turtle Doves fall into both categories and, furthermore, are hunted in large numbers. The total annual hunting bag in European countries is estimated at 2 to 3 million Turtle Doves (Boutin & Lutz 2007). The autumn hunting seasons are differently timed through the European countries, starting earliest at 15th August in Portugal, followed by the other countries until mid-September (Boutin & Lutz 2007). Autumn hunt in some countries ends in February, such as Greece (Boutin & Lutz 2007). These hunting seasons are followed by spring hunt in Malta from 25th March to 22nd May (Boutin & Lutz 2007). To assess influences of hunting and poaching on populations, it is necessary to determine breeding origins of shot Turtle Doves.

Being a popular and legal game species in several European countries leads to increasing reporting probabilities of marked individuals (Kania & Busse 1987). That makes ring-recovery analyses highly appropriate for this study.

For many studies the usage of ring-recovery data was a relevant source to define general migration patterns, such as flyway directions, breeding and non-breeding distribution areas or migratory connectivity (e.g. Reichlin et al. 2009, Korner-Nievergelt et al. 2012, 2014). Furthermore, changes in arrival and departure dates could be registered (Both et al. 2005). Even the impact of hunting on distribution and survival can be evaluated by those analyses (e.g. McCulloch et al. 1992, Aebischer et al. 1999, Péron et al. 2011), because the huge benefit by these databases is the great number of data over a long period of time. That is why we here analysed recovery data on European Turtle Doves from the EURING-database and additional data from national ringing schemes. The aims of this study were to investigate: 1) the use of the flyways by Turtle Doves from different countries, 2) the timing of migration through the different flyways, and 3) the timing and origin of birds at main hunting sites.

MATERIAL AND METHODS

Data sources

The EURING-database contained a total of 897 recoveries of Turtle Doves (EURING-dataset, Table 1), collected between 1913 and 2011. Of these, we discarded all Turtle Dove recoveries with an accuracy of date worse than six weeks (N = 5, Table 1) and individuals with recovery distances less than 100 km (N = 200, Table 1 and 2), leaving a BASIC-dataset of 692 recoveries (Fig. 1). Of these, 50 had one of their capture locations (either ringing site or recapture place) outside Europe (Appendix 1), and 11 Turtle Doves had both capture and recapture locations out of Europe (Appendix 2), while

Table 1. Datasets used for the three presented analyses. Shown are also the datasets on which the analyses datasets are based. We also present the included criteria for the datasets and the total evaluated recoveries in every analyses.

Name of datasets	Analyses	Basis dataset	Included data	N recoveries
EURING-dataset			Birds with recoveries	897
BASIC-dataset	Timing of migration	EURING-dataset	Accuracy of date better than 6 weeks, recovery distances 100 km and more	692
FLYWAY-dataset	Usage of flyways	BASIC-dataset	Ringed in country of ringing scheme, ringed in June or July, recaptured within 5 years, 10 or more recoveries/country, recoveries from August to May, recoveries south of ringing location Discard: 2 birds from UK with recoveries in Mali	262
HUNT-dataset	Hunted birds	EURING-dataset	Capture and re-capture location in Europe, all distances, all date accuracies, countries of recovery where more than 30 recovered individuals were classed as "shot", "hunted" or "dead"	418

631 birds were captured twice in Europe. Total ringing numbers were downloaded in January 2014 from http://www.euring.org/data_and_codes/historic_ringing_totals.html, with additional data provided from curators of national ringing databases (see Acknowledgements).

DATA ANALYSIS

Use of the flyways by Turtle Doves from different countries

For the analysis of used flyways, we only included birds from the BASIC-dataset that were ringed

Table 2. European Turtle Dove ringing (maximum value from either EURING Archival Ringing Totals downloaded from http://www.euring.org/data_and_codes/historic_ringing_totals.html or verbally — see methods) and recovery numbers (according to the EURING database, N = 897) from different ringing schemes, in the period 1913–2011. Recovery rates were calculated for ringing schemes with more than 100 ringed birds. Note: Radolfzell (Germany) also administered Austrian ringing, Ireland was included in the Ringing Scheme UK. * — data not available.

Ringing scheme	Ringed	Recovered	%	recovered dead	alive
Croatia	718	3	0.4	2	1
Czech Republic	5363	65	1.2	58	7
Finland	35	4	-	3	1
France	5603	183	3.3	162	21
Germany-Hiddensee	293	1	0.3	1	0
Germany-Radolfzell	708	15	2.1	14	1
Germany-Wilhelmshaven	1742	52	3.0	44	8
Greece	*	2	-	2	-
Hungary-Budapest	1538	28	1.8	14	14
Italy-Bologna	2976	171	5.7	129	42
Israel	*	7	-	5	2
Malta	*	2	-	-	2
Poland	409	4	1.0	4	-
Portugal	24	4	-	3	1
Russia-Moskwa	1602	13	0.8	13	-
Spain-Madrid	825	57	6.9	31	26
Spain-Madrid Icona	39	28	-	26	2
Sweden-Stockholm Ornis	*	8	-	6	2
Sweden-Stockholm Museum	42	28	-	*	*
Switzerland	133	4	3.0	4	-
The Netherlands	3961	6	0.2	1	5
UK	7629	220	2.9	202	18

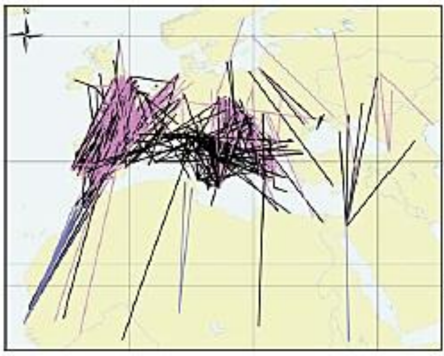

Fig. 1. Overview of validated European Turtle Dove recoveries. Black lines — doves with unknown breeding origin, purple lines — doves with known breeding origin (captured in June–July), blue lines — doves with recovery locations outside of Europe.

in June or July in the country of the ringing scheme and recaptured within 5 years. To consider only migrating birds, we discarded all that were recovered in June and July. Thus, we were able to get insight into their breeding ranges and the localities used during migration or rather wintering. In both occasions, we were able to get an idea about flyway directions. Additionally, we only included countries with 10 or more recoveries of birds ringed in one country and discarded those with recapture locations north of the ringing location. After visual inspection of the ring-recovery data via mapping in ArcGIS 10.2.1 (ESRI, Redlands California) (Fig. 1) and due to different recovery probabilities around the Mediterranean and sub-Saharan belt, we further decided to discard two birds from Great Britain, which were recoveries in Mali leaving the FLYWAY-dataset of 262 recoveries around the Mediterranean from five different countries — UK, France, Germany, Czech Republic and Hungary (Table 1).

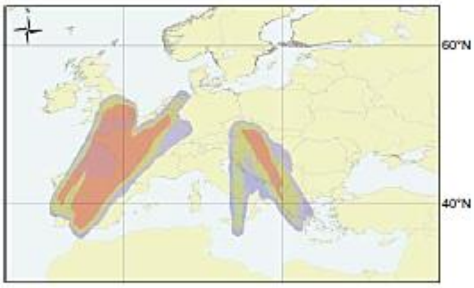

Fig. 2. Line density kernels for 70% (red), 80% (yellow) and 90% (blue) of European Turtle Doves from five different countries using the Western, Central and Eastern flyway.

Firstly, analysis of line density kernels for 70%, 80% and 90% of mark-recapture lines were plotted in ArcGIS 10.2.1 using the line density tool under Spatial Analyst (ESRI, Redlands California) to picture the flyways. Three main flyways along an eastern, central and western route were best distinguished by 80% kernel areas (Fig. 2). For later analyses we used the ranges of 80% kernel to define the positions of flyways according to longitude — afterwards named as western, central and eastern flyway. The western flyway began at ≤ 8° E longitude. The central flyway lay within a longitudinal range between 8° E and 19° E and the eastern flyway was set at a longitude greater than 19° E.

Multinomial mark-recovery models (Bauthian et al. 2007, Thorup & Conn 2009, Korner-Nievergelt et al. 2012) were used to estimate the proportional usage of the western, central and Eastern flyway by different countries while taking into account that ring recovery probability differs between the flyways. It is well described that recovery probabilities are lower in Africa and even differ within Europe, having less recapture probability in East Europe than in West Europe (Kania & Busse 1987, Thorup & Conn 2009).

First, multinomial models were formulated for countries with known numbers of ringed birds (Czech Republic, Hungary, UK — Table 2) (Kania & Busse 1987, Korner-Nievergelt et al. 2010). Second, multinomial models for birds ringed in a given country with unknown ringing numbers (Germany and France) were integrated in the above models. Germany has three ringing schemes and the unknown ringing numbers are caused by an incomplete database in Germany-Wilhelmshaven after 1998 (Geiter 2014 pers. comm.). The unknown ringing numbers in France are caused by unknown data before 2000 in France (Dehorter 2014 pers. comm.).

We assumed that birds using the same flyway have equal recovery probabilities independent of the country the bird belongs to (Korner-Nievergelt et al. 2010). Based on this assumption and given the number of countries with known number of ringed birds is equal or larger than the number of flyways, the recovery probabilities in the different flyways, r_F, become estimable. Then, the expected number of recoveries of country P in flyway F, $E(R_{PF})$, is the product of the number of ringed birds in country P, N_P, the proportion of

Table 3. Description of the most important parameters and indices used.

	Description
Parameters	
p	Cell probability of the multinomial model, based on the number of countries with known numbers of ringed birds and the number of flyways $F + 1$ for never recovered birds
pu	Cell probability of the multinomial model, based on the number of populations with unknown numbers of ringed birds and number of flyways F
m_{PF}	Proportions of birds from country P with known ringing numbers using flyway F
mu_{PF}	Proportions of birds from country P with unknown ringing numbers using flyway F
r_F	Recovery probability in flyway F
Indices	
P	Country: Czech Republic, France, Germany, Hungary, UK
F	Flyway: West, Central, East

birds that use flyway *F*, m_{PF}, and the recovery probability in *F*, *rF*. We can write a multinomial model for the number of recoveries in the different flyways (*W* = West, *C* = Central, *E* = East) for each country *P* with known number of ringed birds:

$$\mathrm{R_P} \sim \mathrm{Multinom}((m_{PW}r_W,\ m_{PC}r_C,\ m_{PE}r_E,\ 1\text{-}\sum_{F=1}^{\delta} m_{PF}),\ \mathrm{N_P})$$

(for description of parameters and indices see Table 3). The last cell contains the probability that a ringed bird is never found. The three multinomial models for each country together form a product-multinomial model. In this model, we integrated two other multinomial models for each of the countries with unknown number of ringed birds. Based on the proportions of birds using each flyway, $\mathrm{m_{PP}}$, and the flyway-specific recovery probabilities, $\mathrm{r_P}$, we can formulate the expected number of recoveries in the different flyways given the total number of recoveries:

$$R_{PF} \sim \mathrm{Multinom}\Big(\Big(\frac{m_{PW}r_W}{\Sigma_{F=1}^{\delta}\ m_{PF}r_F},\ \frac{m_{PC}r_C}{\Sigma_{F=1}^{\delta}\ m_{PF}r_F},\ \frac{m_{PC}r_C}{\Sigma_{F=1}^{\delta}\ m_{PF}r_F}\Big),\ \sum_{F=1}^{\delta} R_{PF}\Big).$$

The integrated model was fitted to the data using Bayesian methods by Markov chain Monte Carlo (MCMC) simulations using WinBugs and its R interface R2WinBUGS (Sturtz et al. 2005) in R 3.3.1 (R Core Team 2014).

Uniform prior distributions were used for the recovery probabilities (r_F). The priors for m_{PF} were constructed by uniformly distributed variables that were constrained to sum to one for each country *P*.

Posterior distributions of the parameters were obtained from the last 1000 sampled values of three Markov chains each of length 10000. Convergence was assessed graphically and by r_hat values and accepted when they were lower than 1.01 (Brooks & Gelman 1998). Means and SD of posterior distributions of r_F and m_{PF} are presented (Table 4).

Timing of migration through the different flyways

The timing of migration through the different flyways was analysed graphically on all birds from the BASIC-dataset (N = 692, Table 1). Note, those are the breeding and non-breeding birds in Table 5. Although knowledge exists about shifts in timing of migration (Glutz von Blotzheim & Bauer 1987), the dataset was too small and unequally distributed through time to divide it into different

Table 4. Proportional usage of flyways (% ± SD) of five different countries. For the analysis, we used Turtle Doves from the FLYWAY-dataset. The ringing numbers of the first three countries are known. The other two do have high ringing numbers, but absolute numbers are unknown.

Country	Western flyway	Central flyway	Eastern flyway
Czech Republic	0.01 ± 0.01	0.56 ± 0.12	0.43 ± 0.12
Hungary	0.05 ± 0.05	0.40 ± 0.12	0.55 ± 0.13
UK	0.94 ± 0.04	0.03 ± 0.03	0.03 ± 0.03
Germany	0.92 ± 0.05	0.06 ± 0.05	0.02 ± 0.02
France	0.62 ± 0.11	0.30 ± 0.11	0.08 ± 0.06
Recovery probability	0.014 ± 0.002	0.006 ± 0.002	0.009 ± 0.003

time periods. That is why all BASIC-dataset birds were assigned to the different flyways West, Central and East. Therefore, the longitudinal point of the most southern location was used to allocate them to a flyway (see description of flyway ranges above). Afterwards, the datasets were introduced to R 3.3.1 to analyse them by boxplots (R Core Team 2014).

Timing and origin of hunted birds

To study the country of origin (here, not necessarily breeding origin) of hunted Turtle Doves, countries with more than 30 individuals classed as "shot", "hunted" or "dead" were evaluated. Here, only recoveries with both, ringing and recovery site in Europe were analysed, and birds were included regardless of the flight distance between ringing and recovery site (HUNT-dataset, Table 1).

RESULTS

Use of the flyways by Turtle Doves from different countries

According to the multinomial mark-recovery models (Table 4), Turtle Doves from Germany and the UK predominantly used the western flyway (92% and 94%, respectively). Birds from France used mostly the western flyway (62%) but also quite frequently used the central flyway (30%). Czech and Hungarian Turtle Doves used both the central and eastern flyways (Table 4). The recovery probabilities were different along all three flyways. It was highest within the western flyway (0.14%) and lowest in the central flyway (0.06%, Table 4). Furthermore, the line density kernel areas of 80% and 90% suggested a possible overlap between the birds using the eastern and central flyway. Particularly the 90% line density kernel included birds crossing between these two flyways.

Timing of migration through the different flyways

The latitudinal pattern of migration was similar along the three flyways (Fig. 3). Turtle Doves from all flyways were captured in their northern distribution ranges from June until July (with a median latitude around 50° N). Spring migration was evident in April and May and autumn migration started in August (Fig. 3).

Timing and origin of hunted birds

Most rings reported by hunters (N = 418) were from doves originally ringed in the UK (28.6%), followed by birds from Italy (23.9%) and France (17.9%). Smaller numbers were from i.e. Albania, Croatia, Poland and Ukraine (Fig. 4, Appendix 3).

The majority of Turtle Dove rings were reported by hunters in September (N = 205, 49%), followed by recoveries in May (N = 66, 15.8%) and August (N = 60, 14.4%). Considerable numbers of ringed birds were shot during spring migration in April and May in France (N = 38, 31%) and Greece (N = 21, 42%) (Fig. 4, Appendix 4).

The majority of ringed doves were reported by hunters in the 1960s (N = 119, 28.5%) and 1970s (N = 98, 23.4%), while very few ringed birds were reported from hunters before 1950 (Fig. 4, Appendix 5).

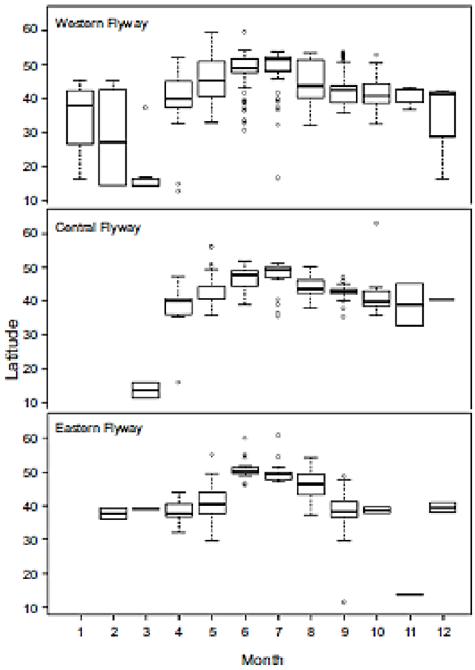

Fig. 3. Timing of migration through the western, central and eastern flyway of European Turtle Doves. All ring and recovery data shown in Fig. 1 were included. The boxes represent the range in which 50% of the data occur (inter-quartile distance from 25% quartile to 75% quartile). The whiskers show extreme values and the black line in the boxes depicts the median. Outliers are shown as circles and extend for more than 1.5 time the inter-quartile distance.

Ring recoveries of European Turtle Doves 61

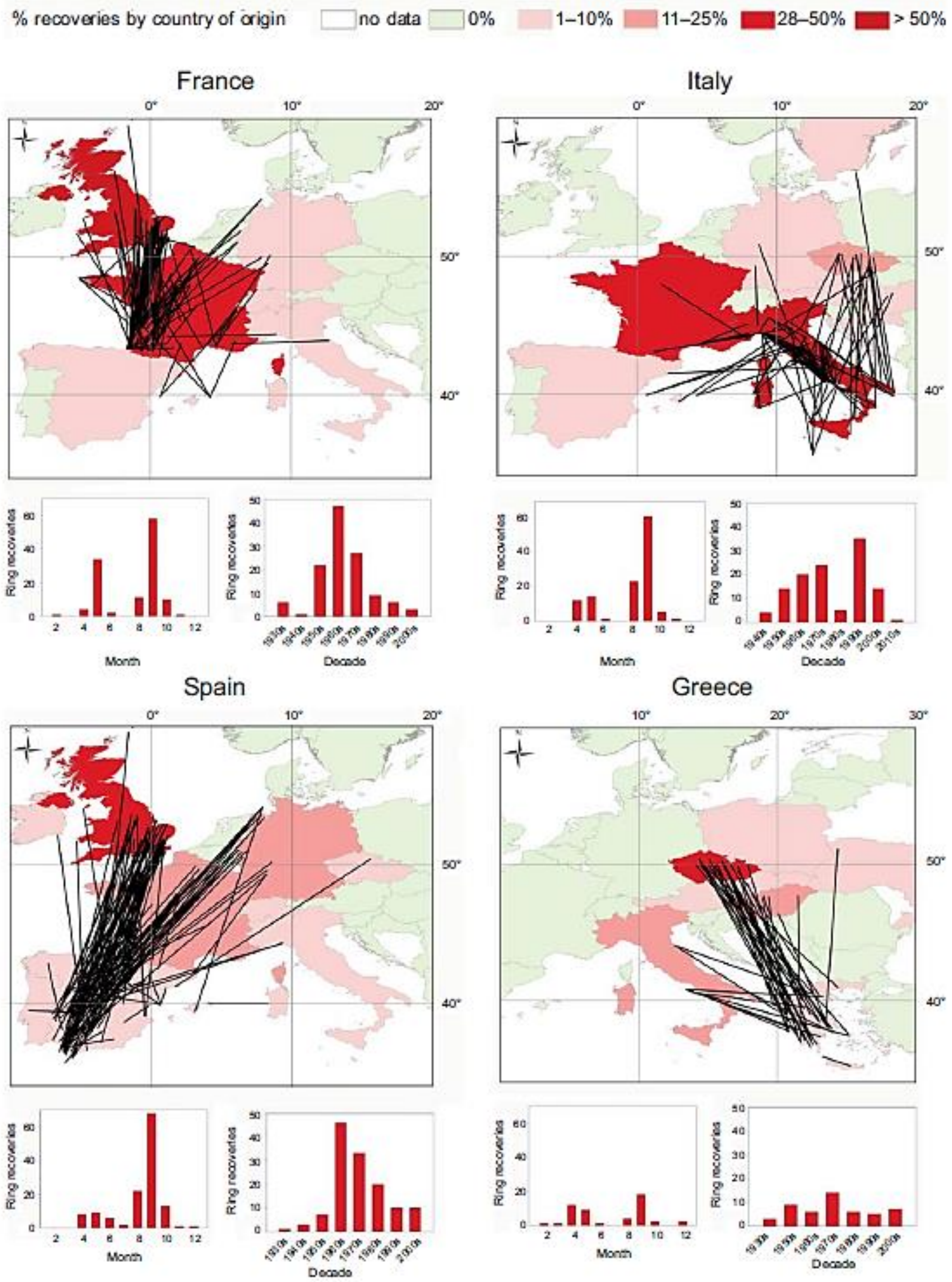

Fig. 4. European Turtle Dove rings reported by hunters in France (western flyway) from 1931 to 2006, in Spain (western flyway) from 1931 to 2009, in Italy (central flyway) from 1941 to 2010 and in Greece (eastern flyway) from 1934 to 2008.

Table 5. European Turtle Dove recoveries from different countries with numbers of excluded recoveries (due to inaccurate dates or short distance) and flyway directions. Each of the recoveries (N = 897) was assigned to the country according to its most northern location. Turtle Doves were counted as "Nonbreeding" when their assigned country was outside Europe or they were not recovered in their actual breeding time (June and July). Breeding Turtle Doves were birds found in June or July. Flyways were assigned to the records of breeding Turtle Doves. Countries with more than 5 breeding bird recoveries are marked in bold.

country	Excluded		Nonbreeding	Breeding	Flyway		
	by date	≤ 100 km			% West	% Central	% East
Albania	-	-	1	-	-	-	-
Austria	-	-	4	3	-	33	67
Bulgaria	-	-	2	-	-	-	-
Croatia	-	1	3	2	-	100	-
Czech Republic	-	3	14	43	-	**47**	**53**
Finland	-	3	-	1	-	-	100
France	-	-	57	101	**99**	**1**	-
Germany	-	18	10	35	**91**	**9**	-
Greece	-	-	4	-	-	-	-
Hungary	**1**	6	10	14	-	**29**	**71**
Ireland	-	-	2	1	100	-	-
Israel	-	-	1	-	-	-	-
Italy	**2**	54	130	11	-	**100**	-
Malta	-	2	2	-	-	-	-
Morocco	-	-	7	-	-	-	-
Poland	-	-	1	2	-	50	50
Portugal	-	3	-	1	100	-	-
Russia	-	3	4	6	-	-	**100**
Serbia	-	1	3	-	-	-	-
Slovakia	-	-	2	2	-	50	50
Spain	**1**	56	15	6	**100**	-	-
Sweden	-	-	2	1	-	100	-
Switzerland	-	1	2	1	100	-	-
The Netherlands	-	6	-	-	-	-	-
Tunisia	-	-	2	-	-	-	-
Turkey	-	-	2	-	-	-	-
UK	**1**	**43**	**67**	**109**	**99**	-	**1**
Ukraine	-	-	5	1	-	-	100

DISCUSSION

Knowledge of the flyways, breeding, stopover and overwintering sites is important for conservation efforts (Trierweiler et al. 2014). This study aimed at summarizing information about Turtle Dove migration patterns and their threats during their migration across Europe.

Use of the flyways by Turtle Doves from different countries

In line with previous suggestions (Murton 1968, Glutz von Blotzheim & Bauer 1987, Aebischer et al. 2001, Eraud et al. 2013), the capture and recapture locations (esp. Fig. 2) supported the idea of three main migration flyways for Turtle Doves. However, the definition of a central flyway (Fig. 1, 2) was more difficult in this study due to smaller sample sizes in Central and Eastern Europe as well as fewer recoveries with assignments of breeding origins than in the West (Table 5). Thus, our data could also indicate a migratory divide between the Czech Republic and Germany. That might be comparable to the one from Central European Hoopoes *Upupa epops*, which led through Germany, Austria, Switzerland and Italy and was set to a range from 10–12° E (Reichlin et al. 2009). In the present study, the migratory divide might be more extended to both sides, regarding the gap width between western and central/eastern flyway. Nevertheless, Fig. 1 shows also regular movements between the central and western flyway. This in turn could also reveal a nomadic pattern of e.g. young Turtle Doves, which seek breeding habitats. Yet, this is only an assumption and needs further investigations, because of the lack of knowledge about ages of the majority of recovered birds and paucity of ring-recoveries from East Europe, including East Germany. Here, the highest number of Turtle

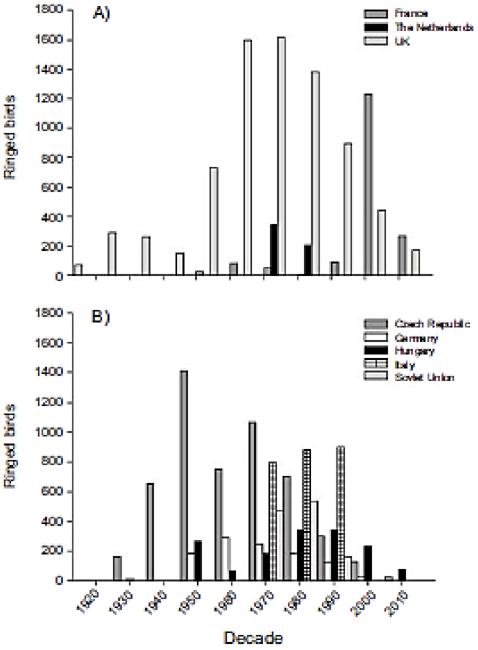

Fig. 5. Changes in the ringing activity over time for ringing schemes with more than 500 ringed European Turtle Doves. A — represents the ringing activity of ringing schemes from West Europe, B — depicts ringing activity of ringing schemes from Central and East Europe.

Doves with origins in Eastern Europe came from the ringing efforts in the Czech Republic, mainly in the 1950s to 1970s (total N = 65 recoveries) followed by Hungary (total N = 28 recoveries) and Russia (total N = 13 recoveries) (Fig. 5). All other East European countries had less than 15 recoveries in total (Table 1). This also reflects the differences in recovery probability among flyways and between areas described by Kania & Busse (1987) and Thorup & Conn (2009).

Two Turtle Dove recoveries from the relevant FLYWAY-dataset were recorded outside Europe. That led to constraints in data evaluation caused by different recovery probabilities in sub-Saharan Africa compared to the Mediterranean (Kania & Busse 1987, Thorup & Conn 2009) and the following discard of those findings. However, regarding Fig. 1 it was obvious that most of the recoveries from outside Europe were recorded along the western flyway. Furthermore, there were some recoveries in Africa along the central flyway. Those localities might indicate the presence of important stopover sites in Morocco, Libya and Tunisia as well as key wintering areas in the sub-Sahara (Appendix 1) (Eraud et al. 2013). Unfortunately, there was only a small number of recoveries on the African continent. Hence, we cannot tell the exact wintering locations. We can only confirm position of wintering locations in the sub-Saharan belt, which matches the results of existing literature (e.g. Cramp 1985, Glutz von Blotzheim & Bauer 1987, Eraud et al. 2013).

According to the usage of flyways, Turtle Doves show a rather strong migratory connectivity (Webster et al. 2002) when looking at the West European population compared to the ones from Central and East Europe. On the other hand, the 80% and 90% line density kernel areas suggested a possible overlap of the central and eastern flyway route. Especially, the 90% line density kernels included several birds crossing between those two flyways (Fig. 1, 2), as has been observed in other birds (Trierweiler et al. 2014). Those flyway crossings of Central and East European Turtle Dove populations need to be interpreted cautiously, because those flyway lines do not depict the actual flight route, but merely two stations of bird migration, which are connected by a non-realistic straight line for simplification of analysis. Nevertheless, those crossing could also show a pattern similar to Purple Martins *Progne subis* (Fraser et al. 2012). Depending on the spatial scale, those birds showed strong connectivity on species level, but no or weak connectivity within the subspecies in the eastern population (Fraser et al. 2012). That further reveals the importance of surveys on the different subspecies of Turtle Doves *Streptopelia turtur*, the European and western Asian type, and *Streptopelia turtur arenicola* as the Balearic subspecies (Cramp 1985).

Timing of migration through the different flyways

We observed a clearly distinguished latitudinal pattern over the course of the year, indicating a stationary breeding time in June and July, mirrored by the months with the most northern distribution areas of Turtle Doves. The graphs (Fig. 3) do not show a clearly defined spring migration start, but first arrivals were registered in April in Great Britain, Italy and Greece (e.g. Aebischer et al. 2001, Croxton et al. 2006, Dimaki & Alivizatos 2014). We determined the start of autumn migration in August, in line with Aebischer et al. (2001) and Eraud et al. (2013). For all flyways, the most southern localities are depicted in the same latitudinal ranges (10–20° N). Along the central and western flyway, there are some findings of Turtle Doves in sub- and north Saharan regions. As described above, those might indicate stopover- or possible overwintering sites. Especially, two recoveries within the central flyway route, which are located in Tunisia, might describe stopover sites during spring migration as they were found in April and May (Eraud et al. 2013).

Due to the small, unequally distributed sample set we could not split the analyses into different time periods. That is why we were unable to investigate changes in timing of Turtle Dove migration or wintering locations through the different flyways. However, Browne & Aebischer (2003b) did not find a change in spring arrival dates for Turtle Doves in England, but suggest an earlier autumn departure since 1970s. This suggestion is supported by Croxton et al. (2006), who showed earlier first arrival dates before 1980s, but also revealed slightly delayed arrival dates from 1982 to 2005 in the UK. Along the eastern flyway, neither arrival nor departure dates changed in Greece (Dimaki & Alivizatos 2014). This might show differences in population development, due to climatic conditions or other differences along the flyways. However, as already mentioned, our data does not support differences in migration timing throughout the different flyways. Also, there is no suggestion for Turtle Doves to have changed their wintering areas further north. This altered migration pattern is known, especially in short-distance migrants (MacLean et al. 2008, Visser et al. 2009). A well-known example are Blackcaps *Sylvia atricapilla*, which usually overwinter in Mediterranean regions, but since the 1970s they also find wintering areas in Great Britain and Ireland (Leach 1981). However, the small sample size, particularly for Central and East Europe might lead to the idea of non-altered phenology. A higher ringing activity in East European countries might change this impression. Nonetheless, since there is lower ringing activity in recent years, which might also depict the decreasing population trend, it seems new methods are needed to find key habitats. To compare and understand the population development of West and East European Turtle Doves, habitat surveys on breeding and overwintering grounds as well as on stopover sites are also mandatory.

Furthermore, the investigation of possible diseases across Europe (Lennon et al. 2013, Stockdale et al. 2014) is highly recommendable. In Turtle Doves from the UK, parasite infections by *Trichomonas gallinae* cause high mortality rates (Lennon et al. 2013, Stockdale et al. 2014) and might be transmitted by different bird species. For instance, *T. gallinae* was detected in different columbids (Villanúa et al. 2006, Bunbury et al. 2007, Sansano-Maestre et al. 2009, Chi et al. 2013), House Finches *Carpodacus mexicanus*, different corvid species (Anderson et al. 2009), Red-legged Partridges *Alectoris rufa* (Stockdale et al. 2014) and several birds of prey (Sansano-Maestre et al. 2009, Chi et al. 2013) and therefore, seems not limited to a single bird group. Furthermore, this threat is positively correlated with warmer temperatures and lower precipitation (Bunbury et al. 2007). Thus, in times of climate change the infection and mortality rates of Turtle Doves might increase even more, which emphasizes the need for studies about disease occurrence. Yet, this epidemic disease has not been studied in East European doves, although it is emerging (Lawson et al. 2011).

Timing and origin of hunted birds

Along all flyways, the highest numbers of ring recoveries from hunting were reported in September. During this month the majority of Turtle Doves migrate south (Glutz von Blotzheim & Bauer 1987), indicating that hunting takes place chiefly at migration stopover sites. Less ring recoveries by hunting were reported in August (Fig. 4). This is in line with the beginning of the legal European shooting season in mid-August (Boutin & Lutz 2007). In Greece and France, ring recoveries from hunting were also reported regularly in April and May. For these countries, hunting is now only allowed until the end of February (Boutin & Lutz 2007), but hunting seasons have changed over the years.

The numbers of ringed birds recovered through hunting clearly depends on the number of birds originally ringed. For example, Italian ornithologists ringed many Turtle Doves in the 1970s to 1990s, and in the same period there were considerable recoveries, mostly from hunting in their own country (Fig. 4, 5). The high numbers of British, Italian and French Turtle Dove recoveries coincide with higher ringing activities in these West European countries (Table 2). This also explains high numbers of recoveries from the Czech Republic, being the East European country with the highest ringing activity. The highest numbers of ring recoveries from hunting were

reported in the 1960s and 1970s, in line with higher ringing activities in several countries in 1950s and 1970s like the Czech Republic, Italy and the UK (Fig. 5). Unfortunately, data are not available from all European countries from the beginning of bird ringing until present. Many data were destroyed due to Second World War, got lost or are unprocessed (Nikolov & Karcza 2014 pers. comm.).

CONCLUSIONS AND FUTURE STUDIES

We here present baseline information on the migration system of European Turtle Doves. The data resulted mainly from a combination of ringing efforts concentrated in some countries and decades, and a high hunting pressure, especially in the Mediterranean countries, during passage. The picture obtained is incomplete due to unequal distribution of the numbers of birds ringed, especially the low numbers of Turtle Doves ringed in Eastern European countries after the Czech efforts in the 1950s to 1970s. Given the decreasing population numbers, it seems unlikely that large numbers of Turtle Doves will be ringed in the future in these countries, and data from the wintering sites themselves are still very scarce.

Therefore, crucial new information about Turtle Dove breeding, stopover and overwintering sites as well as their migratory behaviour will most likely only be attained through alternative methods, additionally to ringing. The study using geolocation in Turtle Doves in France (Eraud et al. 2009) has demonstrated the usefulness of this technique. However, large numbers of Turtle Doves need to be captured in order to have some chance to recover the data loggers. In France, 80 birds were deployed with loggers, and the recapture rate was 10%. Hence, in most places with depleted populations this technique is not practicable, and furthermore no latitudinal data can be obtained during several weeks around equinox, i.e. during migration in late March–early April and late September–early October.

Stable isotope analysis can be applied to gain some insight into breeding and wintering areas. An advantage of this method is the necessary small amount of material like feathers or claws. The effective usage of stable isotope analyses was demonstrated e.g. for European Woodpigeons *Columba palumbus*, Reed Warblers *Acrocephalus scirpaceus* and Teals *Anas crecca* (Procházka et al. 2008, Hobson et al. 2009, Guilleman et al. 2014). A 3-isotope cluster approach can distinguish between large areas in Africa, but not determine specific areas (Hobson et al. 2012). Nevertheless, this method was successfully applied to different bird species, e.g. Marsh Warblers *Acrocephalus palustris* and Barn Swallows *Hirundo rustica* (Hobson et al. 2013, Procházka et al. 2014). Another assignment approach for wintering regions is a spatially explicit multi-isotope assignment, which is also based on three different stable isotopes. Hobson et al. (2014) and Veen et al. (2014) used it, to identify overwintering areas of Wood Warblers *Phylloscopus sibilatrix* and hybrid flycatchers *Ficedula*.

To identify stopover and wintering sites more specifically, the further development of satellite tracking and methods of transmitter attachment in Turtle Doves seems the most promising technique. A French team equipped three Turtle Doves with satellite harnesses and got back two partial migration tracks and one complete (Eraud 2015 pers. comm., see also http://cyrileraud.wix.com/turtle-dove-survey#!vido/crj8). Furthermore, a British team equipped seven Turtle Doves and got back six incomplete and one complete migration cycle (Dunn 2014 pers. comm., see also http://operationturtledove.org/). Abdominally implanted satellite transmitters in Black-tailed Godwits *Limosa limosa limosa* produced useful data about migration phenology, migration routes, staging and stopover areas as well as the migratory behaviour of individuals (Hooijemeijer et al. 2014). However, reproduction and survival rates of godwits were affected by the implants (Hooijemeijer et al. 2014), and godwits have more than double the body mass of Turtle Doves. Thus, the application on the strongly declining Turtle Doves seems risky. Similar problems with harnesses attachment and device recovery would apply to GPS loggers.

Unfortunately, in the EURING-database the age of Turtle Doves was mostly given as "unknown". Thus, estimates of breeding success were not possible from these ring recoveries (Beintema 1995, Thompson et al. 1999). Sometimes, the time difference between ringing and recovery was several years, and these were certainly older individuals. However, for most birds, ages were too uncertain to include age differences into this analysis. Therefore, studies on breeding success of Turtle Doves need to apply other approaches such as the observation of age ratios in migrating groups in autumn based on different plumages (Cramp 1985, Browne & Aebischer 2000, Blasco Zumeta & Heinze 2011). Turtle Doves from all migration flyways pass through countries with legal shooting during migration time (Boutin & Lutz 2007). Based on Browne & Aebischer (2005) millions of Turtle Doves were hunted as legal game species throughout Southern Europe. However, the numbers of shot Turtle Doves have declined greatly since Browne & Aebischer (2005) published the results. For instance, Arnadauc et al. (2011) revealed a decline from 189 000 to 61 000–77 000 shot birds from 1998/99 to 2007/08 in France. Those results might also depict the shrinking numbers of Turtle Doves, since hunting bags might reflect the population size (Cattadori et al. 2003). However, given the fast population declines, there is an obvious need for more protection (BirdLife International 2015). Nevertheless, the stop of hunting might not support population recovery, since Browne & Aebischer (2004) have revealed that Turtle Dove declines were mainly caused by conditions on breeding grounds. Hence, the improvement of conditions and productivity in breeding areas might support the population recovery more effectively than prohibition of hunt.

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STRESZCZENIE

[Szlaki wędrówkowe, terminy przelotów oraz pochodzenie osobników zastrzelonych podczas polowań na podstawie analizy wiadomości powrotnych u europejskiej turkawki]

Dokładna znajomość tras przelotów, lęgowisk oraz miejsc zimowania jest ważna dla podejmowania skutecznych działań ochronnych. W przypadku turkawki – dalekodystansowego migranta, którego liczebność w Europie dość drastycznie spadła w ostatnich dekadach — dane dotyczące jej tras przelotów są nieliczne.

W pracy wykorzystano dane zbierane przez krajowe centrale obrączkowania i dostępne poprzez bazę EURING. Główny materiał do analiz stanowiło 897 wiadomości powrotnych (Fig. 1), których liczbę zawężano w przypadku analiz głównych kierunków wędrówek, terminów migracji, a także pochodzenia ptaków zastrzelonych przez myśliwych w poszczególnych krajach europejskich (Tab. 1, 2). Z analiz wyłączono ptaki, których miejsce zaobrączkowania lub odczytania obrączki znajdowało się poza Europą (Apendyks 1, 2). W analizach, jeśli było to możliwe, uwzględniano dane o całkowitej liczbie zaobrączkowanych turkawek w danym kraju. Większość analiz przeprowadzono dla pięciu krajów o największej liczbie wiadomości powrotnych — Republiki Czeskiej, Francji, Niemiec, Węgier oraz Wielkiej Brytanii (Tab. 2, 3). Oszacowano także proporcje wykorzystania poszczególnych głównych tras wędrówek przez ptaki gniazdujące (zaobrączkowane) w poszczególnych krajach.

Analiza wykazała trzy główne kierunki migracji: zachodni, wschodni oraz centralny (Fig. 2). Znacząca większość turkawek z Francji (62%), Niemiec (92%) oraz Wielkiej Brytanii (94%) leciała na zimowiska zachodnim szlakiem. Część ptaków z Czech (56%) wykorzystywała szlak centralny, a 55% ptaków węgierskich — szlak wschodni. Pozostałe ptaki leciały szlakiem centralnym lub wschodnim, a tylko nieliczne szlakiem zachodnim (Tab. 4, 5). Można więc zakładać istnienie u tego gatunku działu wędrówkowego (ang. migratory divide) znajdującego się na terenach pomiędzy Czechami i Niemcami. Zaskakujące było stwierdzenie, że blisko 30% ptaków francuskich leciało szlakiem centralnym (Tab. 4). Obliczono także prawdopodobieństwo uzyskania wiadomości powrotnej w zależności od wybieranego przez ptaki szlaku przelotu — najwyższe było dla szlaku zachodniego (1,4%), zaś najniższe — dla centralnego (0,6%).

Termin wędrówek był podobny wzdłuż wszystkich tras przelotów. Ptaki były obserwowane na lęgowiskach w czerwcu i lipcu, a od września do kwietnia na najbardziej na południe położonych terenach w obrębie zasięgu występowania (Fig. 3). Wędrówka jesienna rozpoczynała się w sierpniu, a powroty na lęgowiska można było zaobserwować jeszcze w maju.

Większość danych o obrączkowanych ptakach pochodzących od osobników zastrzelonych przez myśliwych dotyczyła ptaków zaobrączkowanych w Wielkiej Brytanii, Włoszech i Francji (Fig. 4, Apendyks 3). Duża liczba wiadomości powrotnych uzyskanych od myśliwych pochodziła z sierpnia i września. Znacząca liczba ptaków została zastrzelona także podczas wędrówki wiosennej, szczególnie we Francji i w Grecji (Fig. 4, Apendyks 4). Większość informacji o zaobrączkowanych ptakach raportowanych przez myśliwych pochodziło z lat 1960-tych i 1970-tych (Apendyks 5), kiedy liczba zaobrączkowanych turkawek w poszczególnych krajach także była wysoka (Fig. 5). Stwierdzono, że myśliwi z Francji i Hiszpanii głównie polowali na ptaki z Wielkiej Brytanii i Francji, myśliwi z Włoch przede wszystkim na ptaki zaobrączkowane w tym kraju, a turkawki zastrzelone w Grecji w większości pochodziły z Czech.

Biorąc pod uwagę trendy populacyjne turkawki, wydaje się, aby było mało prawdopodobne uzyskanie w przyszłości dużej liczby nowych danych pochodzących z obrączkowania. Do uzyskania danych o lęgowiskach, miejscach przystankowych podczas wędrówki i zimowiskach, oraz samej ekologii wędrówki konieczne będzie zastosowanie nowych metod, m.in. geolokatorów i innych nadajników oraz analizy izotopów stabilnych.

Appendix 1. Turtle Doves having one location, i.e. capture or recapture site outside of Europe.

(A) Ringed outside Europe

Flyway	Ringed outside Europe	N	Countries of recovery (N)
West	Morocco	3	France (1), Spain (2)
	Senegal	3	Spain (3)
Central	Tunisia	11	Greece (2), Italy (8), Malta (1)
East	Chad	1	Greece (1)
	Israel	5	Russian (2), Ukraine (3)

(B) Recovered outside Europe

Flyway	Recovered outside Europe	N	Countries of ringing (N)
West	Mali	3	UK (3)
	Mauritania	1	UK (1)
	Morocco	8	France (3), Germany, (3), Spain (1), UK (1)
	Senegal	1	France (1)
Central	Burkina	1	Italy (1)
	Libya	5	Czech Republic (3), Hungary (1), Italy (1)
	Tunisia	1	France (1)
East	Egypt	3	Czech Republic (1), Hungary (1), Italy (1)
	Syria	1	Russia (1)
	Turkey	3	Italy (2), Poland (1)

Appendix 2. Turtle Doves having both locations (capture and recapture site) outside Europe.

Flyway	Capture location	Recapture location	N
West	Senegal	Morocco	7
Central	Niger	Tunisia	2
East	Israel	Sudan	1
	Israel	Turkey	1

Appendix 3. Countries of origin of Turtle Dove rings reported by hunters from France, Spain, Italy and Greece.

Country	N
Austria	2
Albania	1
Croatia	1
Czech Republic	43
France	75
Germany	33
Greece	1
Hungary	8
Italy	100
Ireland	1
Poland	1
Portugal	1
Slovakia	2
Spain	24
Sweden	1
Switzerland	3
UK	120
Ukraine	1
TOTAL	418

Appendix 4. Total monthly numbers of Turtle Dove rings reported by hunters from France, Spain, Italy and Greece.

Month	N
2	2
3	1
4	36
5	66
6	10
7	2
8	60
9	205
10	30
11	3
12	3
TOTAL	418

Appendix 5. Number of Turtle Dove rings reported by hunters from France, Spain, Italy and Greece, by decades.

Decade	N
1930s	10
1940s	8
1950s	52
1960s	119
1970s	98
1980s	40
1990s	56
2000s	34
2010s	1
TOTAL	418

CHAPTER 2

STABLE ISOTOPE ASSIGNMENT OF MIGRATING EUROPEAN TURTLE DOVES (*STREPTOPELIA TURTUR*) TO BREEDING RANGES IN EUROPE

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Stable isotope assignment of migrating European Turtle Doves (*Streptopelia turtur*) to breeding ranges in Europe

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Abstract

European Turtle Doves (*Streptopelia turtur*) are long-distance migrants and experienced a population decline of more than 78 % since 1980. Better knowledge about breeding origins of birds at migration sites is crucial for their conservation. Feathers collected at stopover sites, but moulted at breeding grounds, provide the opportunity to perform stable isotope analysis and assign birds to potential breeding origins, based on a European deuterium isoscape. Here, we isotopically analysed 202 feathers from 16 different countries. 101 samples from 13 different countries were used for isoscape calibration. The remaining samples were assigned to breeding origins and mapped for all samples together as one group, per country and individually. The assignment map for all Turtle Doves together was in line with the known distribution pattern. Bulgarian samples were mostly assigned to Russian breeding areas. Samples from Greece, Italy, Malta, and Spain showed a range of possible origins across southern and central Europe. Using individual assignments, four distribution ranges could be distinguished, corresponding to a cool/humid to hot/dry temperature gradient, from north to south/southwest. Proportions of birds assigned to these ranges and of certain migrants varied among stopover sites. Therefore, results have implications that can be applied for conservation measures in Europe.

Introduction

European Turtle Doves (*Streptopelia turtur*, hereafter called Turtle Doves) are the only long-distant migrating columbids in Europe (Glutz von Blotzheim & Bauer 1994). Unfortunately, despite a severe population decline of more than 78 % since 1980 (EBCC 2017), little is known about the population connectivity during breeding, stopover and wintering, which would improve knowledge about threats at different life cycle stages and would help in conservation management at these stages (Williams & Araújo 2000; Martin et al. 2007; Ocampo-Peñula & Pimm 2014). The International Single Species Action Plan of the European Turtle Dove (Fisher et al. 2018) points out that such information is urgently needed to work on the four main objectives for which specific suggestions are outlined: 1) maintain and increase good quality habitats on the breeding grounds, 2) reduction of illegal killing, 3) hunting only at locally and internationally sustainable levels, 4) maintain and increase good quality habitats at stopover and overwintering sites (Fisher et al. 2018).

Recent ring re-encounter studies highlighted a possible migratory divide at longitudes between Germany and the Czech Republic, with a distinction of a western and eastern flyway (Marx et al. 2016). The results also suggested a strong connectivity for western European Turtle Doves and a rather weak connectivity for eastern European birds, whose flyway was split into a central and eastern route (Marx et al. 2016). Some ring recoveries suggested permeability among migratory divides. This was in line with population genetic studies showing no genetic structure, and thus, indicating gene flow among the eastern and western populations (Calderón et al. 2016).

Using ringing data, important key breeding and wintering areas were not detectable due to low re-encounter numbers in Africa and the agglomeration of ringing records at places with high ringing activity, which do not reflect breeding or wintering habitats necessarily (Marx et al. 2016).

Key breeding habitats inhabited by substantial numbers of Turtle should be protected urgently (Williams & Araújo 2000; Martin et al. 2007; Ocampo-Peñula & Pimm 2014), because climatic and land use changes can affect the reproductive or survival rates of migratory birds, through changes in availability of breeding habitat and food resources (Sillett et al. 2000; Newton 2004). Increased use of herbicides in intensified agriculture and forestry management, already led to a depletion of both, availability and quality, of feeding and breeding habitat (Donald et al. 2001; Browne & Aebischer 2001, 2003; Europäische Umweltagentur 2003).

To track origins of birds and improve information about the population connectivity of migrants, the assignment of breeding and overwintering areas via stable isotopes has become an important tool (Hobson et al. 2009; Hobson et al. 2012 a; Cardador et al. 2015). With feathers of known temporal origin, breeding (e.g. Hobson 2005; Hobson et al. 2006; Szymanski et al. 2006) and wintering ranges (e.g. Pain et al. 2004; Bearhop et al. 2005; Hobson et al. 2012b) can be assigned. This is possible due to the metabolically inactive state of feathers and therefore locked-in geographic isotopic information (Hobson 2005; Wunder et al. 2005; Hobson et al. 2009). Hydrogen, carbon and nitrogen are the most frequently used stable isotopes in assignment studies, because they show predictable patterns over geographic and ecological gradients (Wunder et al. 2012).

Precipitation stable hydrogen (δ^2H_p) varies with altitude and latitude (Rozanski et al. 1993), particularly in the northern hemisphere (Hobson 1999; Bowen et al. 2005; Hobson 2005). Accordingly, δ^2H values in plants and water vary geographically with temperature and humidity gradients (West et al. 2006), and stable hydrogen ratios in feathers (δ^2H_f) can be used to assign origins of European migratory birds (Hobson et al. 2009). The Global Network of Isotopes in Precipitation (GNIP; https://nucleus.iaea.org/Pages/GNIPR.aspx) database is well supplied with many stable hydrogen values across Europe. Therefore, a Pan-European breeding assignment using stable hydrogen seems adequate (Bearhop et al. 2005; Hobson et al. 2009; Hobson et al. 2012b, Hobson et al. 2013; Cardador et al. 2015).

The main emphasis of this study was to compare the breeding areas of Turtle Doves sampled at different migration stopover sites by using δ^2H_f values and assign those to a Pan-European δ^2H_p-isoscape. In particular, we aimed to determine:

1) How well migrating populations and individuals can be assigned to breeding areas based on feather samples

2) If proportions of birds assigned to different areas vary among the migration sites, and
3) If it is possible to determine the number of birds with breeding origins in a foreign country at different migration sites.

Methods

Study species

Turtle Doves breed across Europe except for the most northern European parts (Glutz von Blotzheim & Bauer 1994). They prefer a warm, temperate climate and breed usually below 350 m altitude (Glutz von Blotzheim & Bauer 1994, Bakaloudis et al. 2009). Under favourable conditions they breed in areas with elevations over 500 m in temperate zones and up to 1300 m in southern European regions (Glutz von Blotzheim & Bauer 1994; Del Hoyo et al. 1997).

Suitable breeding habitats are characterised by open lowland regions, which are located at forest edges and are irregularly interspersed with shrubs and hedges (Glutz von Blotzheim & Bauer 1994; Kleemann & Quillfeldt 2015). They feed on crops and seeds either at farmland or in natural areas and nest in woodland, coniferous trees, shrubs or thorny bushes (Murton et al. 1964; Browne & Aebischer 2003, 2004; Kleemann & Quillfeldt 2015).

While in the breeding areas, the majority of adult Turtle Doves start moult in July, renewing the first to occasionally fourth primary feathers before autumn migration begins in August (Glutz von Blotzheim & Bauer 1994; Blasco-Zumeta & Heinze 2011; Marx et al. 2016). They suspend moult for migration and renew the remaining feathers at wintering grounds (Glutz von Blotzheim & Bauer 1994; Blasco-Zumeta & Heinze 2011). Juveniles start a postjuvenile moult in the breeding areas and complete it in the wintering areas (Glutz von Blotzheim & Bauer 1994; Blasco-Zumeta & Heinze 2011).

A ring re-encounter study of Turtle Doves described the two most commonly used flyways - a western and an eastern flyway, the latter being split into a more central and easterly direction (Marx et al. 2016), which is in line with previous literature (both flyways: Glutz von Blotzheim & Bauer 1994; description of western flyway: Aebischer et al. 2001; Eraud et al. 2013). A

genetic approach to study the population connectivity of Turtle Doves described unstructured genetic patterns across Europe, which suggests a panmictic behaviour (Calderón et al. 2016).

Feather sampling

A small subsample of the first (P1; N = 199) or second (P2; N = 3) primary was taken. We collected 202 feather samples, which originated from 16 different countries (Table 1). Based on Marx et al. (2016) and GLS tracking studies (French birds: Eraud et al. 2013; http://turtledoveresearch.com/fr/; British birds: https://www.rspb.org.uk/our-work/conservation/satellite-tracking-birds/tracking-turtle-doves; Italian birds: https://blogs.nabu.de/zugvoegel/category/turteltauben/), feather samples obtained in June and July were defined as samples from local breeders (N = 101). The remaining 101 samples were classified as unknown origin and were sampled during spring migration (April and May) and autumn migration (August to September) (Table 1; Marx et al. 2016). Feather samples came from migrating individuals, rescued birds from wildlife recovery centres, nestlings or breeding birds. Individuals were either hunted, or live captured intentionally by hand (nestlings from Greece, rescued birds from Italy), with special bird trapping equipment (e.g. whoosh or mist net), or they originated from museum collections (Table 1).

Museum samples included also five P1 feather samples of the subspecies *S. turtur arenicola* (Table 1) of known breeding origins. Their native breeding distribution ranges from North Africa, the Mediterranean to Asia including north-west China and Mongolia (Glutz von Blotzheim & Bauer 1994).

Table 1: Overview table about study sites (countries and places), sample origin, subspecies and total number of samples per place as well as the number of calibration- and assignmentdata per place.

country	place	origin	subspecies	collection date	N total	N calibrationdata	N assignmentdata
Algeria	Batna	National History Museum Berlin	*S. t. arenicola*	15 June 1903	1	1	
Austria	Lower Austria	Museum of National History Vienna	*S. t. turtur*	20 July 1996	1	1	
Austria	Vienna	Museum of National History Vienna	*S. t. turtur*	23 June 1980, 31 June 1925, 10 July 1925, 10 July 1937	4	4	
Bulgaria	North of Kavarna	Hunt	*S. t. turtur*	23 August 2014	9		9
France	Auvergne	Live capture	*S. t. turtur*	June/July 2014	19	19	
France	Chize	Live capture	*S. t. turtur*	June/July 2014	18	18	
France	Marne	Live capture	*S. t. turtur*	June/July 2014	20	20	
France	Oleron	Live capture	*S. t. turtur*	June/July 2014	20	20	
Germany	Dahme	National History Museum Berlin	*S. t. turtur*	26 June 1981, 06 July 1981	2	2	
Germany	undefined	National History Museum Berlin	*S. t. turtur*	01 June 1890	1	1	
Germany	Villingen	Live capture	*S. t. turtur*	17 June 2014	1	1	
Greece	Levitta	Museum of National	*S. t. turtur*	01 June 1935	1	1	

country	place	origin	subspecies	collection date	N total	N calibrationdata	N assignmentdata
		History Vienna					
Greece	Dadia Soufli Nationalpark	Live capture at nest (nestlings)	*S. t. turtur*	15 July 2014, 17 – 18 July 2014	3	3	
Greece	Soufli	Hunt	*S. t. turtur*	20 – 25 August 2013	20		20
Iran	Abasabad	National History Museum Berlin	*S. t. arenicola*	09 June 1927	1	1	
Italy	Ferrara	Wildlife recovery center	*S. t. turtur*	29 July 2014	1	1	
Italy	Rieti	Wildlife recovery center	*S. t. turtur*	20 June 2014	1	1	
Italy	Ventotene island	Live capture	*S. t. turtur*	28 – 30 April 2014, 12 May 2014, 14 – 15 May 2014, 21 May 2014, 24 May 2014	16		16
Israel	Haifa	Museum of National History Vienna	*S. t. arenicola*	14 July 1972	1	1	
Israel	Neve Yam	Museum of National History Vienna	*S. t. arenicola*	19 July 1968	1	1	
Kazakhstan	Ilisk	Museum of National History Vienna	*S. t. arenicola*	07 June 1900	1	1	

country	place	origin	subspecies	collection date	N total	N calibrationdata	N assignmentdata
Latvia	Ziemupe	National History Museum Berlin	*S. t. turtur*	18 July 1890	1	1	
Malta	Comino	Live capture	*S. t. turtur*	21 April 2014, 06 May 2014	5		5
Malta	undefined	Hunt	*S. t. turtur*	April 2013, 27 October 2014	10		10
Poland	Borowke	National History Museum Berlin	*S. t. turtur*	01 June 1944	1	1	
Russia	Kasar	Museum of National History Vienna	*S. t. turtur*	01 June 1943	1	1	
Spain	Monfrague	Hunt	*S. t. turtur*	29 August 2013, 31 August 2013	41		41
Turkey	Ankara	National History Museum Berlin	*S. t. turtur*	27 June 1933	1	1	
N total					*202*	*101*	*101*

Stable isotope analyses

Feather samples were washed with a 2:1 chloroform/methanol solution for 24 hours and then dried for 48 hours under a fume hood. For stable hydrogen analyses, 0.27 mg ± 0.1 mg were loaded into silver capsules. Stable isotope analyses were run at the Stable Isotope laboratory of the Leibniz Institute for Zoo and Wildlife Research (IZW). Loaded capsules were placed in a Zero Blank autosampler (Costech Analytical Technologies Inc. Italy) and were flushed with chemically pure helium for minimum 1 hour. Then they were dropped into the elemental analyser (EA; HT Elementanalysator HEKAtech, GmbH, Wegberg, Germany), which was connected to the stable isotope-ratio mass spectrometer (Delta V advantage, ThermoFisher Scientific, Bremen, Germany) by an interface (Finnigan Conflo III, ThermoFisher Scientific Bremen, Germany). Samples were analysed together with three previously calibrated in-house keratin reference materials (Popa-Lisseanu et al. 2012). Measured isotope ratios of δ^2H_f are given in parts per mil (‰) deviation from V-SMOW and scaled to the USGS standard to control for exchangeable hydrogen isotopes (see Soto et al. 2017). Thus, δ^2H_f values report the stable isotope ratio of the non-exchangeable portion of hydrogen in feather keratin. Precision of measurements was always better than 2‰. Isotopic measurements per country were compared visually and statistically for countries with more than one sample by an analysis of variance (ANOVA) followed by a Tukey Honest Significant Difference (TukeyHSD) test. Boxplots and statistic tests were generated for feather samples with known and unknown breeding origins in R 3.3.3 (R Core Team 2016).

Assignment of breeding origins

The R package IsoriX (Courtiol et al. 2016a, Courtiol & Rousset 2017) was used for the assignment of breeding origin probabilities. The package builds isoscapes by using mixed models and computes geographic origins of organisms depending on their isotopic ratios. We followed the instructions specified in the package description and workflow (Courtiol et al. 2016a, b). GNIP data were filtered for the months April to August (i.e. the time when Turtle Doves likely stay in Europe). To fit the isoscape geostatistical model (function isofit), we kept

default settings, but used the option “fitme” in the spaMM method, which works for data with and without a Matern correlation structure (see for details Courtiol et al. 2016a, b). The elevation raster was built by restricting the queried area to Europe with a latitudinal extent of 31°N to 69°N and a longitudinal range from -27°W to 57°E. This area was sufficient according to the longitudinal distribution limits (BirdLife International Handbook of the Birds of the World 2016) and latitudinal limits were based on distinguished flyways (Marx et al. 2016). The number of cells to merge was set to an aggregation factor of 10 and the underlying function was “mean”. To create the isoscape of the mean δ^2H_p distribution in Europe, default settings were used. Then we loaded the calibration data and fit the calibration function by using the standards. The isoscape calibration is based on a linear mixed-effects model, which fits isotopic values of calibration samples to the environment with considered unknown isotopic values by using a linear function (Courtiol et al. 2016b). Then assignment data were implemented into the system and possible origin areas were computed following the workflow (Courtiol et al. 2016b). Individual and group assignments for all samples and by country were conducted. Group assignments followed the Fisher’s method (Fisher 1925), which combines all individual p-value maps to a single group map. Results were exported as rasters to create final maps. According to Courtiol et al. (2016a), we first built the basemap containing countries and borders (embedded in package rgdal (Bivand et al. 2017)) and second, exported the raster files as GTiff-format. Rasters were then clipped according to Turtle Doves’ distribution range (BirdLife International Handbook of the Birds of the World 2016) using ArcGIS 10.2.2. Final maps show most likely origin areas classified by three possibility groups (p-values ≥ 0.7, ≥ 0.8 and ≥ 0.9) and were created at three levels: all assignment samples as one group, grouped by country of collection, and individual maps for each bird.

Results

δ^2H_f values of samples ranged from approximately -80 ‰ to -10 ‰ (Figs. 1 – 2), but the majority had values between -70 ‰ to -40 ‰. The calibration data differed in their δ^2H_f values between countries (ANOVA, $F_{5,88} = 2.35$, $p \leq 0.05$). However, there was only a tending difference for

France compared to Austria (TukeyHSD, p = 0.06, Table 2). Calibration samples had a similar distribution of δ^2H_f values (Fig. 1). Boxplots of assignment data highlighted values mainly ranging from -62 ‰ to -45 ‰, and countries of collection were significantly different (ANOVA, $F_{4,96}$ = 9.74, p < 0.001). Values of Bulgarian samples were most negative (-75 ‰ to -68 ‰, Fig. 2) and significantly different to other collection countries (TukeyHSD, p < 0.5, Table 3).

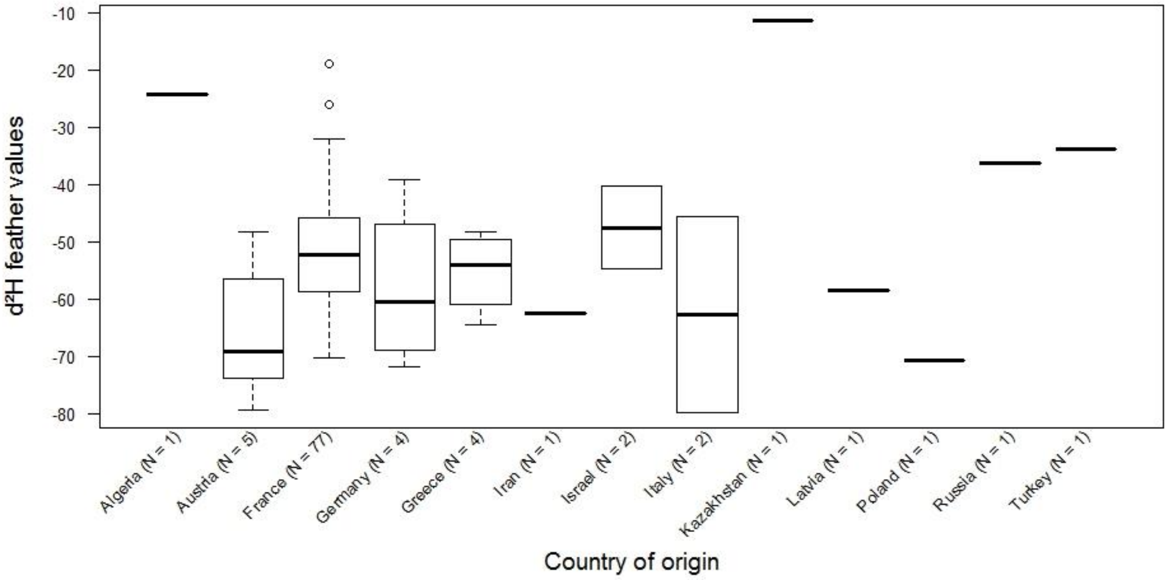

Figure 1: Boxplot graphs for δ^2H_f values only from feather samples per sampling site used as calibrationdata (country of origin) Also the numbers of samples per sampling site are given. The boxes for sampling sites with more than one sample represent the range in which 50 % of the data occur (inter-quartile distance from 25 % quartile to 75 % quartile). Whiskers show indicate extreme values and the median is highlighted as black line within the boxes. The isotopic values for sampling sites with only one sample are represented by a black line only. Circles highlight outliers and extend the inter-quartile distance for more than 1.5 times.

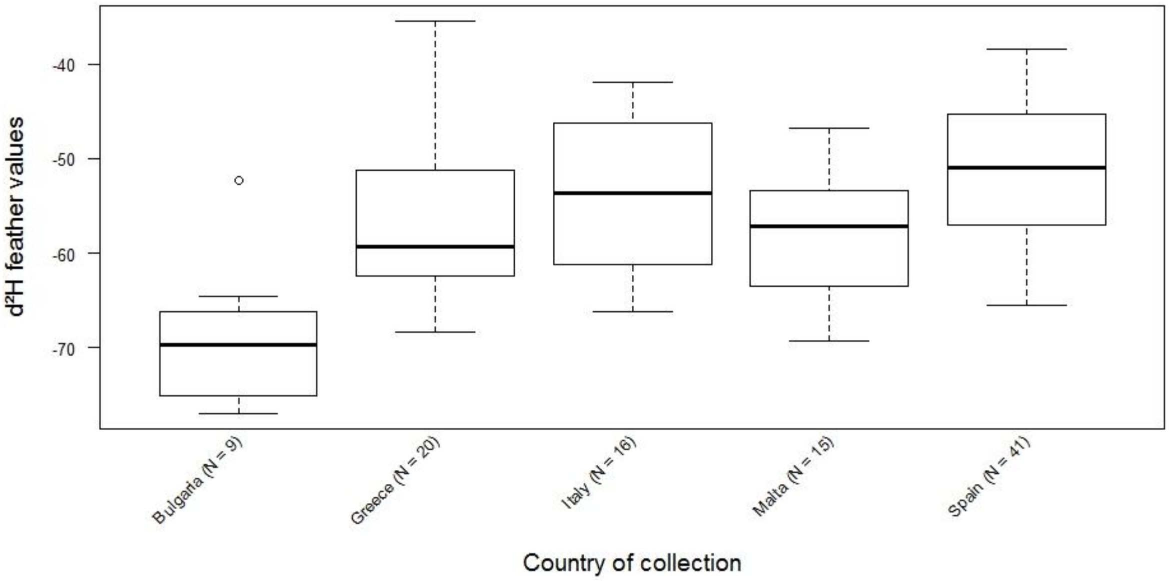

Figure 2: Boxplot graphs for δ^2H_f values only from feather samples per sampling site used as assignment data (country of collection). Also the numbers of samples per sampling site are given. The boxes for sampling sites with more than one sample represent the range in which 50% of the data occur (inter-quartile distance from 25 % quartile to 75 % quartile). Whiskers show extreme values and the median is highlighted as black line within the boxes. Circles highlight outliers and extend the inter-quartile distance for more than 1.5 times.

Table 2: Results of the TukeyHSD test to compare isotopic values of countries of calibration. Significant differences were not detected but a tendentious difference between France and Austria with a p-value = 0.06. P-value were rounded up to the second decimal.

Country	Compared country	p-value
France	Austria	0.06
Germany	Austria	0.90
Germany	France	0.84
Greece	Austria	0.70
Greece	France	0.98
Greece	Germany	1.00
Israel	Austria	0.33
Israel	France	1.00
Israel	Germany	0.86
Israel	Greece	0.96
Italy	Austria	1.00
Italy	France	0.68
Italy	Germany	1.00
Italy	Greece	0.97
Italy	Israel	0.70

Table 3: Results of the TukeyHSD test to compare isotopic values of countries of assignment. Values are significantly different when the p-value is < 0.05.

Country	Compared country	p-value
Greece	Bulgaria	< 0.05
Italy	Bulgaria	< 0.05
Malta	Bulgaria	< 0.05
Spain	Bulgaria	< 0.05
Italy	Greece	0.86
Malta	Greece	0.93
Spain	Greece	0.26
Malta	Italy	0.46
Spain	Italy	0.94
Spain	Malta	0.06

The assignment map for all assigned Turtle Doves together showed a broad possible distributional range. Likely origins lay within southern and central Europe until approx. 55°N latitudinal range, but no likely origins were in high mountainous areas (Alps, Pyrenees, Pindus, Dinaric Alps or Anatolia; Fig. 3).

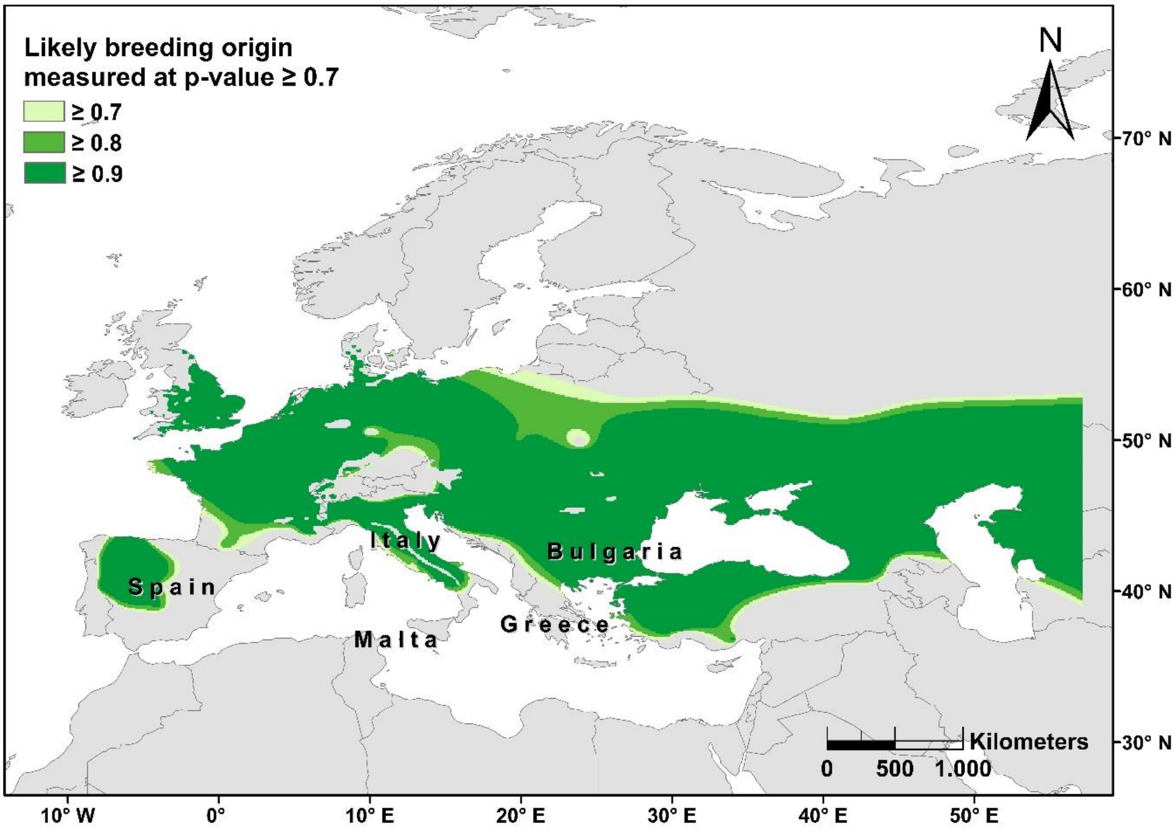

Figure 3: Assignment map showing possible breeding ranges grouped for all Turtle Doves with unknown breeding origins. Drawn are three classified probability ranges in green shades, highlighting areas with origin probabilities of ≥ 0.7, ≥ 0.8 and ≥ 0.9. Countries of collection are written in the map.

Group assignments per country showed the following results:

Bulgarian samples were assigned to Russian and Baltic areas primarily, while the small area at the foothills of the northern and southern alpine regions (Fig. 4) is probably an artefact of similar isotopic values due to the altitude effect. Greek samples were assigned to wide areas ranging from 40°N to 55°N, mainly excluding the Mediterranean as probable breeding origin. In contrast to this, Italian, Maltese and Spanish samples showed likely breeding origins in western Europe from approximately 38°N to 55°N covering Spain, France and the UK but also central and eastern European origins, including Mediterranean areas in Italy, Croatia, Bosnia-Herzegovina and Turkey (Fig. 4).

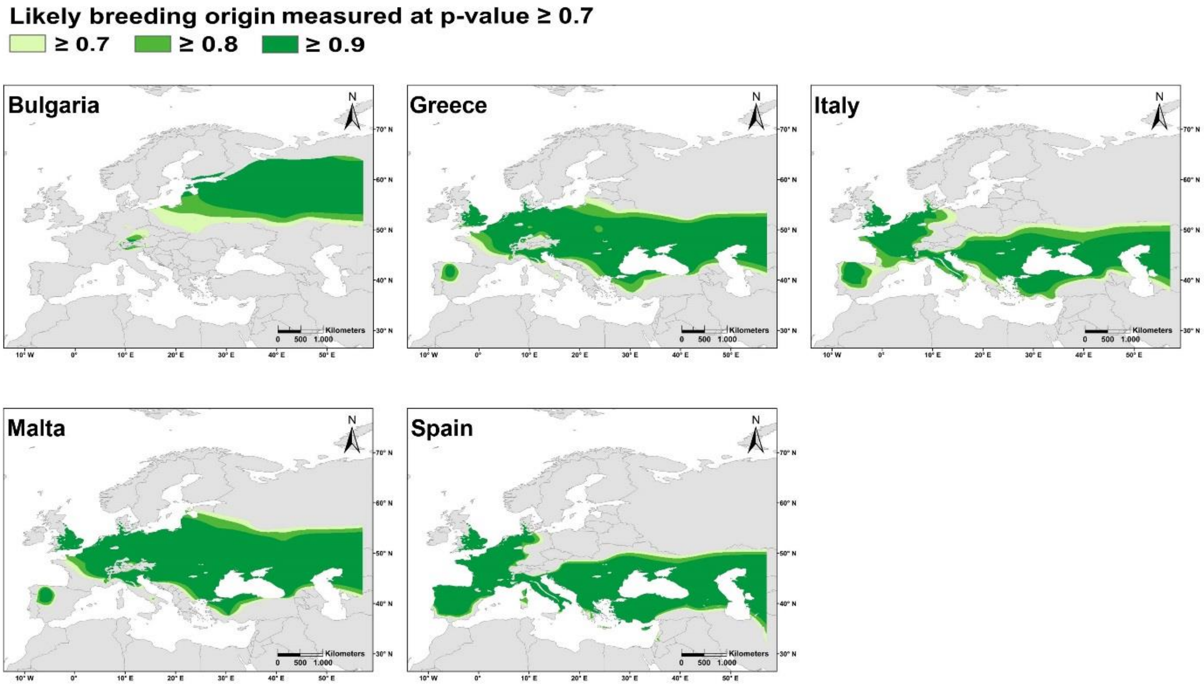

Figure 4: Assignment maps highlighting possible breeding ranges grouped for Turtle Dove samples with unknown breeding origins coming from one stopover site. Per map three classified probability ranges in green shades are drawn, highlighting areas with origin probabilities of ≥ 0.7, ≥ 0.8 and ≥ 0.9

Individual assignments were used to determine proportions of birds coming from different breeding areas. Four large distribution ranges could be distinguished quite well (Figs. S1 - S7 and Table 4), corresponding to a hot/dry to cool/humid temperature/humidity gradient:

1) Stable isotope values between -77 ‰ and -65 ‰ resulted in projected breeding origins in Russia and the Baltic states, and were most common in birds migrating through Bulgaria (89 %), but also observed at all other migratory sites (in Malta (24 %), Greece (10 %), Spain (10 %), and Italy (6 %; Table 4)).
2) Stable isotope values between -64‰ and -59‰ produced projected breeding origins in central eastern Europe (Germany, Poland, Hungary, Czech Republic and Slovakia). These were most common in birds sampled in Greece (45 %), followed by Italy (25 %) and Malta (18 %). This distribution range was absent in Bulgaria and uncommon in Spain (10 %; Table 4).

3) Stable isotope values between -58 ‰ and -49 ‰ resulted in projected breeding origins in France, northern Italy, south-west England, north-west Spain, Romania and Bulgaria. The highest proportions of these origin ranges were found in Maltese samples (48 %), intermediate proportions came from Spain (39 %), Italy (38 %) and Greece (30 %) and low proportions from Bulgaria (11 %; Table 4).
4) Stable isotope values between -53 ‰ and -36 ‰ led to projected breeding origins around the Mediterranean, but also including possible areas at the Atlantic coast and in south-western France. A high proportion was found in birds sampled in Spain (41 %), followed by Italy (31 %) and Greece (15 %), while only two of the samples from Malta and none from Bulgaria fell into this category (Table 4).

Although it was not possible to assign specific breeding origin countries, it was possible to determine a minimal proportion of birds originating from other breeding ranges than the sampled country (i.e. not being national breeders). This proportion was highest in Malta (100 %), and Bulgaria (89 %), rather high in Greece (55 %) and low in Spain (20 %), while none of the birds sampled in Italy could be determined as “foreign” bird (Table 4).

Table 4: Overview table about the number of individuals and percentage of assigned breeding origins of birds sampled in Bulgaria, Malta, Spain, Greece and Italy. Furthermore, numbers of possible national breeders as well as numbers and percentages of sampled birds determined as certain migrants are given per stopover site. Ranges of δ^{2}H$_f$ values per distribution area are highlighted in bold.

Place	**N**	**Russia and Baltic States[1]**	**Central eastern Europe (Germany, Poland, Hungary, Czech Republic and Slovakia)**	**France, N Italy, SW England, NW Spain, Romania and Bulgaria**	**Mediterranean coasts[2]**	**Possible national breeders**	**Certain migrants**
Bulgaria	9	8 (88.9 %)	0	1 (11.1 %)	0	1	8 (88.9 %)
Malta	15	4 (26.7 %)	3 (20 %)	6 (40 %)	2 (13.3 %)	0	15 (100 %)
Spain	41	4 (9.8 %)	4 (9.8 %)	16 (39 %)	17 (41.4 %)	33	8 (19.5 %)
Greece	20	2 (10 %)	9 (45 %)	6 (30 %)	3 (15 %)	9	11 (55 %)
Italy	16	1 (6.3 %)	4 (25 %)	6 (37.5 %)	5 (31.3 %)	16	0
δ^{2}H$_f$		**-77 ‰ to -65 ‰**	**-64 ‰ to -59 ‰**	**-58 ‰ to -50 ‰**	**-53 ‰ to -36 ‰**		

[1] in several birds, the projected area also included the low-Alpine area. [2] in several birds, the projected area also included the Atlantic coasts of France and Portugal, and south-western Portugal.

Discussion

Hydrogen stable isotope values of feathers were modelled across a validated isoscape to assess the potential breeding origins of European Turtle Doves collected at migration sites and thus assign distributional ranges at different levels: overall, by migration site and individually.

Overall, resulting maps primarily describe a large disuse of mountainous areas as Turtle Dove breeding regions (Figs. 3 - 4). This is in line with usual occurrence at elevations up to 350 m and occasionally over 500 m described for continental Europe (Glutz von Blotzheim & Bauer 1994; Browne & Aebischer 2005). In southern Europe, breeding habitats at locations up to 1300 m are also used occasionally (Del Hoyo et al. 1997; Sáenz de Buruaga 2012). Yet, some individuals in the present study might inhabit high elevation areas, especially in southern Europe, because the projected areas included mountain foothills. For future studies calibration samples from those breeding areas might improve assignments.

However, maps generally showed broad ranges of highly likely breeding areas (p-value ≥ 0.9) across entire Europe except the northern limit of approximately 55°N. The results might be affected by small sample sizes of calibration samples per country, but were also probably affected by latitudinal gradients of deuterium values in space (Hobson 2005), which possibly led to major similarity between isotopic values of calibration data per country (Table 2). Deuterium values increase with more southern and south-western latitudes (Rozanski et al. 1993; Hobson et al. 2004; West et al. 2008) and can parallel the known distribution range of a species in such a way, as such, it might be difficult to discriminate populations in more northern or north-eastern directions. Along with gradual distribution of isotopic values, large species distribution ranges, as for Turtle Doves, usually end up in broad assignments of probable origins (e.g. Swainson's thrush *Catharus ustulatus* or Tennessee warbler *Vermivora peregrina*, Hobson & Wassenaar 1997; Hobson 2005). Furthermore, different individual assignments covered wide areas of possible breeding origins, which likely resulted in large group assignments. However, modelled distribution of probable breeding origins are also compatible with the suggested panmictic population genetic structure (Calderón et al. 2016), flyway

crossings (Marx et al. 2016) and the hypothesis of permeability between flyways (e.g. Guillemain et al. 2005).

Despite these large assignment areas it was possible to identify four main distribution ranges by evaluating individual assignments. Because deuterium isotope values depend on the precipitation/evaporation balance, these areas correspond to a latitudinal/climatic gradient and the distribution patterns are in line with previous literature (Glutz von Blotzheim & Bauer 1994; Del Hoyo et al. 1997). More negative δ^2H_f values are found at higher latitudes and towards the interior Eurasian continent (groupings 1 and 2; hot/dry) and heavier δ^2H_f ratios at lower latitudes and at coastal areas (groupings 3 and 4; cool/humid) (Rozanski et al. 1993; Hobson 2005; West et al. 2006).

Individual assignments also add information about origins and possible proportions of passing migrants per country. The results highlighted Malta as main country for "foreign" birds with 100 % of migrants as indicated by very low numbers of 0 – 14 breeding pairs (Fisher et al. 2018), followed by 89 % of Turtle Doves travelling through Bulgaria and 55 % passing Greece. Of these, 89 % and 24 % of birds migrating through Bulgaria and Malta were assigned to Russian and Baltic breeding ranges. This is of particular interest, because recent studies from these breeding ranges are scarce despite a former high ringing activity (Moscow: N > 1600, Marx et al. 2016).

Furthermore, in Italy many of the birds sampled during spring migration period (Table 1) were possible local breeders. However, due to large breeding assignments for Italian birds, their breeding origins can also be in other countries, particularly in the light of a panmictic behaviour (Calderón et al. 2016) and indicated by the Italian migration atlas, which highlighted recoveries from e.g. Czech Republic, Slovakia, Hungary, Sweden, Spain, France and Portugal (Spina et al. 2008).

The presented results have implications that can be applied in the framework of harvest management and protected area planning. In May 2018, the International Single Species

Action Plan for the conservation of the European Turtle dove (Fisher et al. 2018) was adopted by the European Commission. This plan lists four main objectives with specific suggestions (see introduction, Fisher et al. 2018), but as we still lack key knowledge to implement these, further objectives concern international cooperation, stakeholder awareness, and the filling of knowledge gaps. Among the latter, objective 7.7 calls for “Understanding of the country of origin of hunted birds by 2020”, which constitutes basic information to further specify a sustainable level for hunt, locally and internationally (objective 3). To determine countries of origin possible methods are ring re-encounter studies (e.g. Marx et al. 2016), tracking of birds and stable isotope analyses.

Ring re-encounter studies generally have several shortcomings (e.g. Marx et al. 2016), including high trapping effort due to low re-encounter probabilities and an uneven distribution of captures and recaptures in space and time (Kania & Busse 1987; Thorup & Conn 2009). In particular, most Turtle Dove re-encounters were in the form of hunted birds, but the distribution of countries of origin depended heavily on the trapping effort in the different countries (Marx et al. 2016). Furthermore, many ring data remain unreported or unavailable due to loss or they were not processed (Nikolov & Karcza pers. comm.) and the method can have a delay of several years from initial marking to the re-encounter of the bird (Marx et al. 2016).

Tracking studies have a good potential to reveal breeding origins and movements to winter sites, but are limited until now to a low number of individuals by the high costs and the potentially high failure rate of tagged birds (e.g. Bowlin et al. 2010).

The present stable isotope method has some advantages, as a very small feather sample (0.3 mg) can be taken from hunted birds and live captures, thus making the non-invasive method widely applicable and suitable to obtain information quickly on a larger number of birds. On the other hand, the information obtained is relatively coarse, resulting in large possible ranges.

The definition of a sustainable level of hunt on local and international level usually relies on information about numbers and proportions of hunted birds (migrants and national breeders) compared to the population growth rate of a species. However, due to naturally occurring drastic demographic fluctuations and the currently decreasing population size of Turtle Doves, additional anthropogenic threats are suggested to have large negative impacts on population level (Calderón et al. 2016). If legal hunt is practised to an unsustainable level during Turtle Dove migration (Fisher et al. 2018), then this would largely affect foreign and possibly national breeding populations, although due to the uncertainties of the method, respective contributions from local and foreign birds cannot be determined precisely.

Hunting of Turtle Doves is legal in 10 European Union (EU) member states. Our current study was in five of those countries. According to Fisher et al. (2018), Spain has the highest hunting bags (436,807-805,643 birds annually), followed by Greece (273,000-492,800 birds annually), Italy (250-350,000 birds annually) and Bulgaria (145,672 in 2014-15). Malta has the smallest hunting bags of ≤ 6,000 in 2015 and ≤ 1,500 in 2016 and prohibited spring hunt in 2017 (Fisher et al. 2018). Although numbers of hunted Turtle Doves exist for the remaining legally hunting EU member states Austria, Cyprus, France, Portugal and Romania (Fisher et al. 2018), details about breeding origins of Turtle Doves are still needed from these sites. The present study suggests that hunt in Spain affects national breeders probably in high numbers, but also migrants originating from Russia and the Baltic States as well as central eastern Europe (Table 4, Figs. S1 - S3). According to our data, Greece, Malta and Bulgaria mainly hunt migrants (Table 4). Considering Greek samples migrants primarily originate from central eastern Europe and France, northern Italy, south-west England, north-west Spain, Romania and Bulgaria. Birds from all four distribution ranges have stopovers in Malta (Figs. S4 - S5), and individuals passing through Bulgaria mainly originate from Russia and Baltic States (Table 4, Fig. S6). All Italian samples were assigned possible national breeders (Table 4, Fig. S7), which might indicate a high hunting impact on Italian Turtle Doves, and would agree with high hunting numbers of Italian birds in Italy (Marx et al. 2016). Nonetheless, an impact on “foreign” birds cannot be excluded due to large assignment ranges and re-encounters from several other countries (Spina et al. 2008) and to the lack of a specific investigation on the origin of birds migrating through the Italian peninsula during autumn migration, when hunting season is open.

To sum up, the present stable isotope analyses highlighted important breeding origin areas and discussed possible hunting impacts at studied stopover sites on migrating individuals. Although our dataset was limited by the availability of feathers from the different sites, we outline a method that can be used to study origins of migratory Turtle Doves for larger sample sizes and covering more complete time frames. Thus, together with the previous mark re-encounter analyses (Marx et al. 2016), and data from tracking studies, stable isotope analyses may be an important tool to fill knowledge gaps that so far prevent the implementation of an adaptive harvest modelling framework for Turtle Doves in order to improve the species conservation status in Europe.

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Supplementary Materials

Individual assignments of birds from Spain (Figs. S1 to S3), Greece (Fig. S4), Malta (Fig. S5), Bulgaria (Fig. S6) and Italy (Fig. S7) are available online. The authors are solely responsible for the content and functionality of these materials. Queries (other than absence of the material) should be directed to the corresponding author.

Figure S1: Turtle Dove samples from Spain having their assigned origins in Russia and the Baltic states and in central eastern Europe. Certain migrants, which are birds without possible breeding origin assignments in Spain, are highlighted with a red M. Furthermore, the individual isotopic values of δ^2H_f are shown.

Figure S2: Turtle Dove samples from Spain having their assigned origins in France, northern (N) Italy, south-west (SW) England, north-west (NW) Spain, Romania and Bulgaria. The individual isotopic values of δ^2H_f are given. Certain migration cannot be detected, because areas with possible breeding ranges are assigned in Spain.

Figure S3: Turtle Dove samples from Spain having their assigned origins at the Mediterranean coasts. The individual isotopic values of δ^2H_f are given. The individual isotopic values of δ^2H_f

are given. Certain migration cannot be detected, because areas with possible breeding ranges are assigned in Spain.

Figure S4: Turtle Dove samples from Greece ordered by assigned distribution ranges. Migrants are highlighted with a red M. Furthermore, the individual isotopic values of δ^2H_f are shown.

Figure S5: Turtle Dove samples from Malta ordered by assigned distribution ranges. Certain migrants are highlighted with a red M. They have no breeding origin areas in Malta. Furthermore, the individual isotopic values of δ^2H_f are shown.

Figure S6: Turtle Dove samples from Bulgaria ordered by assigned distribution ranges. Certain migrants are highlighted with a red M. They have no breeding origin areas in Bulgaria. Furthermore, the individual isotopic values of δ^2H_f are shown.

Figure S7: Turtle Dove samples from Italy ordered by assigned distribution ranges. The individual isotopic values of δ^2H_f are given. Certain migration cannot be detected, because areas with possible breeding ranges are assigned in Italy.

CHAPTER 3

HIGH PREVALENCE OF *TRICHOMONAS GALLINAE* IN WILD COLUMBIDS ACROSS WESTERN AND SOUTHERN EUROPE

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Parasites & Vectors

RESEARCH **Open Access**

High prevalence of *Trichomonas gallinae* in wild columbids across western and southern Europe

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Abstract

Background: Avian trichomonosis is known as a widespread disease in columbids and passerines, and recent findings have highlighted the pathogenic character of some lineages found in wild birds. Trichomonosis can affect wild bird populations including endangered species, as has been shown for Mauritian pink pigeons *Nesoenas mayeri* in Mauritius and suggested for European turtle doves *Streptopelia turtur* in the UK. However, the disease trichomonosis is caused only by pathogenic lineages of the parasite *Trichomonas gallinae*. Therefore, understanding the prevalence and distribution of both potentially pathogenic and non-pathogenic *T. gallinae* lineages in turtle doves and other columbids across Europe is relevant to estimate the potential impact of the disease on a continental scale.

Results: We examined 281 samples from four wild columbid species for *Trichomonas* infection and determined the genetic lineages. The overall prevalence was 74%. There were significant differences between the species ($P = 0.007$). The highest prevalence was found in stock doves *Columba oenas* (86%, $n = 79$) followed by wood pigeons *Columba palumbus* (70%, $n = 61$) and turtle doves (67%, $n = 65$), while three of five collared doves *Streptopelia decaocto* (60%) were infected. We found seven different lineages, including four lineages present in columbids in the UK, one lineage already described from Spain and three new lineages, one of those found in a single turtle dove migrating through Italy and another one found in a breeding stock dove. Stock doves from Germany and collared doves from Malta were infected with a potentially pathogenic lineage (lineage A/B), which is known to cause lesions and mortality in columbids, raptors and finches.

Conclusions: Generally, turtle doves showed high prevalence of *Trichomonas* infection. Furthermore, the potentially pathogenic lineage A/B (or genotype B according to previous literature) was found in a recovering stock dove population. Both findings are worrying for these columbid species due to the occasional epidemic character of trichomonosis, which can have severe negative effects on populations.

Keywords: *Trichomonas gallinae*, Columbiformes, Stock dove, Phylogenetic analysis, Genetic lineage, Pathogen

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Background

The protozoan parasite *Trichomonas gallinae* infects captive and wild bird species across the world and can lead to the avian disease called trichomonosis. Due to its widespread occurrence and sometimes pathogenic character, it is thought to play a role in the regulation of wild bird populations [1–5]. Bird migrations can contribute to the spread of *Trichomonas*. For instance, the drastic decline observed in greenfinch *Chloris chloris* and chaffinch *Fringilla coelebs* in the UK and Fennoscandia as well as the increased mortality of finches in Germany and Austria was linked to spread of the parasite by bird species during migration [4, 6–9].

Birds belonging to the Columbiformes are the main hosts of *Trichomonas gallinae* [1, 3] and rock pigeons *Columba livia* are thought to be the predominant transmitters of *T. gallinae* worldwide [1]. *Trichomonas* parasites also occur in wild columbid species around the world [10–14]. In Europe, columbids from wild populations in Spain and the UK were surveyed recently. Trichomonosis has been detected in wild European columbid species including collared doves *Streptopelia decaocto*, stock doves *Columba oenas*, wood pigeons *Columba palumbus* and European turtle doves *Streptopelia turtur* (hereafter turtle doves) [3, 12, 15–17].

Turtle doves, stock doves and wood pigeons are migratory birds, which can favour the spread of *Trichomonas* parasites. Turtle doves display three main migration flyways, a western, central and eastern route between Europe and sub-Saharan Africa [18]. Stock doves and wood pigeons from European countries mainly use the western migratory flyway to France and Iberia [19, 20]. As pathogens can be spread by migratory birds, those species might be more vulnerable to parasite exposure [6].

Within the Columbiformes, parasite transmission can occur at shared feeding and drinking sites within and among species and from adults to nestlings while crop milk feeding [1, 21]. Epidemic trichomonosis in finches showed a relatively low host specificity of the parasite [22], with parasite spill-over probably occurring at feeding and watering stations shared by different bird species [3, 23]. If birds are infected by *Trichomonas*, they can develop necrotic lesions in the crop and oropharynx (i.e. trichomonosis), which can lead to death by starvation and suffocation [1]. Moreover, not only the upper digestive tract can be affected, but also less commonly the liver, air sacs as well as parts of the cranium [1]. Nevertheless, there are individual differences in disease response and not all infected birds show clinical signs, because the virulence of the parasite varies among different lineages [4, 14, 24]. For instance, it might happen a bird shows clinical signs of the disease, such as caseous lesions, but recovers after some days [1, 25]. On the other hand, there are highly pathogenic lineages, which weaken birds severely, cause lesions to develop in oropharynx and liver, and lead to death in almost any case [25]. Furthermore, outbreaks can occur even when food shortage or stress is present because birds get more vulnerable to parasite exposure and infection due to limited food sources and crowding with probably infected birds [3]. However, Stabler [26] also showed the possible immunisation with less pathogenic *Trichomonas* lineages in birds. Nevertheless, as reported in finches, the most pathogenic lineages can heavily affect population sizes [6]. Thus, trichomonosis might represent an additional threat to endangered bird species, such as Mauritian pink pigeons *Nesoenas mayeri* in Mauritius [10] or turtle doves in Europe [27, 28]. Likewise, recovering stock dove populations, which showed population declines in the 20th century due to a lack of adequate breeding sites [19, 29], may be threatened again in the future by this emerging infectious disease due to their migratory character and the low host specificity of *Trichomonas* parasites.

Here we examined samples from four different columbid species from Germany, Spain, Italy and Malta. Spanish and UK turtle dove populations are connected through the western European flyway, and turtle doves from western Germany also migrate through Spain [18]. Furthermore, Italian and Maltese turtle doves follow a central/eastern flyway [18]. The aims of the present study were to (i) test whether turtle doves migrating through Italy and Malta, along the central/eastern flyway [18], have similar prevalence and genetic lineages of *Trichomonas* as birds along the western flyway; (ii) obtain baseline data on prevalence and genetic lineages of *Trichomonas* in a recovering population of stock doves in Germany; and (iii) provide information from a larger sample of turtle doves, collared doves (sampled in Malta), stock doves and wood pigeons migrating along the western flyway (sampled in Spain and Germany), with a focus on the potentially pathogenic lineages (in Lennon et al. [12]: lineages 3 and 4, which belong to the genotype B [14]), which are known to cause gross lesions and mortality in columbids, raptors and finches [12, 14].

Methods

Species and study sites

A total of 281 columbids from four different countries (Germany, $n = 180$; Spain, $n = 45$; Malta, $n = 36$; and Italy, $n = 20$) were sampled for *Trichomonas* infections, with the main focus on stock doves and wood pigeons from Germany and turtle doves on migration through Malta and Italy as well as from a breeding population in Spain. We also took samples from Spanish and Maltese collared doves. 80% of sampled birds came from Western Europe, and 20% of birds came from Central Europe. Samples from stock doves included breeding adults ($n = 33$), chicks ($n = 58$) and one first-year bird. Considering stock dove chicks, we had 47 siblings out of 23 nests. Of those nests, 22 included two siblings, and one nest had three siblings. Additionally, we

sampled adult and first-year turtle doves at the end of the breeding season in Extremadura, Spain (39°51′42″N, -6°6′37″E). Other turtle doves were sampled during the non-breeding season, birds during spring migration on Comino, Malta (36°0′36″N, 14°20′8″E) and Italy (on the island of Ventotene: 40°47′54″N, 13°25′55″E) (Table 1). Unfortunately, for almost half of the turtle doves, we could not determine the age, thus they were classified as "unknown" age.

Sampling and parasite culture

The Veterinary Department of the Justus-Liebig University Giessen obtained dead adult wood pigeons from hunting managers from different sites in Germany. Therefore, they constitute a random sample of the population, as they had not died from natural causes. To obtain a tissue sample, we opened the oropharynges of 87 defrosted birds from outside with a sterile scalpel and cut a small tissue sample from the oropharyngeal tract. While we extracted the tissue sample, we also checked the pigeon for lesions. Only one wood pigeon had a yellow plaque in the throat. Furthermore, we obtained 27 samples of oropharyngeal tissue from migrating turtle doves hunted in Malta. The tissue samples were kept at -18 °C until DNA extraction. Since we could not investigate the entire bird, we cannot tell, whether these Maltese birds had lesions in their oesophagus.

Oral swabs were taken from oropharynx and crop after visual inspection of 41 freshly hunted birds in Spain and 126 live birds at all other sites with a dry, sterile cotton tip. No lesions were detected, and swabs were inoculated individually in a *Trichomonas* selective culture medium (OXOID Deutschland GmbH, Wesel, Germany) ($n = 20$) or an InPouch TF culture kit (BioMed Diagnostics, Oregon, USA) ($n = 147$). Both media have similar detection sensitivities for *Trichomonas* parasites and have been used successfully in Finland [30]. The samples were incubated at 37 °C for five to seven days, giving any protozoan parasites sufficient time to multiply [31]. Samples were centrifuged for 5 min at 1,000× *g*, the supernatant was discarded, and the pellet was re-suspended in 1 ml of phosphate-buffered saline (PBS). The samples were centrifuged again for 5 min at 1,000× *g* the pellet was re-suspended in five drops of PBS (approximately 100 μl) and kept at -18 °C until DNA extraction.

DNA extraction

DNA from tissue samples was extracted using the DNeasy blood and tissue kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. For final elution, we used 60 μl of ddH$_2$O to increase DNA concentration in the eluate. *T. gallinae* swab samples were centrifuged for 5 min at 13,000× *g*. The supernatant was discarded, and DNA was extracted as described by Mégia-Palma et al. 2013 [32]. The DNA was re-suspended in 30 μl of ddH$_2$O. The DNA concentration of all samples was measured with a ThermoScientific Nanodrop 2000 micro-volume UV-VIS spectrometer. For samples with a concentration above 70 ng/μl, the sample was diluted with ddH$_2$O to a final concentration of at least 20 ng/μl.

ITS1-5.8S-ITS2 PCR amplification and gel electrophoresis

For infection detection, we amplified the highly conserved ITS1-5.8S-ITS2 ribosomal region of the *T.*

Table 1 *Trichomonas* spp. prevalence found in four columbid species at different European study sites with the use of PCR analysis. The prevalence was calculated according to PCR results

Species	No. of samples	Place (*n*)	Status (*n*)	Sample (*n*)	No. positive	No. negative	Prevalence [%]	No. of sequences
Stock dove	92	Hesse, Germany (90) Brandenburg, Germany (2)	Adult (33) First year (1) Chick (58)	Swab (92)	79	13	86	16
Wood pigeon	87	Bavaria, Germany (50) North Rhine-Westphalia, Germany (24) Lower Saxony, Germany (8) Thuringia, Germany (3) Hesse, Germany (1) Unknown, Germany (1)	Adult (87)	Tissue (87)[a]	61	26	70	23
Collared dove	5	Monfrague, Spain (3) Comino, Malta (2)	Adult (3) Second year (1) Unknown (1)	Swab (5)	3	2	60	2
Turtle dove	97	Monfrague, Spain (42) Comino, Malta (34) Ventotene, Italy (20) Hesse, Germany (1)	Adult (22) Juvenile (23) Second year (2) Unknown (50)	Swab (70) Tissue (27) All	65 0 65	5 27 32	93 0 67	43 0 43
Total	281			Swab (167) Tissue (114) All	154 61 208	13 53 73	93 54 74	61 23 84

[a] One of the wood pigeons (sample WP4) had yellow plaque

gallinae genome [33] with the primers TFR1 (5′-TGC TTC AGT TCA GCG GGT CTT CC-3′) and TFR2 (5′-CGG TAG GTG AAC CTG CCG TTG G-3′) [34], which produce an expected product of 400 bp [4]. We applied two different reagents and reaction volumes for (i) the samples from Malta, and (ii) all other samples due to different laboratory environments, caused by the opportunity to use a new laboratory when Maltese samples became available. For Maltese samples, we used 20 μl reaction volume per sample, including 17.5 μl Dream*Taq* PCR Mastermix (2×) (Thermo Scientific, Germany). The mastermix contained DreamTaq DNA Polymerase, 2× Dream*Taq* buffer, 0.4 mM of each dNTP and 4 mM $MgCl_2$, TFR1 and TFR2 (both 20 μM) and ddH_2O. Furthermore, we added 2.5 μl of template DNA.

For polymerase chain reactions (PCR) of the remaining samples, we used 10 μl reaction volume per sample, including 5 μl of 2× MM Mastermix (Qiagen) (with HotStar*Taq* DNA Polymerase, PCR buffer containing 3 mM $MgCl_2$, 400 μM of each dNTP), 2 μl H_2O, 1 μl loading dye (containing 0.3% Orange G and 25% Saccharose) and 1 μl primer mix, containing 20 μM of both, TFR1 and TFR2 and added 1 μl of template DNA.

The reactions of the Maltese samples were conducted on a peqstar 96Q Real-Time PCR cycler (PEQLAB Biotechnologie GmbH, Erlangen, Germany). PCR reactions of all other samples were conducted on a Biometra TPersonal Thermocycler (Biometra, Göttingen, Germany). All PCR reactions were run with a negative control. We applied following cycling conditions for all PCR reactions: polymerase activation at 95 °C for 15 min, followed by 35 cycles with a denaturation at 94 °C for 30 s, annealing at 60 °C for 90 s and extension at 72 °C for 60 s. Final extension was set to 72 °C for 10 min.

We used gel electrophoresis to visualise PCR products, and positive samples were sequenced at either SEQLAB (Sequence Laboratories Göttingen, Germany) (Maltese samples) or the Konrad Lorenz Institute of Ethology (University of Veterinary Medicine Vienna, Austria) (all other samples). In total, two PCR products from Maltese collared doves, 34 PCR products from stock doves, 39 from wood pigeons and 49 from turtle doves were sent for sequencing. We assumed that most of our samples came from unrelated individuals, except for two pairs of siblings from two nests of stock doves that were included to determine whether they were infected by the same *Trichomonas* lineage.

Furthermore, we applied a Chi-square test using R 3.2.4 [35] to check for differences in prevalence between columbid species. Due to the small sample size of collared doves, those were excluded from statistical analyses.

Fe-hydrogenase PCR amplification and capillary electrophoresis

We performed PCR reactions for all samples that were tested positive and used in the phylogenetic analysis for ITS1/5.8S/ITS2 We used the primers TrichhydFOR (5′-GTT TGG GAT GGC CTC AGA AT-3′) and TrichhydREV (5′-AGC CGA AGA TGT TGT CGA AT-3′) [22]. For Fe-hydrogenase gene PCR amplifications the Multiplex PCR Plus Kit (Qiagen) was used. That leads to higher PCR outputs, when samples were stored in PBS because PBS inhibits PCR reactions due to high chloride concentrations [36].

For polymerase chain reactions, we used 15 μl reaction volume per sample, including 7.5 μl of 2× Qiagen Multiplex PCR Mastermix (Qiagen) (with HotStar*Taq* DNA Polymerase, PCR buffer containing 6 mM $MgCl_2$, and ultrapure quality of dNTPs), 2 μl H_2O, 0.75 μl of each primer (10 μM concentration) and added 4 μl of template DNA.

PCR reactions were conducted on a Biometra TONE Thermocycler (Biometra, Göttingen, Germany). All PCR reactions were run with a negative and a positive control. The positive control originated from a British greenfinch in 2007 [37]. We applied following cycling conditions for all PCR reactions: polymerase activation at 95 °C for 5 min, followed by 35 cycles with a denaturation at 95 °C for 30 s, annealing at 57 °C for 90 s and extension at 72 °C for 90 s. Final extension was set to 72 °C for 5 min.

We used capillary electrophoresis (QIAxcel Advanced, Qiagen, Switzerland) to visualise PCR products and positive samples were sequenced at SEQLAB (Sequence Laboratories Göttingen, Germany). In total, six PCR products were sent for sequencing. Five came from turtle doves, and one originated from a collared dove.

Phylogenetic analysis of the ITS1-5.8S-ITS2 region

Forward and reverse sequences were assembled and trimmed with CLC Main Workbench 7.6.1 (CLC bio, Qiagen). With NCBI Blast [38] we checked every sequence for its closest GenBank match and downloaded these as reference sequences (see Additional file 1: Table S1 for an overview about the percentage of identity to the closest GenBank match). Additionally, we downloaded the closest GenBank matches, obtained for columbids by Lennon et al. [12]. This enables the direct comparison of *Trichomonas* lineages occurring in stock doves, wood pigeons and turtle doves from the UK to Germany, Italy and Spain. *Tritrichomonas foetus* (GenBank accession number DQ243911.1 [39]) was used as outgroup for phylogenetic analysis following previous studies [12]. We aligned all sequences using BioEdit [40]. The nucleotide substitution model that best fitted our alignment was determined with MEGA 6.0 [41] using Bayesian Information Criterion scores. The phylogenetic tree was also inferred with MEGA 6.0 [41] using the Maximum Likelihood algorithm and employing the Tamura 3 substitution model with invariant sites. Node support was

assessed after 1000 bootstrap pseudo-replicates. The GenBank accession numbers of analysed sequences are given in Additional file 2: Table S3.

Phylogenetic analysis of the Fe-hydrogenase gene

Forward and reverse sequences were assembled and trimmed with CLC Main Workbench 7.6.1 (CLC bio, QIAGEN). With NCBI Blast [38] we checked every sequence for its closest GenBank match and downloaded these as reference sequences (see Additional file 3: Table S2 for an overview about the percentage of identity to the closest GenBank match). We also downloaded the reference sequences used in Chi et al. 2013 [42] to specify the sub-lineages of *Trichomonas gallinae*. *Trichomonas vaginalis* (GenBank accession number: XM_001310179.1 [43]) was used as the outgroup for phylogenetic analysis following previous studies [22]. We aligned all sequences using BioEdit [40]. The phylogenetic tree was inferred with MEGA 6.0 [41] using the Maximum Likelihood algorithm and employing the Kimura 2 substitution model with invariant sites. Node support was assessed after 1000 bootstrap pseudo-replicates. The GenBank accession numbers of analysed sequences are given in Additional file 2: Table S3.

Results

Prevalence of *Trichomonas* in columbids

We found an overall prevalence of 74% across all 281 columbid samples with a significant difference between species ($\chi^2 = 91.023$, $df = 3$, $P = 0.007$). Within swab samples, there was a total prevalence of 93% ($n = 154$) and within tissue samples a prevalence of 54% ($n = 61$) (Table 1). The majority of wood pigeons ($n = 61$; 70%) and stock doves ($n = 79$; 86%) were infected. From stock doves, 28 adults showed infection (30%), and 51 chicks were infected (55%). The first-year-old bird was not infected. Also, turtle doves ($n = 65$; 93%) showed high prevalence (Table 1). Additionally, turtle doves from different countries displayed high prevalence for every country (Fig. 1), including the one German turtle dove tested positive for *Trichomonas*. Only turtle doves from Malta had > 50% samples testing negative for *Trichomonas* (Fig. 1).

Phylogenetic analysis of *Trichomonas* lineages

From 124 PCR products sequenced for the ITS/5.8S/ITS2 region, 84 were successfully assembled and used for further phylogenetic analysis (Table 1). Unfortunately, not all PCR products were able to assemble, due to their insufficient quality. The same applies to the PCR products sequenced for the Fe-hydrogenase region. Only three samples were sequenced successfully.

The phylogenetic tree for the ITS1/5.8S/ITS2 region contained seven different lineages (Fig. 2, Additional file 4: Figure S1). The lineage names were given according to first discoverers and where new lineages were found, the lineage names were labelled according to the nomenclature of Gerhold et al. [44]. Most samples clustered in lineages II [12] and C/V/N [42, 44], followed by lineage P (Table 2, Additional file 1: Table S1). Wood pigeons were predominantly infected by lineages II [12] and C/V/N [42, 44]. Turtle doves showed infections mainly caused by the P and III [42] lineage. Most of the Italian turtle dove samples belonged to the P and III [42] lineage ($n = 4$ for each lineage). One turtle dove sample from Italy could not be linked to a previously described *Trichomonas* lineage, but the closest GenBank match was KF993705.1 with 92% maximum identity and 98% query coverage (Table 2, Additional file 1: Table S1). Additionally, another stock dove sample could not be linked to a described lineage. Its closest GenBank match was EU881912.1 with 97% maximum identity and 96% query coverage (Table 2, Additional file 1: Table S1). The Spanish samples mainly belonged to the P and C/V/N [42, 44], lineages ($n = 9$ and $n = 7$, respectively). The Maltese samples occurred mainly in lineage P ($n = 3$).

Collared doves and stock doves were the only species infected by lineage A/B [44]. Besides, six out of seven *Trichomonas* lineages detected in this study occurred in stock doves. The four chicks from two stock dove nests were infected by different *Trichomonas* lineages. The first two siblings showed *Trichomonas* from lineage A/B [44]. The other two were infected by two different *Trichomonas* lineages (P and III [42]).

The samples sequenced for the Fe-hydrogenase gene clustered in sub-lineage P1 distinct to the reference sequences and sub-lineages (Additional file 5: Figure S2).

Discussion

Prevalence of *Trichomonas* in columbids

Stock doves, wood pigeons, collared doves and turtle doves in our study throughout a range of European sites showed a high prevalence of infection by *Trichomonas* sp. These results are in agreement with previous findings in the UK [12].

Although we only had a small sample size of collared doves, the prevalence seems high (67%). Additionally, if we separate the results by countries, Spanish collared doves suggest a much lower prevalence by *Trichomonas* sp. (33%) than in the UK (86%, see [12]) and lie closer to the prevalence shown in Iraq (10%, see [45]), but the samples from Malta showed a prevalence closer to the results shown in the UK (Fig. 1). Both compared studies used swab samples [12, 46] as we did in our survey, thus the differences cannot be linked to the different sample material. Furthermore, Al-Bakry [45] used a larger sample size of collared doves ($n = 40$) compared to Lennon et al. [12], who used seven individuals. Thus, the Iraqi results might be more reliable and a low *Trichomonas* prevalence in collared doves, as shown in

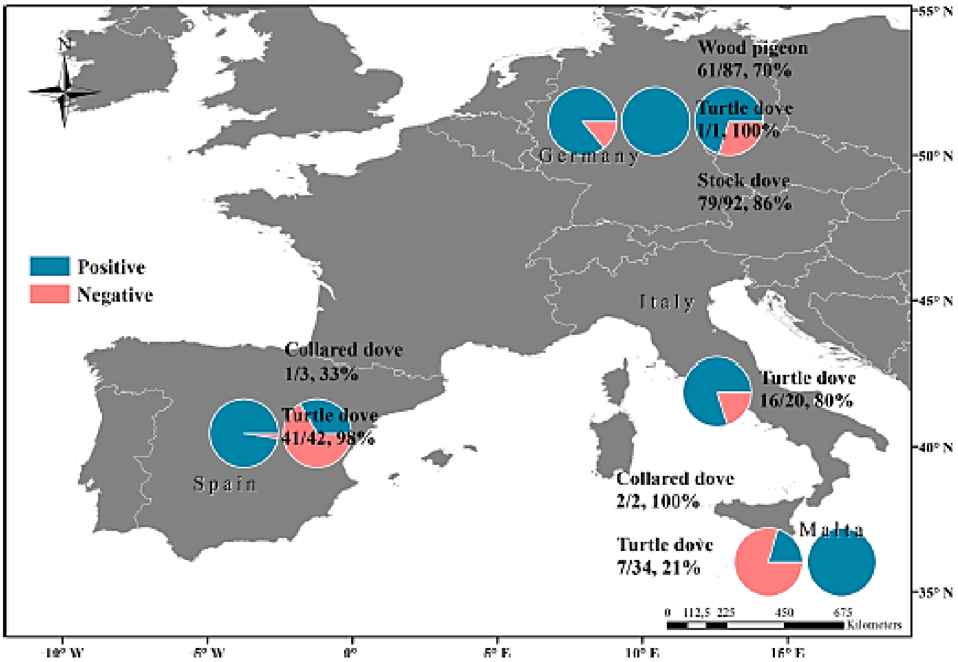

Fig. 1 Map of sample sites and prevalence of *Trichomonas* in columbids from Germany, Italy and Spain. Next to the pie charts, the species name, the number of positive *Trichomonas* samples out of the sample size and percentages of positive *Trichomonas* samples within a species from one country are given. The results for wood pigeons arise from tissue samples. Furthermore, 27 samples of Maltese turtle doves were tissue samples. All of those were tested PCR-negative for *Trichomonas*

Fig. 2 Phylogenetic analysis of the ITS1/5.8S/ITS2 ribosomal region of *Trichomonas* sp. Lineages found within this study together with the identical reference sequences from GenBank analysed using maximum likelihood. Reference sequences are labelled by GenBank accession number and *Trichomonas* species. References to GenBank accession numbers are as follows: JQ030996.1, JQ755275.1 and JQ755278.1 (Peters & Raidal, unpublished; samples from Australia), U86615.1 ([32]; sample from Switzerland), HM579936.1 ([57]; sample from France), KF993705.1 and KF993679.1 (Martínez-Herrero et al. unpublished; samples from Spain), EU881911.1, EU881912.1 and EU881917.1 ([12]; samples from Spain), KC215387.1 ([14]; sample from USA), JN007005.1 ([62]; sample from Austria), KC529665.1 ([42]; sample from UK), DQ243911.1 ([34]; sample from France). Asterisk marks the two single sequences and their sample names, which could not be linked to any of the previously described lineages

Table 2 Summary of *Trichomonas* lineages found in this study compared to sequences described previously

Lineage	No. of infected birds	% infected birds	Species	No. of species	% species	Lineage name by Lennon et al. [12]	Lineage name by Gerhold et al. [44]	Lineage name by Chi et al. [42]
A/B$_{II}^{a}$	6	7.1	SD	4	25.0	3 (TD, WP) and 4 (WP)	A and B	A and B
			CD	2	100			
C/V/N$_{A}^{b}$	23	27.4	SD	4	25.0	1 (TD, WP)	C, D and E	C and V
			WP	10	43.5			
			TD	9	20.9			
O	1	1.2	SD	1	6.3	Not found	Not found	Not found
II	22	26.2	SD	3	18.8	2 (SD, TD, WP)	Not found	II
			WP	12	52.2			
			TD	7	16.3			
P	19	22.6	SD	2	12.5	Not found	Not found	Not found
			WP	1	4.4			
			TD	16	37.2			
Q	1	1.2	TD	1	2.3	Not found	Not found	Not found
III	12	14.3	SD	2	12.5	Not found	Not found	III
			TD	10	23.3			

Notes: For lineages obtained in our study and for the similar study by [12] we displayed the wild columbid host species as well as numbers and percentages of infected individuals. Furthermore, we give the numbers and percentages of lineages found in a species. Note, the percentages in a species (% species) were calculated according to the number of species infected by a certain lineage and divided through the absolute number of infected individuals of a species

Abbreviations: *SD* stock dove, *WP* wood pigeon, *TD* turtle dove, *CD* collared dove

[a] The lineage is synonymous with genotype B described by Sansano-Maestre et al. [14] and is therefore potentially pathogenic

[b] The lineage is synonymous with genotype A described by Sansano-Maestre et al. [14] and is therefore apparently non-pathogenic and widespread

the present study, may be more realistic. However, the small sample size in our study and Lennon et al. [12] should be interpreted carefully.

In our study, stock doves had a much higher prevalence than previously suggested (22–40% [12, 45]). Furthermore, chicks had higher prevalence of *Trichomonas* than adults, which is in agreement with Bunbury [46], who showed the negative impact of trichomonosis in Mauritian Pink pigeon chicks until an age of three months. In the present study, stock dove chicks were between five and 22 days old and therefore, within the most common time for infection with *Trichomonas* parasites in pigeon chicks [46].

In turtle doves, a high infection status of 67% was detected, which is 1/3 lower than previously observed in the UK (95%) [R. C. Thomas, unpublished data]. However, if we disregard the prevalence results of defrosted tissue samples from Maltese turtle doves, we would have a prevalence of 93% in turtle doves from Europe (Table 1), thus very similar to results from the UK.

On the other hand, our results revealed much higher prevalence for wood pigeons than previously suggested (47%, see [12]). However, it needs to be taken into account that the 70% prevalence of *Trichomonas* infection in German wood pigeons and the very low prevalence in turtle doves from Malta likely underestimates the true prevalence, because samples were not cultured directly, but analysed from tissues after freezing and defrosting. This might cause major differences in the detection rate, because of DNA degradation due to several freezing and thawing cycles, especially for Maltese samples during transportation [47–50]. Furthermore, Dunn et al. [51] showed the need to culture *Trichomonas* samples to reliably detect the infection. However, our results suggest high prevalence, at least for wood pigeons, but note that Maltese tissue samples were treated with a different *Taq* DNA Polymerase than wood pigeon samples. Thus, it might be the Dream*Taq* DNA Polymerase was less sensitive to DNA in thawed tissue samples.

Compared to other columbid species like mourning doves (*Zenaida macroura*), the overall prevalence of *Trichomonas* protozoan in the species studied here lies at the top of prevalence ranges. Mourning doves showed a very low prevalence of only 5.6% ranging from 4.4 to 10.6% [52]. In the endangered Mauritian pink pigeon the average prevalence of *Trichomonas* was 50% (ranging from 20 to 82%) [37], and therefore lower than in turtle doves, which are listed as a vulnerable species [28]. However, such prevalence has already been highlighted as a major threat to pink pigeons' population recovery [37]. This might be caused by their small distribution range and population size, but also reveals the importance of gaining knowledge about parasite infection, particularly in recovering and declining birds. In

the UK, trichomonosis has been indicated as a potential additive factor for the reported population decline of turtle doves since the 1970s [12]. Furthermore, Calderon et al. [53] already highlighted the decreasing effective population size of turtle doves, which makes them more vulnerable to threats. Thus, the present study suggests a potentially strong impact of *Trichomonas* on declining turtle doves across western and central Europe and likewise on recovering German stock dove populations.

Turtle doves are likely to be more dependent on anthropogenic food sources at feeding sites than in the 1960s, which was described in detail in the UK [54]. However, this circumstance might not only be limited to Great Britain, since it is linked to increased use of herbicides resulting from agricultural intensification, which has occurred throughout Europe [55, 56]. Furthermore, in the last century a change and intensification in forest management occurred as well [56], which led to a decreased number of natural breeding holes. That is why nowadays stock doves are largely dependent on artificial nest boxes in Germany [19, 56]. In Hesse, the stock dove population recovered locally, through provision of artificial nest boxes. However, besides increased food stress or insufficient natural breeding sites [29], turtle doves, stock doves, but also wood pigeons are migratory birds [19], which is why they may be more exposed to a wider range of parasites and pathogens of different bird species or populations (stop-overs at feeding and water sites) [15, 19, 21, 55]. Moreover, despite the existence of three main migratory flyways [18], European turtle doves show a lack of genetic structure [53]. That increases the probabilities for higher vulnerability and potential fast spread of infection among turtle dove populations.

Resident species, in contrast, may be less prone to infection, as shown for collared doves in Iraq [41] and Spain (this study, but see [12]). On the other hand, collared doves in Malta indicated a 100% *Trichomonas* prevalence.

As the disease occurs worldwide and is rapidly spreading, i.e. among wild finches [4], it seems likely that especially turtle doves and stock doves are also infected in other European countries. Regarding turtle doves, information on the disease in eastern Europe would be especially interesting, since, to our knowledge, no data on prevalence are available yet from the eastern European distribution range. Furthermore, the transmission may be reduced where no supplementary food is provided for endangered or vulnerable species and gamebirds [12].

Phylogenetic relationships among *Trichomonas* lineages

Several attempts have been made to classify the genetic diversity of *Trichomonas* parasites in birds, with ensuing different nomenclatures (Table 2). Out of a total of seven genetic lineages found in the present study, three lineages were found in all columbid species we examined: lineages II [12], P and C/V/N [42, 44] (Fig. 2, Additional file 4: Figure S1). Of those, lineages II [12] and P belong to different *Trichomonas* species (*T. tenax* and *Trichomonas* sp., which grouped within *T. canistomae* [55]). However, species identification, based on morphological methods, may need to be revised and complemented as more genetic data are available. Since several *Trichomonas* species have been reported in birds based solely on morphology [12, 33, 43, 51,], the nomenclature for *Trichomonas* might also need revision as more molecular-based phylogenetic analyses are available. However, judging by the overall occurrence of these three lineages, our findings suggest a widespread distribution of those lineages across columbids [12, 33]. For instance, lineages II [12] and C/V/N [42, 44] were also predominant in species from the UK, Austria and the USA [12, 55]. Lineage II [12] even infected the same host species as shown in the UK [19] (Table 2).

Lineage C/V/N [42, 44] has also been described as "genotype A", an apparently non-pathogenic *Trichomonas* lineage [19] with a global and frequent occurrence. It was previously found in turtle doves and wood pigeons [19], and we here additionally demonstrate infection in stock doves.

Regarding existing literature and previously described lineages, lineages O, P and Q might be newly detected lineages, because they were not described in previous studies [12, 22, 33, 42, 44, 57]. The samples from lineage P sequenced for the Fe-hydrogenase gene also clustered in a distinct and apparently new group of Fe-hydrogenase sub-lineage P1 (S4). Furthermore, lineages O and Q appear distinct to lineages A/B and C/V/N [42, 44], thus they may not be as common or widespread as lineages II, P and C/V/N [12, 42, 44], because they were only found in one German stock dove sample (O) and one Italian turtle dove sample (Q). Additionally, lineage III [42], found in stock doves and turtle doves, was identical to assigned reference sequences isolated from feral pigeons and was only found in Austria and the UK [42, 58]. Thus, we confirm its presence in turtle doves from Malta, Italy and Spain and in stock doves from Germany.

Only, lineage A/B [44] grouped with a potentially fatal lineage (genotype B) [12, 16, 19, 22], which was also responsible for the finch trichomonosis epizootic in the UK [22] (Table 2). This lineage, which is often lethal, has also been detected in turtle doves, wood pigeons and other non-passerines [12, 16, 22, 27] and we here confirm its presence in stock doves from Germany and resident collared doves from Malta. A fatal case of trichomonosis in a stock dove from Germany has been described previously [16] based on necrotic lesions and *Trichomonas* presence in

microscopic analyses [16]. The lethal course of the disease hints to an infection by lineage A/B [44], although it was not genetically confirmed.

The lethal character of trichomonosis was described also in the Mauritian pink pigeon, a resident species on Mauritius similar to the collared doves on Comino (Malta) [52]. Thus, at least on Comino, the collared dove population might decrease in the future due to this potentially pathogenic lineage. Some years ago, the collared dove population already crashed due to an outbreak of a disease, which affected the region around the beak (B. Metzger, personal communication). No further description of the illness is known, but it is possible that it was trichomonosis.

Two genotypes of *T. gallinae* have previously been proposed to exhibit a differential in pathogenicity. Lineage C/V/N [42, 44] is synonymous with genotype A, described by Sansano-Maestre [19] as a wide-spread *Trichomonas* lineage, with mild or no pathogenicity. Lineage A/B [44] is synonymous with genotype B [19] described as possessing a more severe pathogenicity. Regarding all other lineages, pathogenicity has not been assessed. For this purpose, transmission experiments would be very helpful to see, if birds show signs of active trichomonosis and if they recover from infection and acquire possible immunity [25, 26].

Phylogenetic analysis of *Trichomonas* lineages from stock dove siblings

To our knowledge, this is the first report of phylogenetic examination of *Trichomonas* from stock dove nestlings belonging to the same nest. Both findings (siblings), were infected by the same lineage as well as by different lineages; this can be explained by disease transmission *via* crop milk feeding, as both parents share chick feeding [19]. Thus, if both parents are infected by the same *Trichomonas* lineage, chicks receive the same *Trichomonas* pathogen. If both parents are infected by *Trichomonas* but carry different lineages, their chicks might be infected by different pathogens as well. Additionally, studies have shown [14, 59, 60], that individual birds may carry more than one *Trichomonas* lineage, so it is possible we only sequenced one strain when more than one was present. Here, we found a *T. gallinae* and *T. canistomae*-like lineage in two siblings of a stock dove nest. A coinfection of an apparently non-pathogenic *T. gallinae* (genotype A) [14] lineage plus a *T. tenax*-like strain was found previously in pigeons [60].

Conclusions

Our results are in agreement with previous findings of geographically widespread *Trichomonas* lineages. We provide information about diverse lineages from Germany, Spain and Italy across different columbid species. Especially, lineages 2 [12], P and C/V/N [42, 44] were identified in all species and might, therefore, represent the most common *Trichomonas* lineages, at least in Columbiformes. However, only stock doves and collared doves from this study showed infections by the potentially pathogenic and often lethal *Trichomonas* lineages. Furthermore, we detected three newly discovered lineages (O, P and Q) and one additional sub-lineage based on the Fe-hydrogenase gene P1. Additionally, only one German stock dove sample was assigned to lineage O, and one Italian turtle dove sample was assigned to lineage Q. Due to the higher dependency on anthropogenic food sources [19, 54] and artificial nesting sites [19, 29] of turtle doves and stock doves as well as the migratory character of wood pigeons, turtle doves and stock doves these three species might have a higher risk to ingest *Trichomonas* protozoans of different lineages from various bird species and populations at shared feeding and water sites. This might be especially dangerous for recovering stock dove populations and declining turtle dove populations when parasites spread during times of food shortage or stress. Furthermore, it may be particularly worrying when stock doves or turtle doves get infected by potentially pathogenic lineages as we have shown here for stock doves from Germany and has already been shown for turtle doves in the UK [12]. The occurrence of potentially pathogenic lineages of *Trichomonas* in resident collared doves on Comino (Malta) might raise the concern for turtle doves even more since they share watering and feeding sites on the island. However, to estimate an impact on the population level, it would be very important to gain further knowledge about the prevalence of *Trichomonas* protozoans and the occurring lineages in turtle doves from Eastern European countries and to compare these data to turtle doves from Western Europe.

Additional files

Additional file 1: Table S1. Sample names from different columbid hosts with their closest GenBank match for the ITS1-5.8S-ITS2 region, maximum identity and query coverage in % as well as the *Trichomonas* species of the GenBank match, the lineage, host and country in which the reference was found. (DOCX 25 kb)

Additional file 2: Table S3. The GenBank accession numbers (KX459439–KY675299) of the study sequences are listed in the table below. (DOCX 14 kb)

Additional file 3: Table S2. Sample names from different columbid hosts with their closest GenBank match for the Fe-hydrogenase region, maximum identity and query coverage in % as well as the parasite species of the reference. (DOCX 16 kb)

Additional file 4: Figure S1. Expanded phylogenetic tree including information about *Trichomonas* species and origin countries of reference sequences. Furthermore, the host species and sample ID of studied sequences are shown. (PDF 7856 kb)

Additional file 5: Figure S2. Phylogenetic tree based on the analysis of the Fe-hydrogenase gene of *Trichomonas gallinae*. This figure includes information about *Trichomonas* sub-lineages (A1, A1.1-A1.3, A2, C1-C4 and the newly detected sub-lineage P1). Furthermore, information about the origin countries of reference sequences is given, when information was available. Additionally, the host species and sample ID of studied sequences are shown. The break in the direction to sub-lineage P1 equals two substitutions. References to GenBank accession numbers are as follows: AF446077.1 [61], HG008115.1 [8], KC529660.1, KC529661.1, KC529662.1, KC529663.1, KC529664.1, KC962158.1 [42], JF681136.1 and JF681141.1 [22] and XM_001310179.1 [43]. (TIF 7369 kb)

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Availability of data and material
The studied sequences were submitted to the GenBank database under accession numbers KX459439–KY675299.

Authors' contributions
Conceived and designed the study: MM and PQ. Collected the data: MM, GrR, KH, JFM, SK, SLM, BM, JGC and FS. Analysed the data: MM, GeR, HW, SM, JCD, RCT, SJG, KCH, TR and LC. Drafted the manuscript: MM and PQ. Critically revised the manuscript: GeR, HW, GrR, JFM, SLM, JCD, RCT, SJG, KCH, BM, JGC, FS and LC. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Consent for publication
Not applicable.

Ethics approval and consent to participate
In this study, samples were taken from hunted and living columbids. None of the hunted birds was shot exclusively for the present study. Turtle doves in Spain were sampled during the legal annual late summer hunting weekends, at a hunting estate visited after a hunting event. Wood pigeons in Germany were part of independently approved research projects at the Department of Clinical Veterinary Sciences. Swab samples from living wild birds were taken in the field after they were caught in mistnets or whoosh nets during approved bird ringing and migration studies. For those samples, all the methods used were approved by the Animal Welfare Office Justus-Liebig-University Giessen.

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CHAPTER 4

SPECIES DISTRIBUTION MODELS OF EUROPEAN TURTLE DOVES IN GERMANY ARE MORE RELIABLE WITH PRESENCE ONLY RATHER THAN PRESENCE ABSENCE DATA

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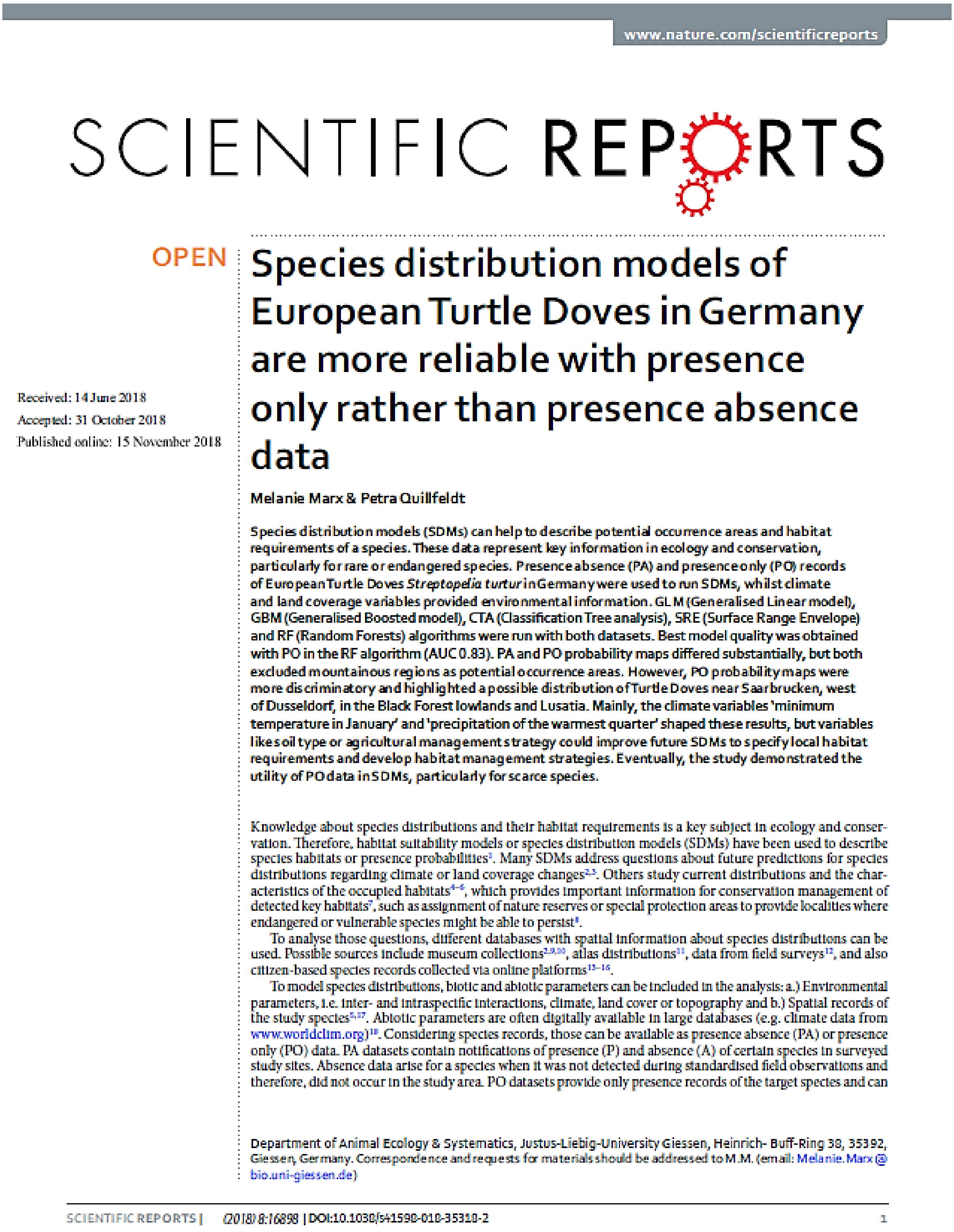

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Species distribution models of European Turtle Doves in Germany are more reliable with presence only rather than presence absence data

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Species distribution models (SDMs) can help to describe potential occurrence areas and habitat requirements of a species. These data represent key information in ecology and conservation, particularly for rare or endangered species. Presence absence (PA) and presence only (PO) records of European Turtle Doves *Streptopelia turtur* in Germany were used to run SDMs, whilst climate and land coverage variables provided environmental information. GLM (Generalised Linear model), GBM (Generalised Boosted model), CTA (Classification Tree analysis), SRE (Surface Range Envelope) and RF (Random Forests) algorithms were run with both datasets. Best model quality was obtained with PO in the RF algorithm (AUC 0.83). PA and PO probability maps differed substantially, but both excluded mountainous regions as potential occurrence areas. However, PO probability maps were more discriminatory and highlighted a possible distribution of Turtle Doves near Saarbrucken, west of Dusseldorf, in the Black Forest lowlands and Lusatia. Mainly, the climate variables 'minimum temperature in January' and 'precipitation of the warmest quarter' shaped these results, but variables like soil type or agricultural management strategy could improve future SDMs to specify local habitat requirements and develop habitat management strategies. Eventually, the study demonstrated the utility of PO data in SDMs, particularly for scarce species.

Knowledge about species distributions and their habitat requirements is a key subject in ecology and conservation. Therefore, habitat suitability models or species distribution models (SDMs) have been used to describe species habitats or presence probabilities[1]. Many SDMs address questions about future predictions for species distributions regarding climate or land coverage changes[2,3]. Others study current distributions and the characteristics of the occupied habitats[4–6], which provides important information for conservation management of detected key habitats[7], such as assignment of nature reserves or special protection areas to provide localities where endangered or vulnerable species might be able to persist[8].

To analyse those questions, different databases with spatial information about species distributions can be used. Possible sources include museum collections[2,9,10], atlas distributions[11], data from field surveys[12], and also citizen-based species records collected via online platforms[13–16].

To model species distributions, biotic and abiotic parameters can be included in the analysis: a.) Environmental parameters, i.e. inter- and intraspecific interactions, climate, land cover or topography and b.) Spatial records of the study species[5,17]. Abiotic parameters are often digitally available in large databases (e.g. climate data from www.worldclim.org)[18]. Considering species records, those can be available as presence absence (PA) or presence only (PO) data. PA datasets contain notifications of presence (P) and absence (A) of certain species in surveyed study sites. Absence data arise for a species when it was not detected during standardised field observations and therefore, did not occur in the study area. PO datasets provide only presence records of the target species and can

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be used to model the distribution of a species, but one needs to consider biases caused by observers (detection bias, recording or reporting bias, geographic bias), due to non-standardised sampling methods[16,19].

Different modelling algorithms can be used in SDMs. Generalised Linear Models (GLM) or Generalised Additive Models (GAM) have been applied frequently to PA data to generate suitability models[20–22]. Other commonly applied model algorithms for PA datasets were e.g. Classification and Regression Tree analysis (CART[23–25]), Artificial Neural Networks (ANN[23]) and Multivariate Adaptive Regression Splines (MARS[23,26]). For PO data Ecological Niche Factor Analysis (ENFA[27,28]) and Maximum Entropy Method (MAXENT[29]) have been widely used, because these algorithms do not require absence data.

Generally, it is recommended to use PA data[1], if available. However, some studies have shown that modelling approaches applied to PO datasets with ENFA or MAXENT provide equal or slightly better SDM performance than models conducted with PA data[5,12,27,30]. Also, the creation of simulated pseudo-absence points in PO datasets is possible and allows application of modelling algorithms usually used on PA data. These pseudo-absences may improve the model quality of the applied algorithm, because they can include background information about non-occupied environments[31,32]. Nevertheless, their application should be carefully assessed, because they might be biased – e.g. when a species is widespread or presence data is rare[31]. Thus, false pseudo-absences may be modelled into a possibly suitable area without species occurrence records, due to the scarcity of the species. However, in PA datasets false absence can also be generated, if a species was not detected during field work and therefore, was noted as absent although it inhabited the survey area[28].

Due to the strong decline of more than 78% from the 1980s until present, the European Turtle Dove (*Streptopelia turtur*, hereafter referred to as Turtle Dove) is listed as a vulnerable species[33,34]. A simulation study from the UK, found an annual population decline of 17.5% due to a decreased number of fledglings[35]. The low breeding success in the UK has been linked to agricultural intensification and an intensified use of herbicides, which led to habitat loss and changes in food availability and quality on breeding grounds, not only in the UK but also in other European countries such as Germany[36–39]. To halt these severe population declines, it is important to discover key breeding habitats for Turtle Doves and to develop management plans for those areas.

In our study, two different datasets with Turtle Dove records from Germany were available – PA data from the 'Monitoring of breeding birds' scheme collected by the DDA (Dachverband Deutscher Avifaunisten e.V.) and citizen-based PO data from the online platform www.ornitho.de[40] (ornitho-data), which is used to record bird sightings. On the website observers can input their bird data as sighting records, but there is no regulation to report every sighted species or to report species using a standardised field protocol[16]. Although observers can add checklists (standardised method) with records about observation time and date as well as presence and absence of certain species (see www.ornitho.de)[40], it seems that these checklists are not commonly used (own observation, judging by the indications of the daily summaries in different regions), but instead non-standardised bird sightings are submitted. Also, there likely is a reporting bias caused by observers, with differing species identification skills, or easily detected birds are reported more frequently[13].

In central Germany, one habitat suitability study was performed in the Wetterau, a small region of Hesse, where Turtle Doves were once known as common breeding birds. In 2012, the study again monitored all habitats known to be occupied in 1998/1999 and recorded presences and absences of Turtle Doves. Results indicated a decrease of breeding pairs by 50% in 2012 compared to 1998/99[6]. The 'Monitoring of breeding birds' scheme confirmed this decline in Germany, highlighting a loss of almost 33% of breeding pairs compared to the mid 1990s[41].

Besides the recorded decline of breeding pairs, a strong positive effect of woodland and grassland was found when the effects of different environmental parameters were examined[6]. The study distinguished positive parameters for feeding and breeding habitat and revealed that dense deciduous forests and middle aged mixed forests were the most important parameters for the breeding areas. Regarding the feeding locations, the most positive parameters were grassland and forest glades.

However, it has been shown in other studies that the results of habitat models or evaluation of habitat preferences may come to other conclusions when processed on different scales[42,43]. For instance, in Great Britain (Ixworth Thorpe and Deeping St. Nicholas) habitat requirements for radio-tagged Turtle Doves were evaluated at two different scales[42]. A small scale assessment was conducted based on recorded positions of radio-tagged Turtle Doves, which were used to define home ranges. A larger scale assessment was based on a 50 m buffer around recorded locations[42]. Results showed a positive effect of pasture and a weaker effect of woodland for breeding grounds on the small scale, but was reversed when evaluated for the larger scale, with woodland as important factor and pasture with weaker effect on Turtle Dove occurrence[42]. However, the scale used depends on the aim of a study[43]. In the present study, German-wide key breeding sites and their environmental characteristics will be evaluated, which is why SDM will be conducted on a large country scale.

In our study, we aim to:

(1) Compare the results of different model algorithms using first, a PA dataset and second, a PO dataset with introduced pseudo-absences
(2) Compare the results of the present study to previous ones for the Wetterau in Hesse[6] and other study sites in Europe[42].

Results

PO and PA data. The model algorithms were run with two different species datasets. Both datasets were distributed across Germany, but only the PA dataset consisted of fixed study sites, which should be checked annually by volunteers. The presence points of PA and PO data showed overlaps in some regions, e.g. Potsdam, Wiesbaden and Mainz (Fig. 1). Nonetheless, there were more Turtle Dove presence points registered in the PO dataset (1168 presence points) than in the PA dataset (293 presence points). Furthermore, PO data were also

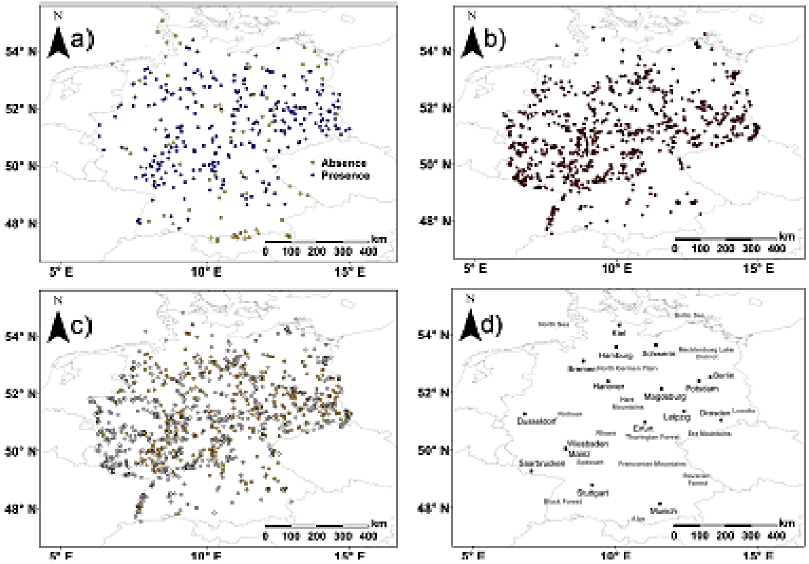


Figure 1. Overview maps. They show the distribution of species records of Turtle Doves for (**a**) PA (presence in blue and absence in yellow) and (**b**) PO (presence records are shown in dark red) data in Germany. (**c**) shows the positions of presence points recorded in PA and PO datasets. Presence points from PO data are given as light grey circles and the ones from PA data as yellow circles. Furthermore, (**d**) a map with Germany's larger cities and landscapes for orientation in space is drawn according to https://www.diercke.de/content/deutschland-physische-karte-978-3-14-100800-5-19-2-190.

Model	AUC PA data	Sensitivity PA data	Specificity PA data	AUC PO data	Sensitivity PO data	Specificity PO data
CTA	0.63	95.64	31.52	0.69	76.03	58.34
GBM	0.75	81.67	61.70	0.77	73.38	67.65
GLM	0.73	75.90	64.70	0.71	71.60	62.10
RF	0.76	82.35	61.96	0.83	72.34	78.07
SRE	0.60	54.65	65.91	0.56	62.97	47.54

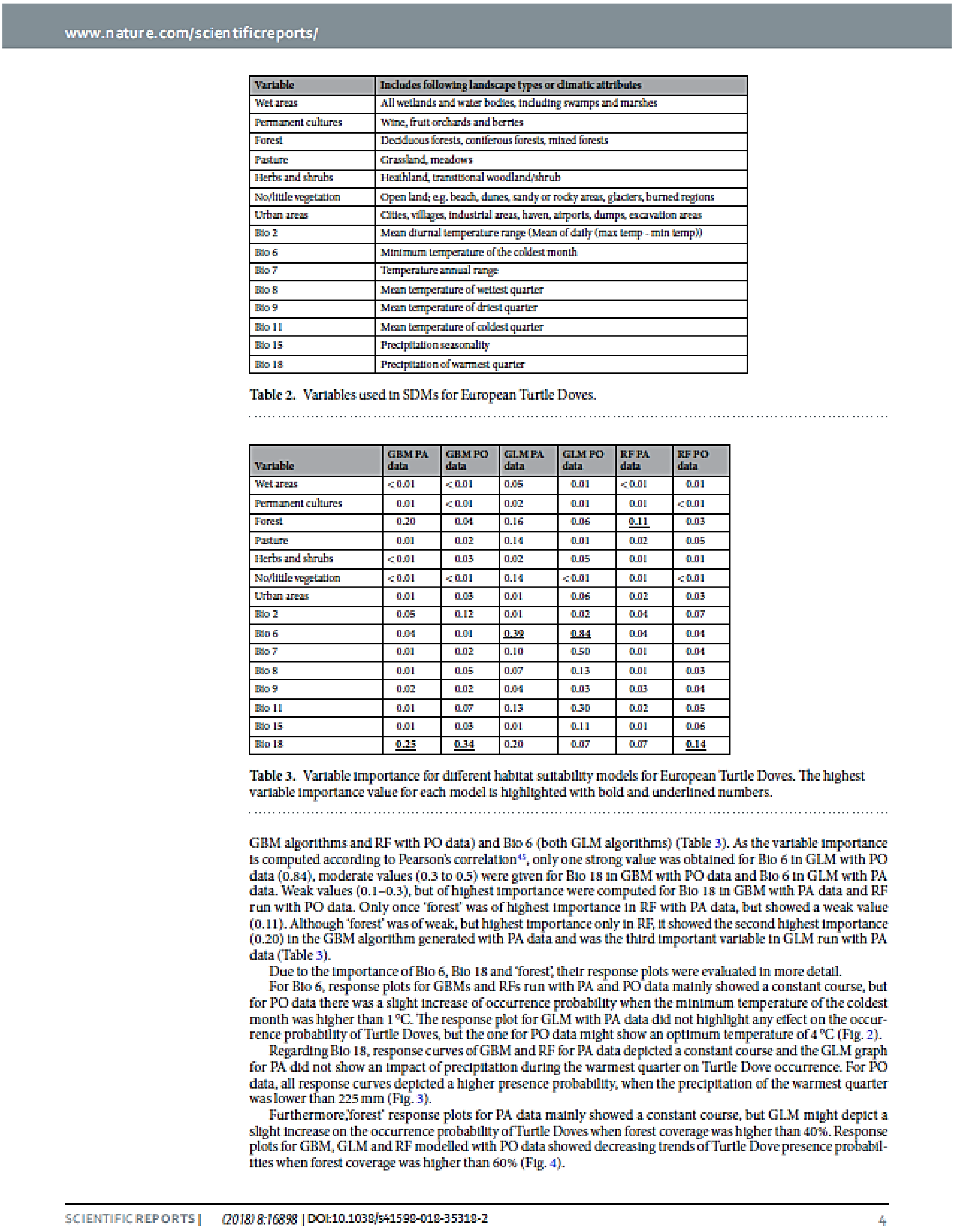
Table 1. Calculated AUC, sensitivity and specificity values of different SDM algorithms for European Turtle Doves.

registered in regions like Lusatia at the border to Poland and in western Germany at the border to France in the Black Forest or close to Saarbrucken. In these regions, the PA dataset had only a few or no registered Turtle dove presence points (Fig. 1).

Model performance. Model performances for PA and PO data, judging by AUC value (Area under Receiver Operating Characteristic (ROC) curve[44]), sensitivity (percentage of presences correctly predicted) and specificity (percentage of absences or pseudo-absences correctly predicted), were similar within same model algorithms. Classification of model quality includes evaluation of all three values. AUC values between 0.5–0.6 describe models that failed, 0.6–0.7 represent poor model quality, 0.7–0.8 are models with fair performance and 0.8–0.9 describe a good model quality[44,45]. Here, models were of higher quality when sensitivity and specificity were similar. Overall, RF with PO data was of best quality (Table 1). The hierarchical model ranking from best to worse was identical between the PA and PO datasets and best quality was achieved with RF, followed by GBM, GLM, CTA and SRE (Table 1). Models showed better sensitivity with PA data, except SRE, but better specificity with PO data, except for SRE and GLM.

For further description and evaluation, we only considered those model results with an AUC ≥ 0.7. Thus, we focus on RF, GBM and GLM in this study.

Importance and influence of variables. Variable importance was evaluated for land cover and climate variables (variable names and attributes included are given in Table 2). The assessment of variable importance revealed climatic variables to be most important in the different model algorithms, particularly Bio 18 (both



Variable	Includes following landscape types or climatic attributes
Wet areas	All wetlands and water bodies, including swamps and marshes
Permanent cultures	Wine, fruit orchards and berries
Forest	Deciduous forests, coniferous forests, mixed forests
Pasture	Grassland, meadows
Herbs and shrubs	Heathland, transitional woodland/shrub
No/little vegetation	Open land; e.g. beach, dunes, sandy or rocky areas, glaciers, burned regions
Urban areas	Cities, villages, industrial areas, haven, airports, dumps, excavation areas
Bio 2	Mean diurnal temperature range (Mean of daily (max temp - min temp))
Bio 6	Minimum temperature of the coldest month
Bio 7	Temperature annual range
Bio 8	Mean temperature of wettest quarter
Bio 9	Mean temperature of driest quarter
Bio 11	Mean temperature of coldest quarter
Bio 15	Precipitation seasonality
Bio 18	Precipitation of warmest quarter

Table 2. Variables used in SDMs for European Turtle Doves.

Variable	GBM PA data	GBM PO data	GLM PA data	GLM PO data	RF PA data	RF PO data
Wet areas	< 0.01	< 0.01	0.05	0.01	< 0.01	0.01
Permanent cultures	0.01	< 0.01	0.02	0.01	0.01	< 0.01
Forest	0.20	0.04	0.16	0.06	**<u>0.11</u>**	0.03
Pasture	0.01	0.02	0.14	0.01	0.02	0.05
Herbs and shrubs	< 0.01	0.03	0.02	0.05	0.01	0.01
No/little vegetation	< 0.01	< 0.01	0.14	< 0.01	0.01	< 0.01
Urban areas	0.01	0.03	0.01	0.06	0.02	0.03
Bio 2	0.05	0.12	0.01	0.02	0.04	0.07
Bio 6	0.04	0.01	**<u>0.39</u>**	**<u>0.84</u>**	0.04	0.04
Bio 7	0.01	0.02	0.10	0.50	0.01	0.04
Bio 8	0.01	0.05	0.07	0.13	0.01	0.03
Bio 9	0.02	0.02	0.04	0.03	0.03	0.04
Bio 11	0.01	0.07	0.13	0.30	0.02	0.05
Bio 15	0.01	0.03	0.01	0.11	0.01	0.06
Bio 18	**<u>0.25</u>**	**<u>0.34</u>**	0.20	0.07	0.07	**<u>0.14</u>**

Table 3. Variable importance for different habitat suitability models for European Turtle Doves. The highest variable importance value for each model is highlighted with bold and underlined numbers.

GBM algorithms and RF with PO data) and Bio 6 (both GLM algorithms) (Table 3). As the variable importance is computed according to Pearson's correlation[45], only one strong value was obtained for Bio 6 in GLM with PO data (0.84), moderate values (0.3 to 0.5) were given for Bio 18 in GBM with PO data and Bio 6 in GLM with PA data. Weak values (0.1–0.3), but of highest importance were computed for Bio 18 in GBM with PA data and RF run with PO data. Only once 'forest' was of highest importance in RF with PA data, but showed a weak value (0.11). Although 'forest' was of weak, but highest importance only in RF, it showed the second highest importance (0.20) in the GBM algorithm generated with PA data and was the third important variable in GLM run with PA data (Table 3).

Due to the importance of Bio 6, Bio 18 and 'forest', their response plots were evaluated in more detail.

For Bio 6, response plots for GBMs and RFs run with PA and PO data mainly showed a constant course, but for PO data there was a slight increase of occurrence probability when the minimum temperature of the coldest month was higher than 1 °C. The response plot for GLM with PA data did not highlight any effect on the occurrence probability of Turtle Doves, but the one for PO data might show an optimum temperature of 4 °C (Fig. 2).

Regarding Bio 18, response curves of GBM and RF for PA data depicted a constant course and the GLM graph for PA did not show an impact of precipitation during the warmest quarter on Turtle Dove occurrence. For PO data, all response curves depicted a higher presence probability, when the precipitation of the warmest quarter was lower than 225 mm (Fig. 3).

Furthermore,'forest' response plots for PA data mainly showed a constant course, but GLM might depict a slight increase on the occurrence probability of Turtle Doves when forest coverage was higher than 40%. Response plots for GBM, GLM and RF modelled with PO data showed decreasing trends of Turtle Dove presence probabilities when forest coverage was higher than 60% (Fig. 4).

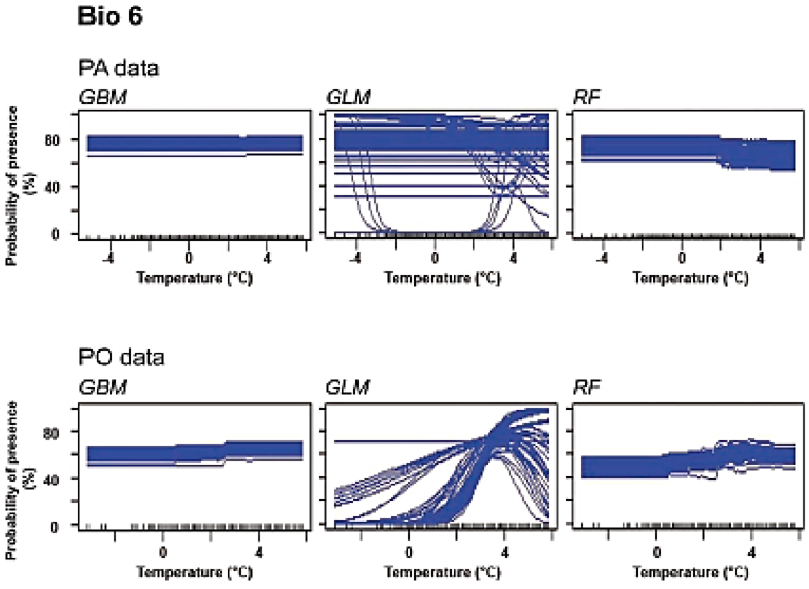


Figure 2. Response curves of variable Bio 6 (Minimum temperature of coldest month). The graphs were created for three species distribution models of Turtle Doves run with PA and PO data.

Probability maps. Probability maps for all models (Figs 5 and 6) highlighted areas with zero or little occurrence probability (<0.5) in the mountainous regions of Germany, but only maps generated for algorithms run with PO data also excluded coastal regions (Fig. 6). Additionally, maps for PO data indicated fewer regions with occurrence probabilities >0.5 than the ones created with PA data. Furthermore, key breeding areas with probabilities >0.8 were much smaller in maps with PO than with PA data (Figs 5 and 6). Generally, maps created with PA data indicated almost all of Germany, except for the south, as areas with high Turtle Dove occurrence probability (Fig. 5). PO based model probability maps mainly highlighted regions with high occurrence probabilities near Saarbrucken, west of Dusseldorf, in the lowlands of the Black Forest and in Lusatia (Fig. 6).

Discussion

In this study, we were able to use two different Turtle Dove datasets (PA and PO) from Germany and run five different SDM algorithms to identify important climatic and land cover variables affecting species' occurrence and we highlighted areas with high species presence probabilities. Evaluation of model performances unveiled an inadequacy of CTA and SRE algorithms for both species datasets (PA and PO). Generally, GBM and GLM performances for both species datasets were similar, but RF ran best with PO data.

Evaluation of variable importance and the corresponding response plots revealed a dependency of Turtle Dove presence on climatic variables. However, it was also obvious that, depending on the species dataset used, the importance of variables changed across model algorithms. Importance values of variables obtained from algorithms run with PO were usually higher than for the same variables obtained by the same algorithms run with PA. Furthermore, response plots for PO data showed clearer effects of variables on Turtle Dove occurrence probability than graphs created with PA data, because those mainly depicted constant response curves. This probably relates to higher numbers of species records in the PO than in the PA dataset, which might represent more evaluable data for modelling algorithms[45].

Bio 6 codes the minimum temperature of the coldest month, which is January in Germany[46]. Regions with modelled favoured temperatures of 1–4 °C in January cover most of Germany except for the regions west of Bremen and north of Dusseldorf, alpine regions, as well as the mountainous areas Harz Mountains and Thuringian forest[46]. Those temperatures support the survival of food sources (weeds, seeds and cereals)[47–49] and hedges as nesting sites, because temperatures lower than 0 °C (and higher than 40 °C) can lead to plant damage due to inhibited physiological processes[50]. Furthermore, temperatures between 0 °C and 10 °C are positively affecting hardening and frost resistance due to cold-acclimation and vernalisation[51–53]. Additionally, the highlighted temperatures were shown to kick-start the development of phenologically early stages of wheat, rape and other crop species[54,55], which may lead to sufficient food availability during the early breeding season.

Bio 18 might be an important factor, because it covers the warmest months July to September[56]. July is one of the major important breeding months of Turtle Doves[57] with high energetic costs for the birds as both adults

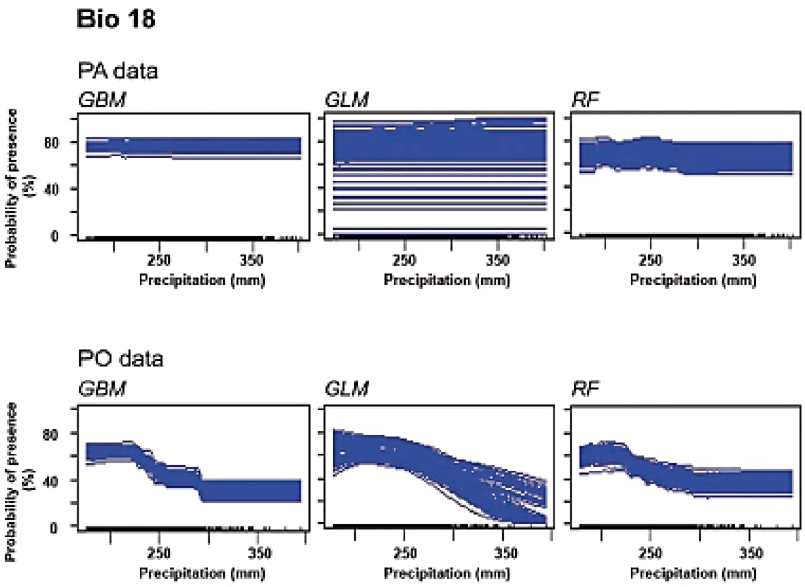


Figure 3. Response curves of variable Bio 18 (Precipitation of warmest quarter). The graphs were created for three species distribution models of Turtle Doves run with PA and PO data.

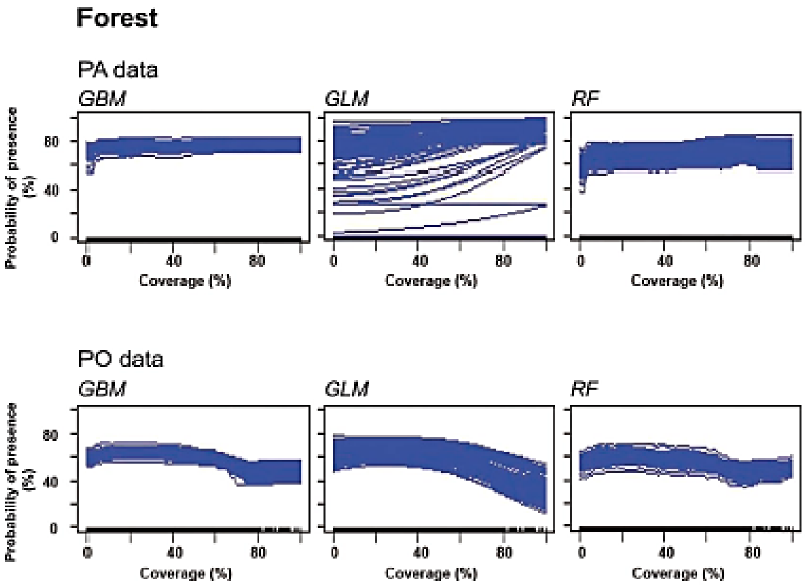


Figure 4. Response curves of the land coverage variable forest. The graphs were created for three species distribution models of Turtle Doves run with PA and PO data.

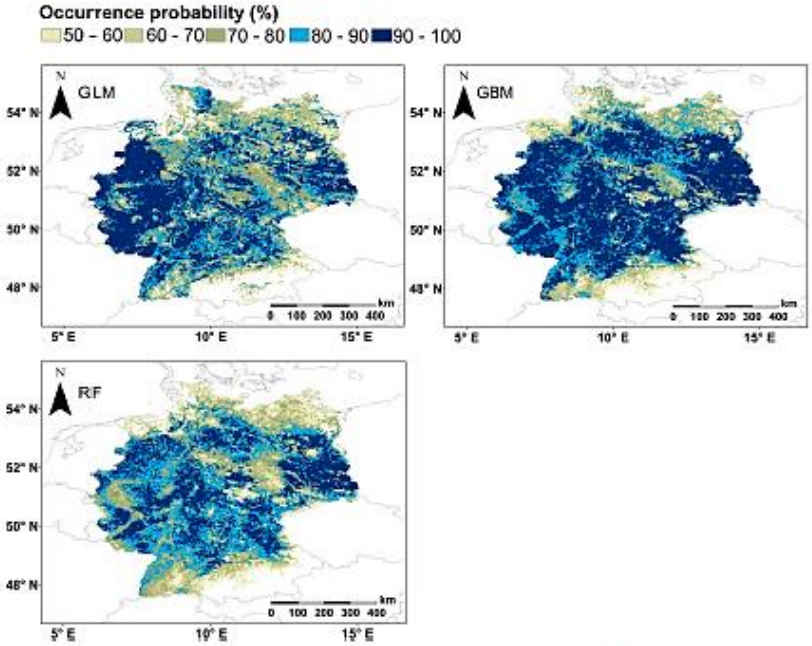


Figure 5. Probability maps generated for three species distribution models of Turtle Doves in Germany run with PA data. Only the areas with a probability ≥0.5 are presented. Probabilities of ≥0.8 were highlighted in blue shades and represent most likely regions for Turtle Dove occurrences and therefore those where adjusted land management would likely support breeding success of the species.

and chicks need to access food frequently. It seems sensible that Turtle Doves would occur in regions with fewer heavy rainfall events. Heavy rain can result in higher mortality rates especially during breeding periods (particularly high mortality rates of chicks and juveniles), longer sheltering periods of adults to regulate the body temperature of their offspring and thus higher energy demands on adults, but also reduced feeding efficiency[58]. These effects have been demonstrated in raptors, Eagle Owls (*Bubo bubo*) and White Storks (*Ciconia ciconia*)[59–63]. Suitable regions with precipitation rates lower than 225 mm cover the western border of Germany, regions around Saarbrucken, Wiesbaden, Mainz, Spessart, Rhoen, the lowlands of the Thuringian Forest and Franconian Mountains, Leipzig, Dresden, Magdeburg as well as the Mecklenburg Lake District[46].

Both climatic variables were able to describe a likely effect on the presence probability of Turtle Doves in Germany reasonably well, but further predictor variables can also heavily impact species occurrence, such as land type or soils[64–66]. For instance, 'forest' would be expected as an important land coverage variable due to its role as nesting site and shelter described in previous literature.[6,35,42,49,67–72] However, only GLM with PA data demonstrated a positive effect on the occurrence probability when coverage was >40% and algorithms run with PO data highlighted negative effects when coverage was >60%. Mainly, forest coverages >60% are distributed in mountainous regions, e.g. the Spessart, the Alps, the Harz Mountains, the Thuringian Forest and the Erz Mountains[46]. The results for PO data were likely driven by the larger distribution and higher numbers of presences in regions with forest coverages of 20–60%[18] than were presence records in PA data (Fig. 1). Furthermore, a forest coverage of >60% might devaluate a Turtle Dove habitat and can negatively affect species occurrence probability, because the spatial availability of feeding sites (farmland or pastures[6]) is reduced.

Probability maps created with PA and PO data showed pronounced differences (Figs 5 and 6). Coinciding with more or less constant response curves created with PA data, resulting probability maps also revealed little variation for Turtle Dove occurrence. Possible key breeding regions in Germany cannot be distinguished clearly and PA data probability maps are hard to interpret.

Probability maps drawn for PO data clearly distinguished areas with likelihoods of >0.8 (Fig. 6). Those areas are of interest for conservation management due to their likely importance for Turtle Dove occurrence. They combine the existence of optimum values for all three variables that were of importance in algorithms. Optimum values include minimum temperature in January between 1 °C – 4 °C (Bio 6), precipitation <225 mm during the breeding months (Bio 18) and forest coverage <60%[18,57]. Therefore, probabilities for survival of food sources, existence of nesting sites and high likelihood for survival of offspring may be highest in the highlighted regions[6,35,42,49,51–55,67–72].

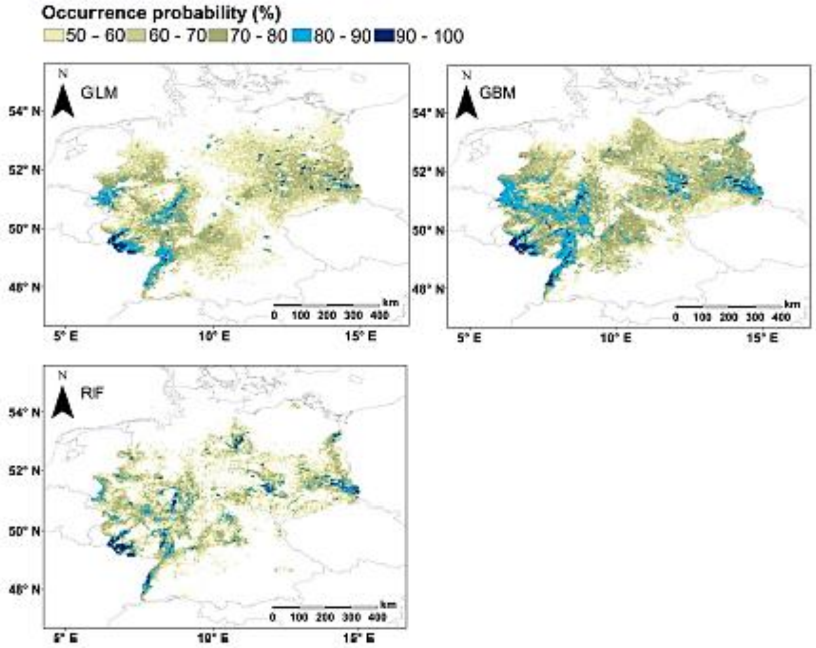


Figure 6. Probability maps generated for three species distribution models of Turtle Doves in Germany run with PO data. Only the areas with a probability ≥ 0.5 are presented. Probabilities of ≥ 0.8 were highlighted in blue shades and represent most likely regions for Turtle Dove occurrences and therefore those where adjusted land management would likely support breeding success of the species.

Conclusions

Generally, model qualities, response curves and probability maps drawn for PA and PO data were different, but results agreed regarding the importance of climatic variables Bio 6 and Bio 18. Previously, climatic variables were used to describe species distributions reasonably well on large scales, either for an entire country like Finland[73] or for a continent such as Europe[74,75]. In this study however, only PO data probability maps were able to specify regions of probably high conservation interest, although in other studies this type of data over-predicted the potential occurrence of species[31,76–78]. Regarding the different approaches with two datasets (PA and PO), citizen-based PO records might become of higher importance for suitability models in the future due to the strong decline of Turtle Doves. This might also be applicable for other scarce species.

Here, modelled important climate variables confirm the description of favoured dry and warm summer conditions of Turtle Doves in central Europe[49]. The temperature in January seems to play an important role for the survival and development of food sources as well as hedges and woodland as nesting sites[50–55]. These conditions support frequent feeding of offspring, prevention of hypothermia and reduced mortality rates of chicks, leading to higher nesting success of Turtle Doves. Although the importance of forests as nesting sites has been shown in other studies[6,35,42,49,67–72], other land coverage variables did not prove to be of highest importance.

Differences between algorithms run with PA and PO data are likely linked to the wider distribution (Fig. 1) and larger numbers of species records in the PO dataset as larger sample sizes usually result in better model accurracy[46]. This supports the assumption that PO data (e.g. citizen-based) can serve as a good data basis and might become of higher importance for suitability models in the future, especially with regard to declining or rare species. Furthermore, data filtering according to e.g. accuracy of recorded location, breeding time and species' territory size[79], as we did here, probably constituted to the quality of used species records and reduced over-representation of one habitat[32]. Additionally, the inclusion of pseudo-absences was already shown to improve model quality when using PO data[31,32,79], and probably also enhanced SDM quality in the present study.

While land coverage categories did not prove to be of highest importance in SDM and climate variables were able to describe the species distribution, but did not present detailed information about the specific characteristics of species' habitats, future SDMs may be improved by the introduction of further variables. Generally, a major change of landscape types and their distribution did not occur across Germany, but management procedures, especially in agriculture, have been modified[35–37,39]. For instance, the intensified usage of herbicides or loss of field margins led to habitat loss and changes in availability and quality of food[35–39]. Therefore, variables like specified land coverage (e.g. forest edge, hedges), soil types, or information about agricultural management (e.g. usage of

herbicides, existence of field margins or herbal vegetation, type of corn, conventional or organic farming, timing of harvest)[6,80–82] could be added. To our knowledge, datasets containing suggested information are not available throughout Germany and investigation of all study sites from PO and PA datasets would require immense effort. Therefore, data could be gained on a smaller (territory sized) scale and conclusions could be drawn for Turtle Dove territories in Germany and implemented in management plans.

Suggestions for Turtle Dove supportive farming practices are already described in previous literature: A delay of harvest times until the end of August could improve the availability of food sources to raise late Turtle Dove broods[35]. Provision of supplementary food in the form of weeds and seeds would support a good body condition of adult Turtle Doves especially in the early breeding season[80,83]. These could be made available through e.g. unfertilised crop field margins, which favour diverse vegetation coverage[81,82]. It has also been shown that chicks in good conditions were mainly fed with seeds from arable plants[80]. This is why foraging sites of Turtle Doves should provide differing weeds and seeds of arable plants for adults and youngs[48,49,50,80]. Also, small patches with variable crops, peas and herbal sites with changing crop rotation and without usage of fertilizers to enrich the food plant diversity[35,81,82] have been suggested to support Turtle Doves during the breeding season.

Methods

Study Species. Turtle Doves are migratory, granivorous birds that feed on weeds and seeds on the ground in agricultural landscapes and build their open nests preferably near the foraging site in protected vegetation such as dense bushes and hedges with a height of at least 4 m, and in forests[6,35,42,49,67–72]. Breeding pairs usually take up territories of ca. 1 km^2, but in some regions up to four pairs per km^2 have been recorded[49]. In general, they tend to breed in warm, temperate regions up to an altitude of 500 m in continental Europe[49,67].

Databases for environmental variables. Environmental variables consisted of climate and land cover data for Germany. Climate variables were downloaded from www.worldclim.org[18]. They were trimmed to the extent of Germany and exported as ascii files in DIVA-GIS following the tutorial for preparation of worldclim files for use in maxent (http://www.lep-net.org/wp-content/uploads/2016/08/WorldClim_to_MaxEnt_Tutorial.pdf)[84]. Corine land cover data (CLC 2006) for Germany came from the European Environment Agency (http://www.eea.europa.eu/legal/copyright)[85]. CLC 2006 codes 37 land cover categories.

ArcGIS 10.2.2 was used to create a fishnet, which based on the extent of trimmed climate grids and equalized the raster cell sizes of both environmental datasets. Therefore, land cover data and the fishnet were intersected. Area sizes and percentages of every land cover variable in each raster cell were calculated and joined to the fishnet according to raster cell IDs in ArcGIS 10.2.2. Furthermore, values of every climate variable in each raster cell were added to the attribute table. The final fishnet was opened in DIVA-GIS to create ascii files for each land cover variable.

All variables were checked for multi-collinearity. For that, we used the vif-function (variance inflation factor) in R 3.3.3[86], which is embedded in the car-package, to check for collinearity between all variables included in the fishnet attribute table and excluded the ones with a vif >8. The procedure was repeated until all remaining variables had a vif of < 8. The minimum threshold for vif to exclude collinearity is 10[87,88], but values lower than 10 are assumed to be more precise[87].

The resulting variable set was applied to each model. It consisted of seven landscape variables and eight climatic variables (Table 2). Land cover variables contained following categories: 'urban areas' included 11 categories (codes 111 to 142), 'permanent cultures' contained two categories (codes 221 and 222), 'pasture' contained only the category 231, 'forest' included three variables (codes 311 to 313), 'herbs and shrubs' contained three categories (codes 321 to 324), 'no/little vegetation' had also three categories (codes 331 to 333) and 'wet areas' contained nine categories (codes 411 to 523).

PA data. The 'Monitoring of breeding birds' database contained PA data from 2005 to 2013. For this monitoring, 1394 study areas of the size of 1 km^2 are randomly distributed across Germany. Monitoring of these sites should be done annually by volunteering observers following a standardised field protocol. The protocol defines four surveys of the sites from March to June by one observer. Surveys start at sunrise under good weather conditions (no rain, low wind speed) and last for two to four hours. Observers follow a strict route of 3–4 km and record all breeding and territorial birds with registry of the position. Species that were not detected during surveys are noted as absent. When study sites were not checked, no data entries were recorded. Here, 1023 study areas contained no data entries throughout the available years, and were deleted. Therefore, we kept only those sites with at least one presence or absence data point during the years 2005 to 2013, obtaining a dataset of 371 sites, which was used for modelling. 293 sites had at least one Turtle Dove presence record and thus were accounted as Turtle Dove habitat. 78 studied sites were recorded for Turtle Dove absence. After filtering, monitoring places in 13 different states remained for model analyses (Fig. 1, Supplementary Table S1).

PO data. The ornitho-dataset consisted of a total of 9064 PO records, in total. Observers can record the sighting localities with exact coordinates (exact locality), per district or municipality. In order to use the most accurate data, we filtered the ornitho dataset for exact localities and the breeding months June and July, because a previous ring re-encounter study showed that Turtle Doves are in their most northern distribution ranges, i.e. breeding areas, in June and July[54]. Furthermore, record locations were filtered for a minimum distance of at least 1 km between Turtle Dove records to remove over-sampled localities[32] and to reduce possible multiple records of a single individual to only one data point, mirroring one record for one Turtle Dove territory (using ArcGIS 10.2.2, assuming a nest density of one per km^2 [49]). After filtering, the final dataset contained 1168 records from 14 states (Fig. 1, Supplementary Table S2).

To obtain an overview about spatial position of presence and absence points of PA and PO data we created another map containing the biggest cities and larger landscapes in Germany (Fig. 1d). Therefore, we depicted the position of cities and landscape names according to https://www.diercke.de/content/deutschland-physische-karte-978-3-14-100800-5-19-2-1[89] in ArcGIS 10.2.2.

Species distribution modelling. SDM was conducted using the Biomod 2 package (based on Biomod[64]) for R version 3.3.3[86]. Therefore, we mainly followed the SDM for Wolverines' (*Gulo gulo*) current disctribution[87] and the setups given in the package description[90]. Biomod 2 is able to build different model algorithms in one run for one species dataset[24,46,91] and no expert knowledge is needed to determine the most appropriate modelling algorithm. Indeed, it is recommended to run a framework of different modelling algorithms[24]. The framework of present SDMs in Biomod 2 consists of three main modelling steps: (1) data formatting, (2) model computation and (3) projection of models[92].

Biomod 2 was run for both, the PA and PO dataset separately, but the first step was equal for both datasets. Datasets were imported into Biomod 2. Then presence and absence or presence only data were defined for each location and environmental parameters were introduced as raster files following[92]. Raster files were stacked and then data were formatted according to the species dataset.

Data formatting for PA data was done using default settings as described in[92]. For PO data, we set the number of pseudo-absences to 1500 and Biomod 2 generated 949, thus the number of pseudo-absences was similar to the number of presences from the PO dataset. Pseudo-absence points had a minimum distance of at least 1 km to presence records and were created using the 'disk' algorithm[92]. The distance factor and the number of pseudo-absences were used to avoid pseudo-replication and also to prevent absences describing the same niche as presences (false absences)[90,93].

Then, five different SDM algorithms were run for both datasets (PA and PO). The algorithms included were: Generalised Linear models (GLM), Generalised Boosted models (GBM), Classification Tree analysis (CTA), Surface Range Envelope (SRE similar to BIOCLIM) and Random Forests (RF). For model calibration, 70% of the data were used. The remaining data were used to test the models[91]. Every model algorithm was run 100 times.

For model evaluation, we calculated AUC-values of algorithms using the fBasics package[94] and sensitivity and specificity using the get_evaluations function described in[92] for both species datasets. Implemented variables were evaluated by variable importance and response curves, which were calculated and created with the associated functions embedded in Biomod 2[46,91,92]. The variable importance is given as a value between 0 and 1 with 1 as the highest possible value. The higher the value of a specific variable, the higher is its influence on the model. However, the calculation technique for variable importance (Pearson's correlation) does not account for interactions between implemented variables and hence does not sum up to 1[46]. Response curves demonstrate the quantitative relationship between environmental variables and the logistic probability of the presence of the species (habitat suitability). Habitat probabilities per model algorithm were projected in Biomod 2[92] using AUC as filter method. Then projections were stacked and subsets for each algorithm were generated. Model averages were built for every algorithm and according raster files were exported as ascii format. Final maps were generated in ArcGIS 10.2.2 for each model. We created maps depicting only those areas that had an occurrence probability of ≥ 0.5. Furthermore, areas with a probability ≥ 0.8 were considered key sites worthy of special management.

Data Availability

All data generated or analysed during this study are included in this published article and its Supplementary Information files. The raw datasets are from the DDA and restrictions apply to these data, which is why they are not publicly accessible, but can be obtained upon reasonable request and with permission from DDA and its teams responsible for the 'Monitoring of breeding birds' scheme and ornitho-data (Supplementary data S1 'Monitoring of breeding birds' request date: 28.10.2014, permission date: 26.05.2015; Supplementary data S2 ornitho.de application numbers 2013.006 and 2013.006a from 29.01.2014 and updated at 06.11.2014).

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Author Contributions

M.M. Analysed the data by running the different model algorithms and drafted the manuscript. P.Q. Helped with data analyses, interpretation and critically revised the manuscript.

Additional Information

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Appendices

The following appendices are saved on CD-Rom-device (attached to this thesis). The files are saved in folders, which are named according to chapter or supplementary publication.

Supplement: Chapter 2

- **Supporting Information**
  S1: Turtle Dove samples from Spain having their assigned origins in Russia and the Baltic states and in central eastern Europe. Certain migrants, which are birds without possible breeding origin assignments in Spain, are highlighted with a red M. Furthermore, the individual isotopic values of δ2Hf are shown.
- **Supporting Information**
  S2: Turtle Dove samples from Spain having their assigned origins in France, northern (N) Italy, south-west (SW) England, north-west (NW) Spain, Romania and Bulgaria. The individual isotopic values of δ2Hf are given. Certain migration cannot be detected, because areas with possible breeding ranges are assigned in Spain.
- **Supporting Information**
  S3: Turtle Dove samples from Spain having their assigned origins at the Mediterranean coasts. The individual isotopic values of δ2Hf are given. The individual isotopic values of δ2Hf are given. Certain migration cannot be detected, because areas with possible breeding ranges are assigned in Spain.
- **Supporting Information**
  S4: Turtle Dove samples from Greece ordered by assigned distribution ranges. Migrants are highlighted with a red M. Furthermore, the individual isotopic values of δ2Hf are shown.
- **Supporting Information**
  S5: Turtle Dove samples from Malta ordered by assigned distribution ranges. Certain migrants are highlighted with a red M. They have no breeding origin areas in Malta. Furthermore, the individual isotopic values of δ2Hf are shown.
- **Supporting Information**
  S6: Turtle Dove samples from Bulgaria ordered by assigned distribution ranges. Certain migrants are highlighted with a red M. They have no breeding origin

areas in Bulgaria. Furthermore, the individual isotopic values of δ2Hf are shown.

- **Supporting Information**

  S7: Turtle Dove samples from Italy ordered by assigned distribution ranges. The individual isotopic values of δ2Hf are given. Certain migration cannot be detected, because areas with possible breeding ranges are assigned in Italy.

Supplement: Chapter 3

- **Additional file 1**

  Table S1: Sample names from different columbid hosts with their closest GenBank match for ITS1/5.8S/ITS2 region, maximum identity and query coverage in % as well as the *Trichomonas* species of the GenBank match, the lineage, host and country in which the reference was found.
- **Additional file 2**

  Table S3: The GenBank accession numbers (KX459439 – KY675299) of the study sequences are listed in the table below.
- **Additional file 3**

  Table S2: Sample names from different columbid hosts with their closest GenBank match for Fe-hydrogenase region, maximum identity and query coverage in % as well as the parasite species of the reference.
- **Additional file 4**

  Figure S1: Expanded phylogenetic tree including information about *Trichomonas* species and origin countries of reference sequences. Furthermore, the host species and sample ID of studied sequences are shown.
- **Additional file 5**

  Figure S2: Phylogenetic tree based on the analysis of the Fe-hydrogenase gene of *Trichomonas gallinae*. This figure includes information about *Trichomonas* sub-lineages (A1, A1.1-A1.3, A2, C1-C4 and the newly detected sub-lineage P!). Furthermore, information about the origin countries of reference sequences is given, when information was available. Additionally, the host species and sample ID of studied sequences are shown. The break in the direction of sub-lineage P1 equals two substitutions. References to GenBank accession numbers are as follows: AF446077.1 [61], HG008115.1 [8], KC529660.1, KC529661.1, KC529662.1, KC529663.1, KC962158.1 [42], F681136.1 and JF681141.1 [22] and XM_00131079.1 [43].

Supplement: Chapter 4

- **Supplementary Table S1:** List of analysed PA data from the ‘Monitoring of breeding birds’ dataset (Supplementary data 1_TT_DDA_bioclim.pdf).
- **Supplementary Table S2:** List of analysed PO data from the ornitho-dataset (Supplementary data 2_Presencedata_ornitho.pdf).

Supplement: Calderón et al. 2016_BMC Evolutionary Biology

- Calderón L, Campagna L, Wilke T, Lormée H, Eraud C, Dunn JC, Rocha G, Zehtindjiev P, Bakaloudis DE, Metzger B, Cecere JG, **Marx M,** Quillfeldt P (2016): Genomic evidence of demographic fluctuations and lack of genetic structure across flyways in a long distance migrant the European Turtle dove. BMC Evolutionary Biology. 16: 237.
- **Additional file 1:** Details of the samples used. A table with additional information on the blood and tissue samples obtained for our work.
- **Additional file 2:** Further details on materials and methods. Additional information on the laboratory methods related to the RAD-seq procedures and mtDNA sequencing. Also, further details on the demographic history modelling performed with DIYABC, and the niche modelling analysis conducted in MAXENT.
- **Additional file 3:** Analysis of molecular variance (AMOVAs). Results of the AMOVAs performed, testing for significant genetic differences among the three main flyways (using mitochondrial and nuclear DNA data).
- **Additional file 4:** fastSTRUCTURE analysis indicating the number of genetic clusters suggested for the turtle dove, $K = 1$. Barplot graphics representing $K = 1$ and 3 are shown.
- **Additional file 5:** Principal component analysis to test the goodness-of-fit of each evaluated scenario against the simulated data in DIYABC. PCA plots show that Scenario 4 had the best goodness-of-fit among all tested scenarios.
- **Additional file 6:** Posterior probability of scenario choice in DIYABC. Direct estimation approach that uses 0.1 % of the closest simulations to the observed data. (b) The logistic regression uses 1 % of the closest simulations.
- **Additional file 7:** MaxEnt habitat suitability models for European Turtle doves. Niche modelling analysis based on additional climate models: CC (CCSM4), MR (MIROC-ESM) and MI (MPI-ESM-P). Models available on http://www.worldclim.org/.

SUPPLEMENT: QUILLFELDT ET AL. 2018_PLOS ONE

- 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(8):e0200798.
- **S1 Table.** *Trichomonas* samples (N = 71) with their closest GenBank match for ITS1/5.8S/ITS2 region, maximum identity and query coverage in % as well as the *Trichomonas* species of the GenBank match, lineage of *Trichomonas gallinae*, host and country in which the reference was found.
- **S1 Fig.** Decline in greenfinch number in Hesse, Germany, according to the spring (https://www.nabu.de/tiere-und-pflanzen/aktionen-und-projekte/stunde-der-gartenvoegel/index.html) and winter garden bird counts (https://www.nabu.de/tiere-und-pflanzen/aktionen-und-projekte/stunde-der-wintervoegel/index.html) of the NABU (Nature and Biodiversity Conservation Union, Germany).

List of publications

PEER-REVIEWED PAPERS

Marx M, Rocha G, Zehtindjiev P, Peev S, Bakaloudis D, Metzger B, Cecere JG, Benedetti MB, Frahnert S, Gamauf A, Voigt C, Quillfeldt P (submitted to Conservation Science and Practice): Stable isotope assignment of migrating European Turtle doves (*Streptopelia turtur turtur*) to breeding ranges in Europe.

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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(8):e0200798.

Marx M, Reiner G, Willems H, Rocha G, Hillerich K, Masello JF, Mayr SL, Moussa S, Dunn JC, Thomas RC, Goodman SJ, Hamer KC, Metzger B, Cecere JG, Spina F, Koschkar S, Calderón L, Romeike T, Quillfeldt P (2017): High prevalence of *Trichomonas gallinae* in wild columbids across western and southern Europe. Parasites & Vectors.

Calderón L, Campagna L, Wilke T, Lormée H, Eraud C, Dunn JC, Rocha G, Zehtindjiev P, Bakaloudis DE, Metzger B, Cecere JG, **Marx M**, Quillfeldt P (2016): Genomic evidence of demographic fluctuations and lack of genetic structure across flyways in a long distance migrant the European Turtle dove. BMC Evolutionary Ecology. 16: 237.

Marx M, Korner-Nievergelt F, Quillfeldt P (2016): Analysis of Ring Recoveries of European Turtle Doves Streptopelia turtur – Flyways, Migration Timing and Origin Areas of Hunted Birds. Acta Ornithologica. 51(1):55-70.

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CONFERENCE CONTRIBUTIONS

Marx M, Lormée H, Eraud C, Dunn J, Rocha G, Zehtindjiev P, Peev S, Bakaloudis D, Metzger B, Cecere JG, Voigt C, Kramer-Schadt S, Quillfeldt P (2015): Brut- und Überwinterungsgebiete Europäischer Turteltauben *Streptopelia turtur*. Deutsche Ornithologen-Gesellschaft und Partner. Schwerpunktthema “Vogelzug”. Konstanz, Germany. (Talk)

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Erklärung

Ich erkläre: ich habe die vorgelegte Dissertation selbstständig und ohne unerlaubte fremde Hilfe und nur mit den Hilfen angefertigt, die ich in der Dissertation angegeben habe. Alle Textstellen, die wörtlich oder sinngemäß aus veröffentlichten Schriften entnommen sind, und alle Angaben, die auf mündlichen Auskünften beruhen, sind als solche kenntlich gemacht. Ich stimme einer evtl. Überprüfung meiner Dissertation durch eine Antiplagiat-Software zu. Bei den von mir durchgeführten und in der Dissertation erwähnten Untersuchungen habe ich die Grundsätze guter wissenschaftlicher Praxis, wie sie in der „Satzung der Justus-Liebig-Universität Gießen zur Sicherung guter wissenschaftlicher Praxis“ niedergelegt sind, eingehalten.

Ort, Datum   Unterschrift