Migration patterns of European bats assessed with stable isotopes

Cecilia Kruszynski de Assis

MIGRATION PATTERNS OF EUROPEAN BATS

ASSESSED WITH STABLE ISOTOPES

Inaugural-Dissertation

to obtain the academic degree

Doctor rerum naturalium (Dr. rer. nat.)

submitted to the Department of Biology, Chemistry, Pharmacy

of Freie Universität Berlin

by

CECILIA KRUSZYNSKI DE ASSIS

Berlin, 2021

This dissertation was conducted in the Department of Evolutionary Ecology of the Leibniz Institute for Zoo- and Wildlife Research in Berlin between October 2016 and October 2021 under the supervision of PD Dr. Christian C. Voigt.

1st Reviewer: PD Dr Christian C. Voigt

2nd Reviewer: Prof. Dr Heribert Hofer

Date of defence: 19.05.2022

I dedicate this thesis to all Brazilian scientists,

that are still trying to make science in the country.

Genocides will not pass. Science will prevail.

"Até lá, vivo as perguntas sabendo que elas são tudo que eu tenho."

"Until then, I live the questions knowing they are everything I have."

(freely translated from "A Linha", Corrano, D. 2021)

ACKNOWLEDGEMENTS

It has finally come to an end this Ph.D. journey and I have so many people to thank. I have so many people close to my heart that deserve a piece of this Ph.D. because without them it would not be complete. This was obviously not done overnight, and it took every single piece of support that I had, from academic advice to sharing wine glasses. I think this is the only section of the thesis where we are reminded that this scientist is not a machine and did not go through this path alone. I have joined the impostor syndrome club and I had special people that reminded me how good I was at doing this and pushed me forward to get this thesis completed.

Firstly, thank you to my family, Rita, Antonio, and Tiago that supported my path choice and have held my hand not only for this Ph.D. but for everything. I miss you so much.

I would like to thank my supervisor, PD Dr. Christian Voigt, for all the teaching and support through the years. Even though a few things deviated from Plan A, we managed to do a good job together.

I would like to thank José Wagner "Xuleta" and Silvia who on a very particular night full of tequila and too many beers instigated me to start this path. Even though we were afar for most of these years, I always felt you were very much present in my life.

I want to thank the Bat lab members for the insights and field trips where I learned so much about the species that would become my thesis subject. Special thanks to Kseniia Kravchenko. You were there for me right from the start, and I could see you grow and improve in your science. I am so very thankful for this, my friend. I am looking forward to seeing everything you will accomplish in your career. Special thanks go to Dr. Shannon Currie, who not only became one of my closest friends but has improved me in so many parts of my life. I could not describe this in detail, because it would take maybe a book-length letter.

Talking about friends, many thanks to the ones the Leibniz Institute brought to me: Lorena Derežanin, Leon Barthel, Sónia Fontes, Renata Martins, Sanatana Soilemetzidou, Morgane Gicquel, Miguel Veiga, Arjun Dheer, Patricia Calderón, Saba Mottaghinia, Sara Troxel, Michał Hryciuk, Nicolas Fasel, Marcela Uliano da Silva, Daniel Lewanzik, Tanja Straka, and Lysanne Snijders. Even though a pandemic has separated us in the last two years, I still have you in my heart and hope for a similar glory (a Ph.D. end) for you all. To the more senior IZW members, Prof. Jörns Fickel, Meike Mathews, and all the lab technicians deserve my recognition. Thank you for making time for me. For Dr. Alexandre Courtiol, special thanks to you for the advice and all the help in numerous analyses. I hope our friendship goes beyond my Berlin years and we meet each other again in real life.

A special thanks to my Berliner family Carise, Débora, Andressa, Bárbara, Melissa, Maíra, Fernando e Felipe. You made every day lighter and happier.

I would like to thank Dr. Liam Bailey for all his help, patience, teaching, supervision, cuddles, and laughter. I am so very thankful for this delightful partnership and again, I would need another book to put into words everything you meant for me during the past years.

Finally, thank you to all the reviewers that took the time to revise and proofread this thesis.

[5] Faunistica - Büro für ökologische & faunistische Freilanduntersuchungen, Jaguarring 4, 23795 Bad Segeberg, Germany

[6] Avitec Research GbR, Sachsenring 11, Germany

[7] Vorweden 1, 18069 Rostock, Germany

[8] Büro Nachtschwärmer, Augustenstr. 77, 18055 Rostock, Germany

[9] NABU Mecklenburg-Vorpommern, Wismarsche Str. 146, 19053 Schwerin, Germany

[10] Institut für Mineralogie, Technische Universität Bergakademie Freiberg, Brennhausgasse 14, 09599 Freiberg, Germany

[11] Büro für faunistische Fachgutachten, Berlin, Germany

TABLE OF CONTENTS

LIST OF FIGURES

APPENDICES

SUMMARY

Migration is an essential process in animals' lives, which they use to avoid adverse weather conditions and resource scarcity. Migratory animals are considered particularly vulnerable to anthropogenic changes to their environment because they require a specific temporal and spatial progression of appropriate habitats for foraging and reproduction, and a refuge from harsh environmental conditions. Anthropogenic activities that change the environment such as habitat loss, habitat fragmentation, and climate change have had a global effect on migratory species resulting in widespread population declines. Climate change is a particularly serious threat for migratory species as it can have impacts across all the habitats within which migratory animals must move. Efforts to mitigate climate change may also have adverse effects on migratory species. The trade-off between renewable energy use and wildlife conservation is not always straightforward. Changes in energy systems have the potential to alter the functioning of ecosystems and wildlife populations by affecting species' access to resources, habitat availability, and connectivity. The use of wind energy poses a particular threat for many migratory species. Studies have shown that wind energy farms pose a mortality risk to flying migratory individuals both inland and on the coast, and new concerns are being raised around offshore establishments.

Conservation of migratory species in the face of such threats is of vital importance and will likely require the protection of the multiple habitats that are used during the migratory journey. However, there is often limited knowledge about the migratory pathways and stopover sites used by migratory species, posing a major challenge for effective conservation. Stable isotope analysis can be used to trace terrestrial migratory routes and identify migratory origins. The development of such techniques to determine the corridors and pathways migratory animals are using to move between habitats has assisted us in further honing conservation efforts. Stable isotope analysis allows the use of a small number of samples, which can be collected in one sampling event, to detect environmental

tracers that are related to the spatial-temporal movement of animals. The application of stable isotopes in conservation biology is growing rapidly and shows great promise for the conservation of endangered species.

Bats are one of the most taxonomically diverse groups of mammals, however, only a few species are known to migrate long distances. These species have evolved a combination of physiological and morphological traits to allow long-distance migration. The European Nathusius' pipistrelle (*Pipistrellus nathusii*) is one of the most well-studied migratory bat species worldwide. The species is known to maintain a long-distance migration with both coastal and offshore pathways. Recent studies have shown that Nathusius' pipistrelles have been increasing their geographical range, even reaching 60° N in latitude. Their long-distance migratory behaviour and the increase in European wind farms make them highly vulnerable to environmental changes. Considering their broad European range, high vulnerability to environmental changes, and the current need to protect migratory species, Nathusius' pipistrelles are an interesting model species in which to apply stable isotope analysis to investigate migration patterns.

In this thesis, I use stable isotope analysis to identify the northern migratory corridors of Nathusius' pipistrelles (**chapter 1**) and differentiate the mortality risk posed by wind turbines on different demographic groups during migration (**chapter 2**). In **chapter 1**, I use a dual-isotope approach (δ^2H and ^{87}Sr/^{86}Sr) to determine the origin of bats found on three islands in the north of Germany. Although δ^2H analysis suggested a possible Fennoscandian origin, ^{87}Sr/^{86}Sr analysis refuted this possibility and proposed they would be originating in Russia and the Baltic states. In **chapter 2**, I use a comprehensive dataset of fur samples from carcasses collected beneath wind turbines and living individuals across Germany to assess the vulnerability of different demographic groups to wind turbine mortality. Compared to adults, juveniles were more vulnerable at low wind turbine densities; this effect was minimised at high density, with both ages equally affected. In

addition, I found more females and regional migrants in both living and carcass populations. Overall,

this dissertation demonstrates the importance of stable isotope analysis in wildlife research and

provides a practical example of how it can help inform species conservation.

ZUSAMMENFASSUNG

Migration ist ein essentieller Prozess im Leben von wandernden Tierarten, der dazu dient, ungünstige Wetterbedingungen und Ressourcenknappheit zu vermeiden. Wandernde Tiere gelten als besonders anfällig für anthropogene Veränderungen ihrer Umwelt, da sie eine bestimmte zeitliche und räumliche Abfolge geeigneter Lebensräume für eine erfolgreiche Nahrungssuche, Fortpflanzung und zum Schutz vor rauen Umweltbedingungen benötigen. Anthropogene Umweltveränderungen, wie zum Beispiel der Verlust und die Fragmentierung von Lebensräumen als auch der Klimawandels, haben sich weltweit auf wandernde Arten ausgewirkt und zu einem weit verbreiteten Rückgang sowie zur Verschiebung der Verbreitungsgebiete geführt. Der Klimawandel ist eine besonders ernste Bedrohung auch für wandernde Arten, da er sich auf alle wichtigen Lebensräume entlang der Wanderrouten auswirken kann. Bemühungen zur Abschwächung des Klimawandels können allerdings zusätzliche negative Auswirkungen auf wandernde Arten haben. So ist zum Beispiel die Abwägung zwischen erneuerbaren Energien und deren Wirkung auf die Natur und Wildtiere nicht trivial und es können neue Konflikte entstehen. Veränderungen in der Energiegewinnung können Ökosysteme stören und Wildtierpopulation beeinflussen, indem sie Lebensräume beschränken und deren Vernetzung sowie die Ressourcenverfügbarkeit verringern. Studien zeigen, dass Windenergieanlagen ein Mortalitätsrisiko für fliegende Tiere besonders während der Migration sowohl im Landesinneren als auch an der Küste darstellen. Auch bezüglich Offshore-Anlagen gibt es Bedenken.

Der Schutz wandernder Arten ist angesichts solcher Bedrohungen von entscheidender Bedeutung und erfordert insbesondere den Schutz der verschiedenen Lebensräume, welche während der Migration genutzt werden. Während lokale Lebensweisen besser verstanden sind, ist das Wissen über die von wandernden Arten genutzten Wanderrouten und die dabei genutzten Lebensräume oft begrenzt, was eine große Herausforderung für einen wirksamen Schutz darstellt. Mit Hilfe der

Analyse stabiler Isotope lassen sich die terrestrischen Wanderrouten zurückverfolgen und die Ursprungsorte der Wanderungen ermitteln. Die Entwicklung solcher Techniken zur Bestimmung der Korridore und Zwischenstationen von wandernden Tieren hat dabei geholfen Schutzbemühungen weiter zu verbessern. Die Stabilisotopenanalyse ermöglicht es mit einer geringen Probenmenge, die einmalig entnommen werden kann, Umweltmarker nachzuweisen, die mit der räumlich-zeitlichen Bewegung von Tieren in Zusammenhang stehen. Die Verwendung stabiler Isotope im Naturschutz etabliert sich immer weiter und ist für die Erhaltung gefährdeter Arten sehr vielversprechend.

Fledermäuse sind eine der taxonomisch vielfältigsten Säugetiergruppen, aber nur von wenigen Arten ist bekannt, dass sie wandern. Diese Arten haben eine Kombination von physiologischen und morphologischen Merkmalen entwickelt, die Langstreckenwanderungen ermöglichen. Die Europäische Rauhautfledermaus (*Pipistrellus nathusii*) ist eine der am besten untersuchten wandernden Fledermausarten weltweit. Es ist bekannt, dass diese Art über weite Entfernungen wandert und dabei sowohl küstennahe als auch küstenferne Flugrouten nutzt. Jüngste Studien haben gezeigt, dass diese Fledermäuse ihr Verbreitungsgebiet erweitert haben, welches jetzt sogar bis zum 60. Breitengrad (60° N) reicht. In Anbetracht ihres weiten europäischen Verbreitungsgebiets, ihrer hohen Anfälligkeit für Umweltveränderungen und der Notwendigkeit wandernde Arten zu schützen, sind Rauhautfledermäuse eine interessante Modellart für die Untersuchung von Migrationsmustern mittels der Analyse stabiler Isotope.

In dieser Arbeit verwende ich die Analyse stabiler Isotope, um die nördlichen Wanderkorridore von Rauhautfledermäusen zu identifizieren (**Kapitel 1**) und das Mortalitätsrisiko durch Windkraftanlagen für verschiedene demografische Gruppen während der Migration zu bestimmen (**Kapitel 2**). Im **Kapitel 1** verwende ich einen dualen Isotopen Ansatz (δ^2H und ^{87}Sr/^{86}Sr), um die Herkunft von Fledermäusen zu bestimmen, die auf drei Inseln in Norddeutschland gefunden wurden. Obwohl die δ^2H-Analyse einen möglichen fennoskandischen Ursprung nahelegt, widerlegte die

^{87}Sr/^{86}Sr-Analyse diese Möglichkeit und legt nahe, dass sie aus Russland und den baltischen Ländern stammen. Im **Kapitel 2** verwende ich einen umfassenden Datensatz basierend auf Haarproben, um das Mortalitätsrisiko verschiedener demografischer Gruppen durch Windkraftanlagen zu bewerten. Die Proben stammen sowohl von unter Windkraftanlagen gesammelten Kadavern als auch von lebenden Individuen aus ganz Deutschland. Jungtiere waren im Vergleich zu adulten Tieren bei einer geringen Dichte von Windkraftanlagen stärker gefährdet; bei einer hohen Dichte verschwand dieser Unterschied und beide Altersklassen waren gleichermaßen betroffen. Darüber hinaus zeigen die Totfunde und Daten von lebenden Individuen, dass die Populationen weiblich dominiert sind und dass es mehr regional ziehende Individuen als Langstreckenzieher in Deutschland gibt. Insgesamt zeigt diese Dissertation, wie wichtig die Analyse stabiler Isotope in der Wildtierforschung ist und sie liefert ein praktisches Beispiel dafür, wie die Methode zum Artenschutz beitragen kann.

This thesis aims to apply stable isotope analysis to elucidate the pathways of migratory bats and understand the threat posed to them by wind turbines during migration. The introduction to the thesis gives a background to the topic, presenting key terms and concepts essential for its comprehension. In the first section, I provide a broad introduction to migration and examples of different migratory animals. In the second section, I explain several terms related to stable isotope analysis and the theories behind the method when used to determine the origin of animals. Finally, in the last section, I present migratory bats, their unique characteristics that enable long-distance journeys, and introduce Nathusius' pipistrelles (*Pipistrellus nathusii*) as the model species used in this thesis.

1. A walk on the wild side – Migration patterns of animals

1.1 To be or not to be – Migration concepts

Migration is a vital component of the ecology and life history of animals and is present in a variety of taxa, ranging from invertebrates to vertebrates (Dingle 2014) and across many environments (e.g., terrestrial, aquatic, and aerial). The earliest evidence of animal migration dates to the Stone Age, with rock paintings showing the movement of African savannah animals, circa 20,000 years ago (Hoare 2009). Ancient Greek philosophers were the first to document a theory of migratory behaviour in animals. Aristotle theorised about the movements of several seasonal birds (Aristotle, circa 350 BC). However, his explanation for their sudden disappearance was that the birds seen in summer morphed into different species that were seen in the winter (Hoare 2009). The first actual record of animal migration was reported in fish (herrings – family Clupeidae) in the late 18th century (Gilpin 1786). Since then, our knowledge of the drivers, causes, and cues for migration has increased dramatically (Dingle 2006; Bauer and Klaassen 2013; Shaw 2016).

Although migration is a widespread phenomenon, no single definition has garnered consensus among scientists. The definition of animal movements may include terms such as one-way, two-way, partial, continuous, complete, return, exploratory, etc. Conceptually, migration definitions are either focused on the individual (physiological, behavioural, genetic) or the population (ecological, evolutionary) levels (Dingle and Drake 2007). For example, a behavioural definition consists of the relocation of the animal on a larger scale with a longer duration than those arising in its normal daily activities (Dingle and Drake 2007). The ecological view of migration is defined as a yearly, round-trip, seasonal movement of animals between two (or more) locations (Shaw 2016). Other definitions include factors, such as geographical direction, periodicity, distance, and spatial locomotion (see a review of definitions in Dingle and Drake (2007)). In some cases, there is still a discussion of what is a migratory individual based on the distances the animals travel. The scale varies depending on the animal; hence, a short distance movement could imply an individual is sedentary or migratory. For bats and birds that move large distances daily for foraging, the scale can be several kilometres. For example, following Kunz and Fenton (2005), a sedentary bat moves less than 50 km, a regional migrant, between 100–500 km, and a long-distance migrant more than 500 km.

Migration is considered adaptive for most animals, and migrants play an important role in many ecosystem processes. As they travel between locations, they transport nutrients, plants, pathogens, and parasites and link habitats that otherwise would not be connected (Bauer and Hoye 2014). This behaviour is often associated with the success of populations in a variety of environmental conditions, such as harsh winters (Dingle 2006). The underlying drivers of animal movement can be summarised into three categories: "refuge", "breeding", and "tracking" (Table 1) (Shaw 2016; Alerstam and Bäckman 2018). Refuge migration occurs when organisms must find an alternative habitat in response to temporarily unfavourable conditions (e.g., harsh winters, droughts, and predation). These animals usually have one primary habitat that they leave due to unfavourable

27

conditions. Monarch butterfly (*Danaus plexippus*) migration is an example of two-way refuge migration, even though not the same individual that starts the journey finishes it. They migrate from several locations in the U.S. and Canada to Mexico every year in order to avoid the harsh winter and limited resource availability in their northern habitats (Reppert and de Roode 2018). Breeding migration refers to the movement of individuals between the habitat they forage and another habitat specifically for breeding, thereby they have two preferential habitats; this behaviour is not necessarily linked to temporal changes in abiotic/biotic factors. The common cuttlefish (*Sepia officinalis*) presents seasonal movements, migrating closer to shore to spawn, and then moving offshore during feeding and development (Keller et al. 2014). Organisms that undergo tracking migration will consistently follow food resources (e.g., migratory prey), and tend to have no primary habitat. Some carnivores that follow prey that are themselves migratory are considered to perform tracking migration. Barren-ground wolves (*Canis lupus*) seem to follow migratory caribou (*Rangifer tarandus*) in the Northwest Territories, Canada, during winter (Walton et al. 2001).

Individuals can exhibit different migration strategies. When only a fraction of the population moves and the other remains either in its breeding or non-breeding area, this is termed partial migration (Dingle and Drake 2007). Differential migration is when all individuals of the same population migrate but differ in their migration distances (Shaw 2016); it is often presented as different sexes or ages moving different distances (Dingle and Drake 2007). These migratory strategies might be affected by changes in environmental conditions. For example, the common noctule bat (*Nyctalus noctula*), which is a migratory species with a large distribution range across Europe, has been reported to have partial and differential migration. For common noctules, females travel longer distances than males during spring migration (Lehnert et al. 2018). Noctule bats have been reported to be expanding their winter range northward, with more individuals roosting at high latitudes in response to milder climates (Godlevska 2015; Kravchenko et al. 2020). Whether or not an

28

individual migrates or remains a resident is based on a trade-off between the potential for increasing survival or holding a competitive advantage for later seasons. American dippers (*Cinclus mexicanus*) spend winter in low-elevation streams in British Columbia, Canada; some individuals breed there, while the rest migrate to higher elevation streams to breed. In this case, partial migration seems to be caused by competition for limited breeding sites forcing some individuals to breed elsewhere (Gillis et al. 2008). Therefore, different migratory strategies will be selected as an individual seeks favourable environmental conditions and optimal energy balance to increase its growth, survival, and reproduction.

Several triggers can be associated with an individual's decision to start the migration. The decision consists of a complex combination of cues, such as food supply shortage, competition for resources, individual's body condition, photoperiod, temperature, and precipitation patterns (for examples and reviews see Beebee (1995); Jenni and Kéry (2003); Lehodey et al. (2006); Van Buskirk et al. (2009); Chapman et al. (2015)). For example, populations of green turtles (*Chelonia mydas*) seem to vary the time between the migration events depending on their body conditions each year, stopping or not to reproduce in Costa Rica (Hays 2000). The migration of *Aphrissa statira* butterflies was reported to be correlated with El Niño Southern Oscillation in Panama because the drought and rain periods interfered with larval food production (Srygley et al. 2010). The spring departure dates of American redstarts (*Setophaga ruticilla*) in Jamaica were associated with the amount of rainfall during the non-breeding period and not with the photoperiod change (Studds and Marra 2011). Once an individual begins migration it must be able to orientate in a particular direction by using a few features or properties of the environment. This "compass", which has evolved to enable animals to perform accurate long-distance movements, may use cues, such as the Earth's magnetic field (review in Lohmann et al. (2007)), the sun (Alerstam and Bäckman 2018), the stars (Alerstam and Bäckman 2018), ocean (Lohmann et al. 1999) and river (Peake and McKinley 1998) current flows, etc. With

these cues, animals navigate based on a compass-map system, which helps them to know their relative location and the direction to travel to their destination (Rozhok 2008).

1.2 Bridge over troubled water – Vulnerability and conservation of migratory animals

Habitat loss is the greatest threat to the conservation of animals. Breeding, feeding, and resting sites have declined by more than 50% in the last century (WWF 2020). Some habitats are particularly vulnerable, such as coastal areas, which have been highly affected by increased coastal developments. Coastal developments are expected to impact 91% of all temperate and tropical coasts by 2050 (Usaid 2009). Migratory species, particularly those who cover long distances, are often highly susceptible to environmental change, such as climate change, and the destruction and alteration of landscapes as they rely on many different habitats along their journey (Wilcove and Wikelski 2008). Because the life cycle of migratory animals is dependent upon access to specific areas along the migratory pathway, individuals have fewer opportunities to simply shift to alternative habitats (Berger 2004).

Conservation focus for migratory species must take into account differences in migratory behaviour and its effects on population connectivity, i.e., the extent to which individuals from the same breeding populations migrate to another site. Weak connectivity occurs when individuals from one breeding population can migrate anywhere across the whole species' wintering range or to various wintering sites (Shaw 2016). This is also the case in breeding populations with individuals originating from different wintering sites (Shaw 2016). American white pelicans (*Pelecanus erythrorhyncos*) are known to maintain random mating within a breeding population and often do not return to their birth area (limited philopatry), producing a weakly connected population (Reudink et al. 2016). Strong connectivity occurs when individuals from one breeding site migrate mainly to one

wintering site. An extreme case of strongly connected populations is the Kirtland's Warbler (*Dendroica kirtlandii*), which breeds in Michigan, U.S., and winters exclusively in the Bahamas (Ewert et al. 2012). Therefore, disrupting any of these locations would be extremely detrimental to this bird population. The degree to which migratory populations are connected is an important facet to consider for species conservation.

Populations with strong connectivity are more vulnerable to declines since they are mainly composed of one major population and rarely connect with peripheral populations (Bowlin et al. 2010; Marra et al. 2019). Strongly connected populations, however, may benefit the most from conservation efforts because the connections between two or more areas are well established. While populations of weakly connected individuals may appear inherently 'safer' considering that the risks are divided among more locations, these populations can be harder to manage. Thus, conservation measures must be implemented over large regions, which encompass a significant percentage of subpopulations. Information on population connectivity can, therefore, result in radically different decisions on how to allocate resources for the conservation of species (Shaw 2016). For instance, long-distance migrants may cross different geopolitical jurisdictions where conservation measures are absent or inadequate. Because migratory connectivity is important to conservation, its efforts need to be established beyond political borders to protect suitable corridors and habitats for these highly mobile species (Dallimer and Strange 2015; Voigt et al. 2015). The loss of critical migration corridors or stopover sites may jeopardise an entire population, as the migrant capacity to refuel, rest, or reproduce may disappear (Kurvits et al. 2011).

Environmental conditions within different habitats along a species' migratory route will have important consequences on the ability of migrants to refuel and seek refuge. Anthropogenic changes may cause disruptions to intact breeding and wintering locations as well as to a sequence of suitable habitats along the migratory route (O'Connor et al. 2020; Cardenas-Ortiz et al. 2020; Barbosa et al.

2020). The ability to adapt and change their behaviour may be the only thing preventing population extinction. The accelerated shift in climate conditions due to human-induced changes to the environment is altering resource availability at a pace that animals may be unable to cope with (Robinson et al. 2009). Consequences of these changes include alterations in migration timing (Dunn and Winkler 1999; Parmesan and Yohe 2003), frequency, and even migratory status – with some individuals ceasing to migrate; it has been described for birds, insects, and fish (Moore et al. 1998; Gresh et al. 2000; Wikelski et al. 2007; Wilcove and Wikelski 2008; Jepsen et al. 2015; Flack et al. 2016; Kravchenko et al. 2020). In the Arctic, for example, climate change is causing the permafrost to thaw, shifting the caribou (*Rangifer tarandus*) habitat boundaries, consequently making the caribou travel increasingly longer distances in search of food and causing negative effects on their body conditions (Joly et al. 2011). The effects of climate change, however, are far from uniform. In some cases, it may increase the quality of a migratory habitat. For example, tree lines are shifting northwards into the habitats of migratory species that are dependent on them (Cazzolla Gatti et al. 2019). These species are thus able to shift their stopover and breeding locations further north because the quality of the habitat has changed.

Many migratory species such as birds and bats pass through aerial habitats, however, little research focus has been applied to those other types of habitats. Aerial habitats remain understudied and are largely absent from environmental policy, hindering the protection of aerial biodiversity (Davy et al. 2017). Animals that fly at high altitudes not only face direct threats on land but also in the air, either by direct collisions with anthropogenic structures or physical barriers, such as aircraft and pollution (Voigt et al. 2018b). Aerial habitat conservation is difficult and requires more information on high-altitude flying animals in order to shift the efforts of policymakers. A recent study discussed the possibility that aerial species might be compensating for the lost ecosystem functions previously provided by terrestrial wildlife and thus their presence can be even more

important to maintaining ecosystem functions (Zuluaga et al. 2022). Understanding migration drivers and establishing tools to verify such behaviour are essential to allow us to improve the conservation efforts applied to migratory animals.

1.3 Blowin' in the wind – Wind turbine impacts on wildlife

Rising energy demand and efforts to minimise climate change require a significant decrease in fossil fuel use. In an effort to use alternate sources of energy and reduce the negative effects of CO_2 emissions on the global climate, many countries are promoting energy production from renewable sources, such as wind (IRENA 2020). While these power sources have a clear positive value for energy production and are considered environmentally friendly, they may negatively affect wildlife, thus the trade-off between renewable energy use and wildlife conservation is not always straightforward (Arnett et al. 2008; Saidur et al. 2011; Baerwald and Barclay 2011; Schuster et al. 2015; Zimmerling and Francis 2016; Thaxter et al. 2017; Thaker et al. 2018). While it is possible to utilise renewable energy sources without adversely affecting wildlife, this requires careful planning. The changes in energy systems have the potential to alter the functioning of ecosystems and wildlife populations by affecting species' access to resources, habitat availability, and connectivity. For example, studies have shown that wind energy farms pose a mortality risk to migratory species inland and on the coast (Thaxter et al. 2017); new concerns are being raised around offshore establishments and their possible impacts remain understudied. Animals may be killed by operating turbines, either by blunt-force trauma when colliding with blades or by barotrauma in the tailwind vortices of the spinning blades (Baerwald et al. 2008; Voigt et al. 2015). In addition, animals may suffer from habitat loss during wind turbine constructions in sensitive areas, such as forests or wetlands.

The exact number of bat and bird fatalities at wind turbines is currently unknown. A recent review has tried to summarise the different records of animals killed, but the numbers continue to vary over different countries and periods (Winder et al. 2019). One of their main conclusions was that the 300 bird species and 22 bat species recorded in collisions in the U.S. have had their populations affected, most likely via a loss of connectivity between summer and wintering habitats of migratory species. It is therefore important that additional funds and efforts are established to improve risk evaluation. In addition, the appropriate location of wind farms to avoid future impacts should be continuously evaluated.

Recent population trend analyses suggested that some species with high collision risks are in decline (Zahn et al. 2014; Frick et al. 2017), yet the effects on populations are difficult to monitor because wind turbines can kill individuals that originate from both local and distant populations of unknown location (Popa-Lisseanu et al. 2012; Lehnert et al. 2014). It has been suggested that the number of fatalities at wind farms could be reduced substantially by temporarily stopping turbines at night at certain times of the year and under certain climatic conditions (Arnett et al. 2008). However, these mitigating measures have not been applied systematically, generating potentially major conservation issues for migratory species. Understanding where and when individuals migrate is essential for deciding on new wind farm locations and to help establish adequate mitigation schemes.

2. Let's get chemical – Stable isotopes as an alternative tool to track migratory animals

Several techniques have been developed to track migratory animals (see a review of several methods in Table 1.1. from Hobson et al. (2019)). The method applied differs depending on the species and the environment in which it travels. One broadly applicable method is extrinsic markers, which have been used for many years and are well established. This method relies on fixed markers,

such as banding/marking (e.g., tags, collars, streamers), or phenotypic plasticity (morphological markings), which enable the identification of individuals at any given location along their migratory route (Hobson et al. 2019). It is usually the cheapest method; however, it requires capturing the animal at least two times in order to retrieve low-resolution information, and sometimes it can take several years of recaptures to assemble adequate data (Hobson et al. 2019). With the development of new onboard tracking technologies, such as radio and satellite transmitters, the quantity and quality of the information increased. However, these bio-loggers still have several limitations, such as transmitter size/weight (in the case of small animals), distance to triangulate the position with a receiver, battery life, etc (Davis 2008). Therefore, extrinsic methods require the knowledge of several points in the animal's route (e.g., the origin and the final destination) to recapture the animal and retrieve the data, yet for the vast majority of migratory species recapture rates are extremely low (< 0.01% (Hobson 2003)).

Many migratory bird and bat species are too small to be studied with the bio-logging technology that is currently available. Therefore, we needed to establish alternative methods to gain information on their phenology, migratory corridors, and connectivity between summer and wintering habitats. A few intrinsic methods have a major potential to infer animal migration, such as contaminants (e.g., heavy metals), parasites and pathogens, genetics, etc (Hobson et al. 2019). A useful and powerful intrinsic method such as stable isotope analysis offers an advantageous alternative to existing methods because all necessary information is obtained from a single sample without the need to recapture the animal (Hobson et al. 2019). Additionally, minimum quantities of samples are sufficient for most analyses, which do not necessarily require invasive sampling methods and can be used in any size animal. The application of stable isotopes in conservation biology is growing rapidly and shows great promise for the conservation of endangered species (Bond and Diamond 2011; Pietsch et al. 2011). Stable isotope analysis can be used to trace terrestrial migratory routes, identify migratory

origins, and determine the vulnerability of different demographic groups (Hobson 2003; Martínez del Rio et al. 2009; Caizergues et al. 2016; Wieringa et al. 2020).

Stable isotopes are atoms with an equal number of protons but a different number of neutrons in their nuclei, conferring them different relative atomic masses with nearly identical chemical properties (Albarede 2011). Isotopic abundances are commonly reported using the delta (δ) notation, which represents the isotopic ratio of an element (Pinti 2011). The ratio represents the differences in abundance between the most abundant stable form (usually the "light" isotope) to the less abundant one (usually the "heavy" isotope) in a sample, relative to the same ratio in a reference material (international standards for hydrogen – Vienna Standard Mean Ocean Water (V-SMOW); for nitrogen – N_2 in the air; for carbon – Pee Dee Belemnite (PDB)); the ratio is often reported in per mille (‰) or percent (%) (Equation 1).

$$\delta X = \frac{R_{SAMPLE} - R_{STANDARD}}{R_{STANDARD}} \times 1000 \qquad (1)$$

where X represents the least abundant isotope of an element, R_{SAMPLE} is the isotopic ratio of the sample and $R_{STANDARD}$ is the ratio of the standard.

Stable isotope analysis has been used as a multidisciplinary tool and is widely applied in physics, Earth sciences, biogeochemistry, animal and plant physiology, ecology, anthropology, and archaeology (Fry 2006). Stable isotope analysis was first conducted by ecologists to assess diet, trophic level, and source of nutrients in food webs (Fry 2006). This method can help unravel the parameters of cryptic animals. For example, an analysis of $\delta^{13}C$ in common vampire bats (*Desmodus rotundus*) showed that the species was able to survive in a forested area (which is uncommon for the species) by preying upon species of capybaras in open habitats (Gonçalves et al. 2020).

To apply the method to migration, a few conditions must be followed. First, as the stable isotopic values reflect the animal's (consumer) diet, what they eat and drink will influence directly the ratios we find. Therefore, the food web must differ either in space or its isotopic signature for us to use this information to access the consumer origin. The food items themselves can be the same, however, they must present different isotopic values (Hobson et al. 2019). For example, in the breeding area, the animals feed on food with a distinct isotopic value from the food they eat in the wintering area (Voigt et al. 2012a). Researchers found that *Lasiurus curasoae* bats were travelling more than 500 km from the U.S. to feed on agave plants on the south coast of Mexico (Fleming et al. 1993), which is currently known as the "nectar corridor" that bats use during migration. During winter, these bats feed on C3 plants (e.g., forest plants) and during migration, switch to CAM plants (e.g., cacti).

Moreover, the tissue used for the analysis will determine the time period evaluated. Metabolically active and inactive tissues differ in the spatial information they can provide. The time taken for tissues to change their isotopic value to match that of their diet is termed "turnover rate" (Hobson et al. 2019). For example, European bats moult in summer before they migrate (Fraser et al. 2013). As fur is a metabolically inactive tissue, if we analyse a fur sample taken any time before the following summer, it will reflect the food web the bat ingested before migration, i.e., the time of fur growth. The isotopic signatures may vary according to the tissue-specific turnover rate (Martínez del Rio and Carleton 2012). Stable isotope ratios in bat blood integrate the diet over past days and weeks, liver and muscle over several weeks, and wing membrane tissue over weeks or even months (Voigt et al. 2003; Mirón M. et al. 2006). The turnover rate of different tissues is usually quantified using diet-switch experiments (e.g., Bearhop et al. (2002); Voigt and Speakman (2007)). Therefore, different tissues can be used to access different periods of the animal's life cycles.

Finally, physiological and metabolic processes influence isotopic signals in the consumer's tissues, e.g., discrimination factor, exercise, and pool source. Discrimination factor or isotopic

fractionation is an offset between the ingested food and drinking water and the isotopic signals in tissues due to physical or metabolic processes (Hobson 2008; Voigt et al. 2013). This offset is because living organisms tend to have a preference in the assimilation of a particular isotope to another isotope of the same element (Wassenaar 2018). The molecular bonds are facilitated when made between light isotopes (Caut et al. 2008a). A well-known example in nature is the preferential fixation by photosynthetic organisms of the lighter (and vastly more abundant) isotope ^{12}C compared with the heavier ^{13}C.

To correlate the tissue values to the environmental data, first, we must establish a transfer function. Transfer functions are regression models that calibrate the environmental isotopic ratios to predict the values for the animal tissues. This calibration uses the discrimination factor to account for the variation of isotopes in animal tissues. Currently, robust transfer functions remain scarce because they are species-, age-, sex-, and time-specific (Caut et al. 2008b; Hobson and Wassenaar 2019), and they depend on baseline data across a large geographical range (Voigt and Lehnert 2019). A few transfer functions have been established (see review in Table 5.3 in Voigt and Lehnert (2019)), however, for example, for carnivorous animals establishing this function seems particularly difficult because of physiological characteristics (e.g., low dependency on drinking water (Pietsch et al. 2011)). Therefore, stable isotope analysis requires a deep knowledge of biology and the natural history of the animal, for one to shed light on what stable isotope values actually reflect.

Stable isotope analysis has been used as environmental tracers to elucidate the geographic origin of animals (Hobson and Wassenaar 2019) through isoscapes origin models. Isoscapes are models that predict the spatial distribution of stable isotopes across continents, linking isotopic signatures of the environment to animals. The isoscapes can be calibrated with the transfer functions to assign the origin of animals more accurately. The isoscape we use to assign the origin of animals is predicted with mean values of the environment in each measured location (Figure 0-1a). This isoscape presents

a temporal variation in each measured location (Figure 0-1b) and uncertainty in each point prediction (Figure 0-1c) ("IsoriX" package version 0.8.2 in R (Courtiol et al. 2019)). With the application of isoscapes, we have greatly expanded our understanding of migratory pathways. For example, past studies have successfully applied the stable isotope approach to the study of bat migration for tracing elevational (Voigt et al. 2014), latitudinal (Meehan et al. 2004; Popa-Lisseanu et al. 2012; Sullivan et al. 2012; Ossa et al. 2012; Voigt et al. 2012a; Lehnert et al. 2014; Pylant et al. 2016; Lehnert et al. 2018), and longitudinal migration (Weller et al. 2016). In addition, in Eurasian Golden Orioles (*Oriolus oriolus*) this method has even been used to investigate past migratory behaviour in relation to rainfall patterns from museum specimens, enabling us to recreate migratory patterns from almost 200 years ago (Milano et al. 2021). Researchers have used several isotopes for understanding aspects of migration, I will mainly discuss hydrogen, oxygen, strontium, carbon, and nitrogen.
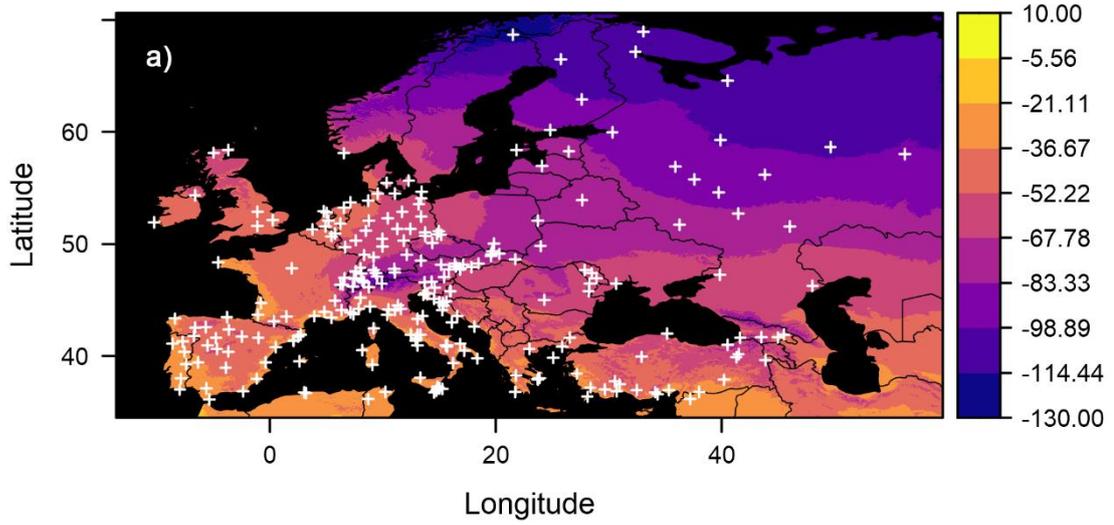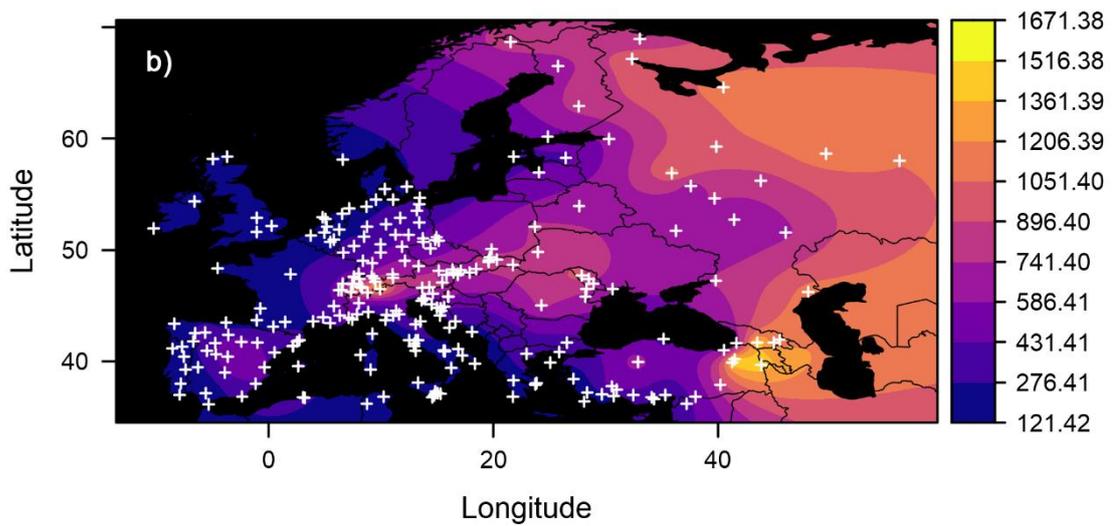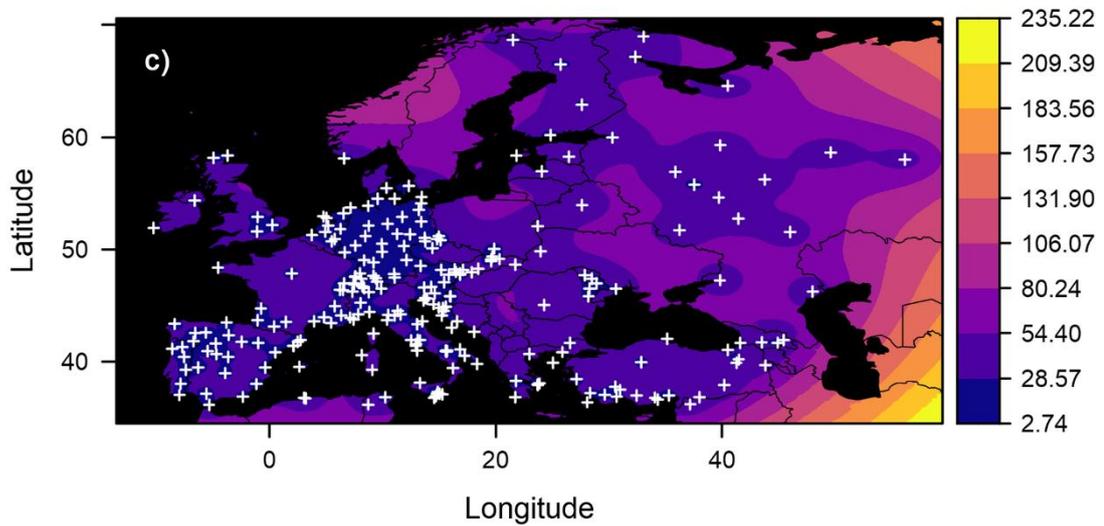
Figure 0- 1: European isoscapes of stable hydrogen isotopes. (a) Mean variation predictions from each point. (b) Residual variation to predict the temporal variation at each location. (c) Uncertainty in each point prediction. The uncertainty increases as the distance from the measured location increases. White crosses represent each source point.

2.1 When it rains, it pours – Stable hydrogen isotopes

Stable hydrogen isotope ratio (δ^2H), mainly measured in water, is the most used biomarker to determine the long-distance origin of migratory animals (Hobson 1999). In geographic assignments, tissue samples are usually compared to δ^2H recorded in precipitation. The cycle of water and the different chemical bonds the water molecule produces can lead to a variation in δ^2H over space and time, generating a gradient of values that can be distinguished over massive geospatial scales (regional, continental, global) (Courtiol et al. 2019). δ^2H varies inversely with latitude, elevation, and distance from the coast (Wassenaar 2018).

On one hand, it is relatively easy to obtain water samples from around the world and map extensive landscapes. On the other hand, the explanatory power of these models is limited because they return relatively large regions of potential origin, encompassing several hundreds of kilometres (Wassenaar 2018). The lack of precipitation data in some regions (e.g., Russia) further reduces the resolution of the predictions. δ^2H is often measured in samples from animals that have biologically inert materials with a low turnover rate, such as feathers, fur, or chitin, to depict the origin of animals where those tissues were formed (moulted, grown, or shed, respectively). Due to their low turnover rate, these materials retain their isotopic composition until the next moulting event occurs (Hobson 2005).

Hydrogen analysis has a major disadvantage because organic hydrogen can exchange with the ambient water vapour (Wassenaar and Hobson 2000). This exchange is dependent on temperature, type of tissue (C–H bonds are mainly fixed, but H bonds to other functional groups are not, e.g., –COOH, –NH2, and –SH; Wassenaar and Hobson (2000)), and methods used for tissue preparation (grinding, whole tissues, etc). Therefore, the comparison between laboratories where different air moistures might be present is an issue (Wassenaar 2018).

2.2 Breath in, breath out – Stable oxygen isotopes

Similar to δ^2H, stable oxygen isotopes ($\delta^{18}O$) are also used to determine geographical origins on a global scale (Wassenaar 2018) and are suitable markers to differentiate between terrestrial and marine biomes and between continents (Pearson et al. 2020). Figure 0-2 shows the oxygen isoscape for Europe. Even though oxygen has a key advantage over hydrogen because it does not have exchangeable parts, the analysis increases in complexity because there are more oxygen sources (air O_2, H_2O, dietary O) and sinks (H_2O, CO_2) in biological dietary systems (Wassenaar 2018). Most studies involving $\delta^{18}O$ have tried to distinguish the influence of food and ambient water, growth effects, relationships within and among tissues, etc (Kirsanow and Tuross 2011; Storm-Suke et al. 2012; Coulter et al. 2017). Pietsch et al. (2011) used δ^2H and $\delta^{18}O$ in hair to verify the provenance of wild cats that are traded illegally in the U.S. and Canada, however, found that felid bulk hair does not correlate to expected isoscapes' predictions. Further studies are necessary to understand the relationship between tissues from different animals and environmental data. Therefore, its use in migration studies remains largely unexplored.

$\delta^{18}O$ (‰)

□ -3.5 to -1.6
□ -5.5 to -3.5
□ -7.5 to -5.5
□ -9.4 to -7.5
□ -11.4 to -9.4
□ -13.3 to -11.4
□ -15.3 to -13.3
□ -17.3 to -15.3
■ -19.2 to -17.3

http://waterisotopes.org
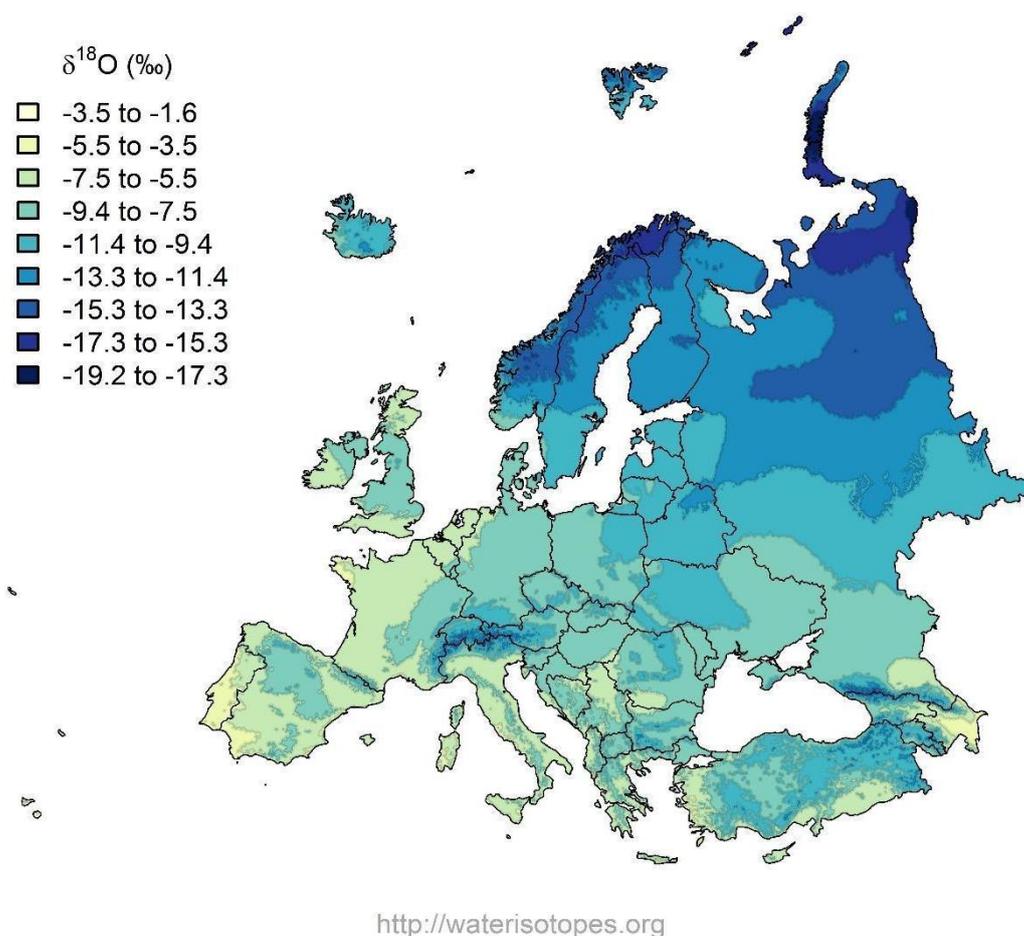
Figure 0- 2: European isoscape for stable oxygen isotope. The figure was obtained from *waterisotopes.org* archives.

2.3 We will rock you – Stable strontium isotopes

Recent studies have tried to overcome the issue of the broad-scale possible origins from δ^2H and $\delta^{18}O$ by adding other stable isotope elements, such as strontium, to isoscape origin models (Chamberlain et al. 1997; Oelze et al. 2012; Vautour et al. 2015). Stable strontium isotope ratio ($^{87}Sr/^{86}Sr$) can also be used as a complementary geochemical marker since it varies with bedrock type, soil geology, and soil age, making the variation stable (Hoogewerff et al. 2019). Figure 0-3

shows the strontium isoscape for Europe. Several studies have used $^{87}Sr/^{86}Sr$ as a marker for spatial tracking of animals (e.g., Koch et al. (1995); Sellick et al. (2009)) and humans (e.g., Bentley (2006); Font et al. (2012); Tipple et al. (2013)), as well as for provenance studies (e.g., Vogel et al. (1990); Frei et al. (2009); Voerkelius et al. (2010)). Recently, a study established a continuous-surface geographic assignment for monarch butterflies (*Danaus plexippus*) using $^{87}Sr/^{86}Sr$, which should aid in future studies using this isotope (Reich et al. 2021).

Differently from other isotopes, $^{87}Sr/^{86}Sr$ in tissues is believed to match the ecosystem values without a fractionation between trophic levels, tissues, or different species (Wassenaar 2018). This facilitates its use to match samples with environmental data. However, studies that used $^{87}Sr/^{86}Sr$ have only expressed the lack of fractionation between tooth enamel and bone tissue (Vogel et al. 1990; Clementz 2012), which have the slowest turnover period (Cerling et al. 2007), and the studies have not been performed with other tissues. More studies are required to understand the link between $^{87}Sr/^{86}Sr$ and other tissues.

Strontium Isoscape
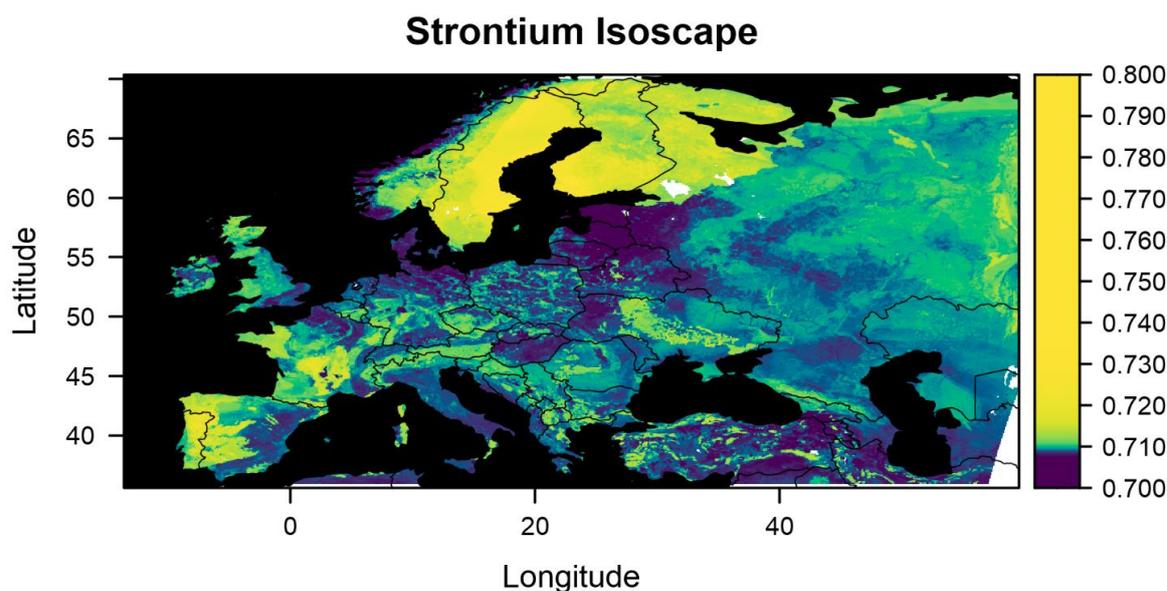
Figure 0- 3: European isoscape for stable strontium isotope. The distribution of ^{87}Sr/^{86}Sr values is heavily skewed, therefore we used a non-linear colour palette following Bataille et al. (2020).

2.4 Safe and sound – Stable carbon and nitrogen isotopes

Stable carbon (δ^{13}C) and nitrogen (δ^{15}N) isotopes have been considered for local-scale geographical assignments; however, their application to migration studies is not as straightforward as δ^2H. Similar to other isotopes, animals must move between habitats that have isotopic signatures with different C and N profiles for these isotopes to be effective at determining geographic assignments; however, variation in C and N is determined by the presence of different wild plants and often plantations in the environment (Tieszen et al. 1983), which can change depending on human activities. δ^{13}C and δ^{15}N are the most common isotopes used to determine diet, trophic level, and habitat preference.

δ^{13}C is directly linked to the three primary photosynthetic pathways of plants: C3 (e.g., most plants), CAM (e.g., succulents), and C4 (e.g., grasses). When we analyse tissues from herbivorous

animals, we can identify which type of plants they feed on (Fry 2006). $\delta^{13}C$ is also enriched by trophic level. The relative proportion of plants with different photosynthetic pathways is linked to precipitation and temperature and varies latitudinally (Fry 2006). The use of $\delta^{13}C$ for tracking animals requires confidence about the relationship between the animals and their food sources in order to obtain solid isoscape results (Hobson 2019), which is currently lacking. Therefore, $\delta^{13}C$ still requires further exploration.

$\delta^{15}N$ in consumer tissues is a way to trace the protein derived from the diet, thus it varies largely with the animal's trophic position (Vanderklift and Ponsard 2003; Caut et al. 2008a; Rex et al. 2010; Siemers et al. 2011), and to a lesser extent with the $\delta^{15}N$ values of local ecosystems (Hartman and Danin 2010). N is mainly present in lipids and carbohydrates. One disadvantage of using $\delta^{15}N$ is that it depends largely on the method of excretion an animal uses, and thus the discrimination factor of this isotope varies depending on the animal's physiology status (e.g., fasting) (Post 2002; Vanderklift and Ponsard 2003). In addition, with the introduction of fertilisers used in crops and other soils, $\delta^{15}N$ can fluctuate annually (McCutchan et al. 2003). This makes it difficult to create an isoscape for this isotope. With the use of machine learning, isoscapes for carbon and nitrogen are starting to be developed (Bowen, G. pers. comm.).

3. You can sometimes get what you want – Migratory bats

Bats, an ecologically and taxonomically diverse group, comprise approximately 20% of the mammalian diversity worldwide, with more than 1,400 recognised species (Burgin et al. 2018). However, only a few species are known to perform any type of migration, including partial (i.e., part of the population migrates) or whole population migration, and regional or long-distance migration (for more details of each species-particular migration see Table 15.1 in Krauel and Mccracken

(2013)). Considering their small size, bats are exceptional among terrestrial mammals in their ability to migrate (Krauel and McCracken 2013). Their migratory behaviour is much less common than in other flying animals, such as birds (Gauthreaux 2019). Bats cover shorter distances, rarely undergo continental migration (except *L. cinereus cinereus* (Cryan et al. 2014)), and require many stopover intervals for refuelling and rest compared to birds (Hedenström 2009; McGuire 2012).

Migratory bats have evolved a combination of physiological and morphological traits to assist their travels. They are adapted for rapid and energetically efficient flight because their wings are long and narrow (high aspect ratio), with pointed wing tips and high wing loading (Fleming 2019). Because of their ability for powered flight, they may cover several thousand kilometres during their annual journeys (Popa-Lisseanu and Voigt 2009; Alcalde et al. 2021). Yet, powered flight is energetically expensive, which forces bats to optimise their migration behaviour by minimising the energy expended in relation to the distance travelled per unit of time (maximum range speed) (Troxell et al. 2019). In addition, migratory bats consume insects while migrating (Voigt et al. 2012a), thus reducing the need for prolonged stopovers for refuelling. Lastly, they save energy by entering torpor during daytime resting, known as torpor-assisted migration (McGuire et al. 2014). Bats face several trade-offs on their journeys, between acquiring sufficient fat deposits (energy reserves) to fuel flight and maintaining optimal body conditions (weight, size) for flight with low energetic costs (Fleming et al. 2003).

In tropical bats, migration tends to occur due to seasonal fluctuation in food availability (Fleming 2019). Conversely, temperate zone bats tend to migrate to avoid low food availability and extremely low air temperatures during harsh winters, which are physiologically stressful (Fleming 2019). These species will overwinter in less harsh temperature areas, hibernating through winter as a means of energy conservation. The annual cycle of temperate zone bats continues in spring when individuals meet for reproduction (i.e., spring migration). There is often a difference between the distances

individuals migrate and the locations they spend summer in, with females migrating longer distances to form maternity colonies. Finally, in autumn both sexes restart their journeys to their hibernation sites (i.e., autumn migration). In some species (e.g., *N. noctula*, *P. nathusii*), mating occurs along the female migratory pathways with males defending roost sites that females then visit (McCracken and Wilkinson 2000). In other species (e.g., *Myotis* species in North America), individuals of both sexes form 'swarms' at the entrances of hibernation sites for mating. Fertilisation seems to occur differently depending on the female hibernation status: hibernating females store the viable sperm in their oviducts during winter, then ovulate and undergo fertilisation in the spring prior to migrating to their summer maternity roosts (Pfeiffer and Mayer 2013); non-hibernating females mate with fertilisation occurring simultaneously, prior to, or during spring migration (e.g., *L. borealis*, *L. yerbabuenae*, and *Tadarida brasiliensis*) (Orr and Zuk 2013).

Efficient conservation measures for the protection of migratory bat populations are often impaired by the lack of information on migratory corridors and stopover sites (Cryan and Brown 2007; Dzal et al. 2009; Taylor et al. 2011). Little is known about the behaviour of bats during their migration, i.e., whether they stop for extended periods or simply forage along the way. The definition of a migratory stopover is scale- and species-specific (Taylor et al. 2011). Researchers have found a few bat species that prolong their stay in specific places during autumn migration (Cryan and Brown 2007) but could not quantify how long one individual stayed. In contrast, another study showed that most individuals stayed only one night except when weather conditions forced a delay; these individuals had sufficient fat reserves to complete the migration without any additional foraging (McGuire et al. 2012). Therefore, more efforts to understand the behaviour of different species while migrating are needed.

Currently, it is widely assumed that coastal habitats and river valleys represent suitable pathways for migratory bats (Furmankiewicz and Kucharska 2009; Šuba et al. 2013). Additionally, peninsulas

have been identified as suitable stopover sites (Taylor et al. 2011). However, our understanding of where bats migrate remains largely speculative. Despite decades of banding efforts (Hutterer et al. 2005), and recent advances in GPS bio-logging methods (Richter and Cumming 2008; Weller et al. 2016) and stable isotope approaches (Cryan et al. 2004; Popa-Lisseanu et al. 2012; Fraser et al. 2012; Voigt et al. 2013, 2014, 2016, 2012c; Sullivan et al. 2012; Ossa et al. 2012; Baerwald et al. 2014; Kravchenko et al. 2015; Pylant et al. 2016; Lehnert et al. 2018), our current knowledge regarding the connectivity of different habitats is still unclear for most migratory bat species (Popa-Lisseanu and Voigt 2009; Ciechanowski et al. 2010). Because their annual ranges often encompass substantial geographic areas that usually cross different federal or international boundaries, the conservation of migratory bats can be challenging (Flaquer et al. 2009; Mas et al. 2021). Consequently, conservation efforts need to be geographically and politically broad in scope. Even though migratory animals are internationally protected by law (Conservation of Migratory Species of Wild Animals), often the conservation efforts applied to migratory animals vary between countries (Voigt et al. 2012b). Therefore, conservation efforts must involve protecting a variety of different roost sites, including those used for mating, migration, maternity colonies, foraging habitats around critical roost sites, as well as habitats used *en route* during migration (Racey and Entwistle 2003; Wiederholt et al. 2013; Voigt et al. 2018b).

3.1 Europe's next top model – Nathusius' pipistrelles (Pipistrellus nathusii) as a model for the study of migration

The European Nathusius' pipistrelle (order Chiroptera, family Vespertilionidae, *P. nathusii* (Keyserling & Blasius, 1839)) is one of the most well-studied migratory bat species worldwide. This species holds the world record for the longest migratory distance of any bat, covering more than 2,200 km one way between the Baltic states and southwestern Europe (Alcalde et al. 2021). The

species presents a head and body length of 46–55 mm and a wingspan of 220–250 mm, the forearm length ranges from 32–40 mm, and the weight is approximately 6–15.5 g (Paunović and Juste 2016). In summer, the species roosts in tree holes, buildings, and bat boxes, while in winter it prefers to hibernate in crevices in cliffs and cave entrances (Sachanowicz et al. 2019). *P. nathusii* maintains a generalist diet, feeding mainly on insects connected to aquatic habitats, such as Diptera and Chironomidae (Flaquer et al. 2009; Voigt et al. 2012a; Krüger et al. 2014). During migration, they have been found to feed while travelling ('fly-and-forage' strategy) to replenish the energy loss due to flight (Voigt et al. 2012a; Šuba et al. 2013). As for mating, males defend territories or resources against conspecific males (resource-defence polygyny) as many other European bats; however, small male aggregations seem to be common as well (Jahelková and Horáček 2011). The males are known to perform complex loud acoustic displays combined with songs (Smotherman et al. 2016) and present high fidelity to their mating roosts (Russ and Racey 2007).

Currently, the species is in the least concern category of the IUCN Red List (Paunović and Juste 2016). However, a recent study in France identified a decline of 46% in the French *P. nathusii* population (Bas et al. 2020). For more than 30 years, researchers have aimed at identifying the migratory corridors of Nathusius' pipistrelles (summary in Figure 0-4) (Kurvits et al. 2011)). Initially, they engaged in large banding campaigns at the places of summer origin or along the migratory route, hoping to receive recaptures from the area where bats hibernate. These efforts revealed that Nathusius' pipistrelles from North-eastern and Central Europe move in the Southwestern and Western directions to reach their hibernation area in France and Belgium, the Netherlands, and Luxembourg (Figure 0-4a) (Hutterer et al. 2005). Limpens and Schulte (2000) reported that Nathusius' bats may use a corridor starting in Eastern Europe, migrating along with the Polish, German, and Dutch coastline to the southwest of Germany, southeast of France, north of Italy, and Switzerland (Figure 0-4b). However, with a broader dataset, Hutterer et al. (2005) challenged this

assumption and suggested that wintering habitats should be extended along the Atlantic and Adriatic coasts. Lastly, there is evidence of current changes in the wintering ranges in Finland (Blomberg et al. 2021), which might indicate that instead of taking the route from Baltorussia (Estonia, Latvia, Lithuania, Belarus, and Russia), bats may migrate through Fennoscandia (Finland, Sweden, and Norway) and cross the Baltic Sea to continental Europe (Figure 0-4c). Evidence for this movement is weak since it is based on acoustic data, which does not inform the direction of movements. Migratory pathways between Sweden and Germany would conflict with recent offshore wind turbine developments in the Baltic Sea. Despite evidence of these migratory routes, the relative abundance of Nathusius' pipistrelles with Fennoscandian origin at the German coastline is yet unresolved. The knowledge of the location, sizes, and composition of maternity colonies and source population is essential to monitor how the species is coping with adverse challenges such as climate change and to demand proper conservation efforts from the countries in which *P. nathusii* occurs.

Currently, *P. nathusii* areas of summer residency and migratory corridors have often been associated with wetlands, such as forests adjacent to bogs, lakes, and marshlands (Flaquer et al. 2009; Dietz et al. 2009; Furmankiewicz and Kucharska 2009; Voigt et al. 2016; Ijäs et al. 2017). Multi-year recaptures have demonstrated high site fidelity at summer grounds, maternity roosts, and mating sites (Hutterer et al. 2005), suggesting that a significant proportion of the European population of Nathusius' pipistrelles crosses Germany twice each year. Consequently, Germany has an essential responsibility for protecting this and other migratory species as a central location in the flyways of European migratory bats (Voigt et al. 2015).

Nathusius' pipistrelles are the most recorded species in fatalities from wind turbines (Lehnert et al. 2014; Măntoiu et al. 2020). Bat collisions most commonly occur on nights with low wind speed (< 6 m/s), and before/after the passage of storm fronts when a large number of bats are likely to be migrating, showing a peak of fatalities occurring during migration (Rydell et al. 2010; Măntoiu et al.

2020). Estimates obtained from systematic environmental impact assessments suggest that a single turbine may lead to 2–30 bat fatalities per year if no mitigation scheme is implemented (Brinkmann et al. 2011; Korner-Nievergelt et al. 2013; Măntoiu et al. 2020). The total may sum up to hundreds of thousands of bats killed per year in countries with high wind energy production, such as Germany (Fritze et al. 2019) and the U.S. (Hayes 2013). Considering the low reproductive rate of most bat species, with only about 1–2 offspring per year (Garbino et al. 2021), wind turbine fatalities may lead to population declines because species are not able to compensate for the additional losses of individuals at wind turbines.

Nathusius' pipistrelles have been extensively studied using stable isotope analysis. Information concerning the exact moult pattern is important to ensure accurate determination of when and where the animal acquired the isotopes. However, this is still poorly documented for this species and other mammals. Fraser et al. (2013) reviewed the moulting pattern in bats, showing that in European species it mainly occurs in a single event in summer or autumn; however, it may vary between species and demographic groups. For example, the moulting period of *P. nathusii* fur was defined between late June/early July and mid-August (Voigt et al. 2016), which suggests that the species have new fur when migrating to their wintering sites in autumn. This information can be used to determine the time period the tissue analysed will reflect. In addition, because transfer functions are species-specific, to date, no transfer function for δ^2H has been established for *P. nathusii* using non-migratory individuals of this species. Previous studies that have assigned the origin of *P. nathusii* individuals have used a pool of five different bat species (Popa-Lisseanu et al. 2012; Lehnert et al. 2014). Consequently, potentially imprecise geographical origins have been assigned to those individuals.

Considering their broad European range, high vulnerability to environmental changes, and the current need to protect migratory species, Nathusius' pipistrelles are an interesting model to determine migration patterns through the application of stable isotopes.
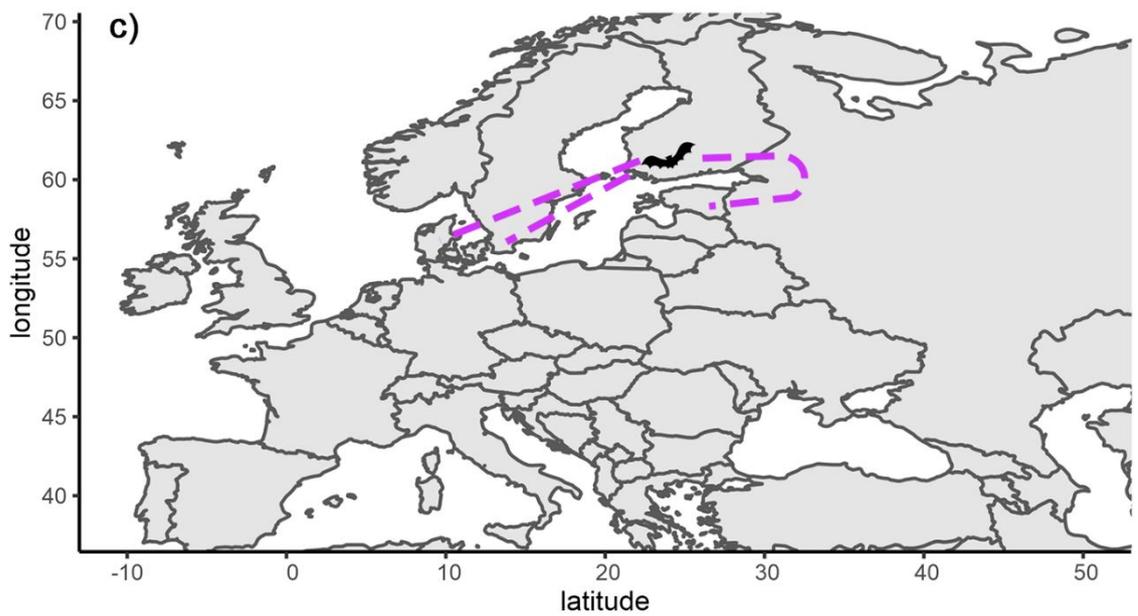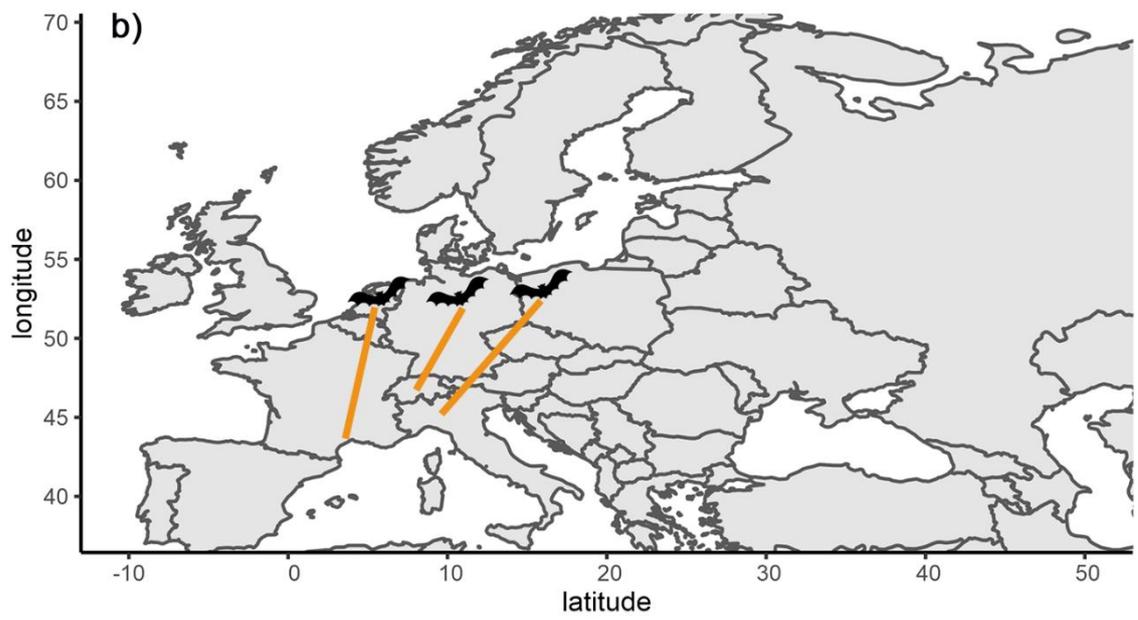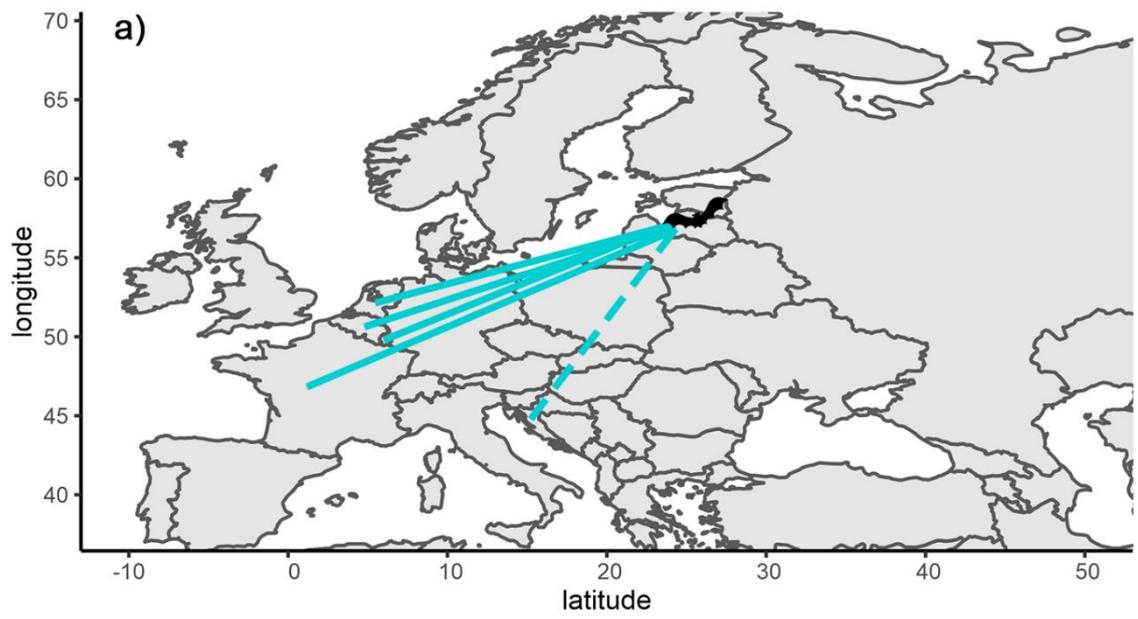
Figure 0- 4: Possible migratory routes of Nathusius' pipistrelles (*Pipistrellus nathusii*). a) Bats moving from North-eastern and Central Europe to their hibernation area in France, Belgium, the Netherlands, and Luxembourg (blue solid lines); possibly reaching the Adriatic and Atlantic seas (blue dashed lines) (Hutterer et al. 2005); b) bats migrating along with the Polish, German, and Dutch coastline to the southwest of Germany, southeast of France, north of Italy, and Switzerland (orange solid lines) (Limpens and Schulte 2000); c) bats wintering in Finland and moving through Fennoscandia (Finland, Sweden, Norway), crossing the Baltic Sea to continental Europe (purple dashed lines) (Blomberg et al. 2021).

THESIS OUTLINE

This thesis aims to apply stable isotope analysis to identify migratory pathways in *P. nathusii* and use this knowledge to help inform more adequate conservation measures for migratory bat species. In **chapter 1,** I applied a dual-isotope approach to distinguish between different Northern European pathways the *P. nathusii* could migrate (Fig. 0-5). This represents the first study using $^{87}Sr/^{86}Sr$ in fur keratin to improve probability maps of the origin of bats and establish connectivity for *P. nathusii* bats captured during migration. I analysed the summer origin of *P. nathusii* bats found in three islands in the north of Germany. As a critical stopover site for the species, the bats found in Germany may use different pathways to migrate to their winter grounds. Detailed information about winter and summer ranges, as well as corridors used for their seasonal journeys, is important to understand the bat migration cycle. Following recent summer acoustic observations of *P. nathusii* in Fennoscandia, I hypothesised that the individuals observed during spring and autumn migration on German offshore islands originated from Fennoscandia rather than the more commonly assumed

Baltorussian origin. I used stable strontium isotopes to detect a possible Fennoscandian summer origin and thus refine the broad geographical scale that stable hydrogen isoscapes provide. I predicted that the western capturing sites (Fehmarn and Heligoland) would include more bats from Fennoscandian, presenting higher ^{87}Sr/^{86}Sr values compared with the eastern capturing site (Greifswalder Oie). This would either postulate that bats of Fennoscandian origin cross Denmark and the Baltic Sea along a northeast-southwest trajectory between Sweden and Germany or bats could originate from Baltorussian populations but make use of the Fennoscandian route to reach Germany. I reported the migratory possible origins of 59 Nathusius' pipistrelles captured on Northern German islands.

Then, I present in **chapter 2** an assessment of the vulnerability of different demographic groups of *P. nathusii* bats to wind turbine mortality (Fig. 0-5). This is the first study to present the mortality risk of different groups based on carcasses and the living population. I combined the stable isotope analysis with demographic data to determine the vulnerability of specific groups of bats to wind turbine mortality in Germany. A few studies have provided information on this matter for other species (Lehnert et al. 2014; Thaxter et al. 2017; Cardenas-Ortiz et al. 2020); however, they lacked the comparison with the living population. The comparison with the living population is important to verify if such results are due to unequal ratios of sex, age, and origin in the population or are truly driven by a higher vulnerability in certain demographic groups. Hence, the data shows a unique point of view that removes a possible bias that would result from only evaluating the individuals found dead below wind turbines. I evaluated the possible mortality risk around wind turbines of different sex, age, and migratory status groups. I used 119 carcasses and 524 living individuals. I hypothesised that long-distance migrants would be more affected because they migrate using corridors with a high density of wind turbines (e.g., coast and rivers). In addition, juveniles would be more vulnerable because of their strong exploratory behaviour (Horn et al. 2008) and lack of experience in responding

to different environmental cues (Lindecke et al. 2019), such as a limited perception of danger (Hunt et al. 2017). Female bats would be at higher collision risk because of their observed attraction towards wind turbines, i.e., search for social partners or roosts for mating at wind turbines (Roeleke et al. 2016). Sex- or age-skewed mortality can gradually change the demographic structure of animal populations, leading to accelerated declines or even population collapses (Coulson et al. 2001). The impact of wind turbines on females could affect the establishment of maternity colonies and for males of leks and territories. Juveniles are a key group to quickly respond to climate change by shifting the ranges the species breed and hibernate (Kravchenko et al. 2020). The effects of wind turbines on adult long-distance migrants can minimise the migratory behaviour thus disrupting the genetic pool and increasing the genetic drift (Winder et al. 2019).

In conclusion, this project provides evidence of the functionality of different isotopes to determine the origin of migratory bats, thus the method could be applied for the conservation of a European species. This dissertation confirms the use of stable isotopes for studying migratory animals and contributes with a practical application to necessary conservation measures.

Figure 0- 5: Overview of the topics, methods, type of data, and main analyses integrated with this thesis.
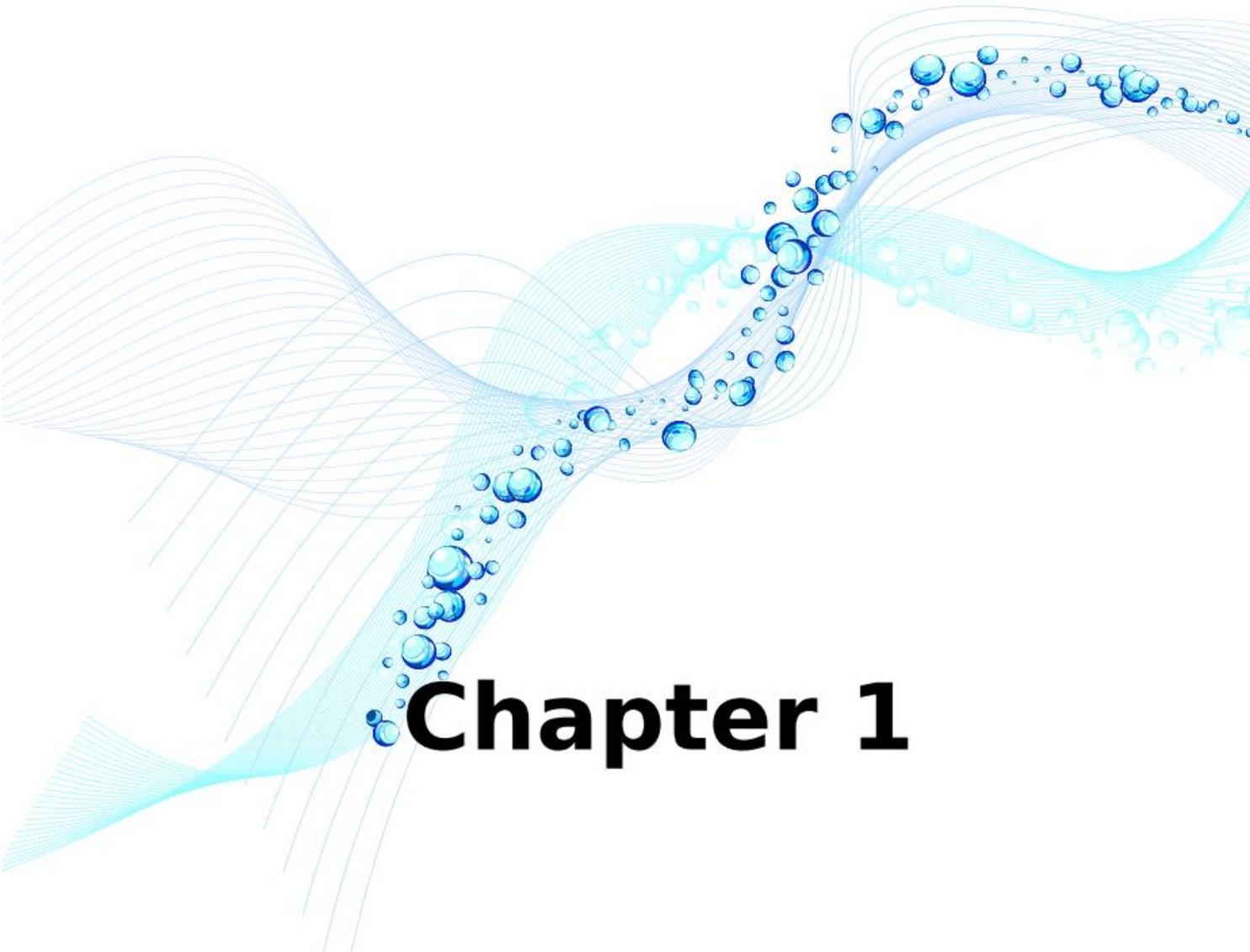
Chapter 1

Identifying migratory pathways
of Nathusius' pipistrelles
(*Pipistrellus nathusii*)
using stable hydrogen
and strontium isotopes

IDENTIFYING MIGRATORY PATHWAYS OF NATHUSIUS' PIPISTRELLES

(*PIPISTRELLUS NATHUSII*) USING STABLE HYDROGEN AND STRONTIUM ISOTOPES

Rapid Communications in Mass Spectrometry WILEY

Identifying migratory pathways of Nathusius' pipistrelles (*Pipistrellus nathusii*) using stable hydrogen and strontium isotopes

Cecilia Kruszynski[1,2] | Liam D. Bailey[1] | Alexandre Courtiol[1] | Lothar Bach[3] | Petra Bach[3] | Matthias Göttsche[4] | Michael Göttsche[5] | Reinhold Hill[6] | Oliver Lindecke[1,2] | Hinrich Matthes[7] | Henrik Pommeranz[8] | Ana G. Popa-Lisseanu[1] | Antje Seebens-Hoyer[9] | Marion Tichomirowa[10] | Christian C. Voigt[1,2]

[1]Leibniz Institute for Zoo and Wildlife Research, Alfred-Kowalke-Str. 17, Berlin, 10315, Germany

[2]Institute of Biology, Freie Universität Berlin, Königin-Luise-Str. 1-3, Berlin, 14195, Germany

[3]Freilandforschung, zoologische Gutachten, Hamfhofsweg 125b, Bremen, 28357, Germany

[4]Sodwiese 1, 24326 Stocksee, Germany

[5]Faunistica - Büro für ökologische & faunistische Freilanduntersuchungen, Jaguarring 4, Bad Segeberg, 23795, Germany

[6]Avitec Research GbR, Sachsenring 11, Germany

[7]Vorweden 1, Rostock, 18069, Germany

[8]Büro Nachtschwärmer, Augustenstr. 77, Rostock, 18055, Germany

[9]NABU Mecklenburg-Vorpommern, Wismarsche Str. 146, Schwerin, 19053, Germany

[10]Institut für Mineralogie, Technische Universität Bergakademie Freiberg, Brennhausgasse 14, Freiberg, 09599, Germany

Correspondence
C. Kruszynski, Institute of Biology, Königin-Luise-Str. 1-3, Freie Universität Berlin, 14195 Berlin, Germany.
Email: ceciliak.assis@gmail.com

Funding information
Bundesamt für Naturschutz; Conselho Nacional de Desenvolvimento Científico e Tecnológico, Grant/Award Number: 290079/2015-2

Rationale: Identifying migratory corridors of animals is essential for their effective protection, yet the exact location of such corridors is often unknown, particularly for elusive animals such as bats. While migrating along the German coastline, Nathusius' pipistrelles (*Pipistrellus nathusii*) are regularly killed at wind turbines. Therefore, we explored the paths taken on their annual journey.

Methods: We used isotope ratio mass spectrometry to measure stable hydrogen and strontium isotope ratios in fur keratin of 59 Nathusius' pipistrelles captured on three offshore islands. Samples were pre-treated before analysis to report exclusively stable isotope ratios of non-exchangeable hydrogen. We generated maps to predict summer origins of bats using isoscape models.

Results: Bats were classified as long-distance migrants, mostly originating from Eastern Europe. Hydrogen analysis suggested for some bats a possible Fennoscandian origin, yet additional information from strontium analysis excluded this possibility. Instead, our data suggest that most Nathusius' pipistrelles migrating along the German coastline were of continental European summer origin, but also highlight the possibility that Nathusius' pipistrelles of Baltorussian origin may travel offshore from Fennoscandia to Germany.

Conclusions: Our findings demonstrate the benefit of using complementary isotopic tracers for analysing the migratory pathways of bats and also potentially other terrestrial vertebrate species. Furthermore, data from our study suggest an offset of fur strontium isotope ratios in relation to local bedrock.

1 | INTRODUCTION

In the era of the Anthropocene, human-induced changes to the environment, such as land use and global climate changes, threaten a number of species, leading ultimately to a worldwide biodiversity crisis.[1–3] Migratory species are particularly vulnerable to anthropogenic changes because they depend not only on intact breeding and wintering locations, but also on a sequence of suitable and intact habitats along their migratory routes.[2,4,5] Thus, conservation efforts need to be established beyond political borders to protect suitable corridors and habitats for these highly mobile species.[6,7]

Migratory behaviour is observed in a range of vertebrate taxa, including ungulates, birds, cetaceans, sea turtles and bats.[8] Considering their small size, bats are outstanding among terrestrial mammals in their ability to cover long distances when migrating.[9,10] Yet, efficient conservation measures for the protection of migratory bats are impaired by the lack of information on migratory corridors and stopover sites.[11] Currently, it is widely assumed that coastal habitats and river valleys represent suitable pathways for migratory bats in Europe.[8,12–15] In addition, peninsulas have been identified as suitable stopover sites.[16] Other than that, our understanding of where bats migrate remains largely speculative. This lack of knowledge has emerged as a major conservation problem since thousands of migratory bats are killed by wind turbines,[6,17,18] which are increasingly erected along coastal habitats, both onshore and offshore.[13] Thus, understanding where and when bats migrate is essential for deciding where to avoid wind parks and where to establish adequate mitigation schemes.

The European bat, Nathusius' pipistrelle (*Pipistrellus nathusii*), holds the world record for long-distance migration among bats, covering more than 2200 km one-way between the Baltic countries and southwestern Europe.[8,9,19,20] Large banding campaigns along the presumed migratory routes helped to identify wintering areas based on the recapture of banded bats.[9,19,20] These banding efforts support the existence of a coastal migratory pathway of Nathusius' pipistrelles with a northeastern origin (e.g. Russia, Baltic countries, Belarus) to central, western and southwestern Europe (e.g. Spain, France, Benelux countries, Germany) in late summer[9] (Figure 1). Alternatively, acoustic recordings suggest a possible migratory pathway of Fennoscandian individuals across the Baltic Sea from Sweden via Denmark to Germany[8,21,22] (Figure 1). Recently, it was also suggested that Baltorussian bats move via Finland, Sweden and Denmark to Germany.[13,14] Migratory pathways between Sweden and Germany would conflict with recent offshore wind turbine developments in the Baltic Sea. Despite evidence of these migratory routes, the relative abundance of Nathusius' pipistrelles of Fennoscandian origin at the German coastline is as yet unresolved.

Stable isotope analysis provides a powerful tool to expand our understanding of migratory pathways and the likely origin of migratory animals. Stable isotopes have been repeatedly used as environmental tracers to shed light on the geographic origin of animals using isoscape origin models, i.e. models based on the spatial distribution of stable isotopes across continents.[23–25] Isoscape origin models involving bats are usually based on stable hydrogen isotope ratios of fur, since it is a biologically inert matrix that, once formed, carries the same isotopic composition from the moulting area to the wintering area without changing until the next moulting event occurs prior to summer migration.[26–28] In the past, isoscape origin models were based mostly on stable hydrogen isotope ratios (depicted in the delta notation δ^2H in relation to an international standard), since the δ^2H values of meteoric water follow latitudinal gradients.[29] Yet, the explanatory power of these models is limited because they return
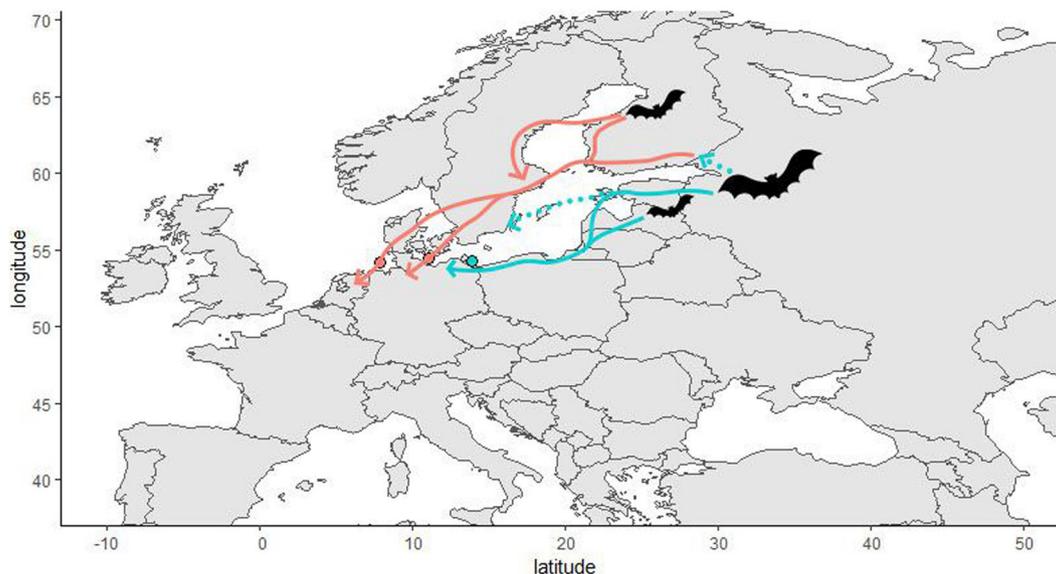
FIGURE 1 Map of potential migratory pathways of Nathusius' pipistrelles along the coast of the Baltic Sea. Blue lines highlight the pathway from Baltorussian populations to Poland and Germany. Red lines highlight the pathways of Fennoscandian populations via Denmark to Germany. Dashed blue lines represent alternative pathways of Baltorussian bats via Fennoscandia to Germany. Western offshore islands in Germany (Heligoland and Fehmarn) are indicated by red circles and the eastern island (Greiswalder Oie) by a blue circle

relatively large regions of potential origin, encompassing usually several hundreds of kilometres on the latitudinal scale.[23] Recent studies have tried to overcome this problem by adding stable isotope ratios of other elements, such as nitrogen and carbon, to the isoscape origin models.[27,30–35] Strontium stable isotope ratios (^{87}Sr/^{86}Sr) can also be used as a complementary geochemical marker since ^{87}Sr/^{86}Sr values vary with bedrock geology and age,[36] and since consumer ^{87}Sr/^{86}Sr values match closely ecosystem ^{87}Sr/^{86}Sr values.[37] For example, ^{87}Sr/^{86}Sr values were about 0.737 in tooth enamel and 0.728 in bone material of wildlife from Sweden,[38] whereas values in the same matrix were lower for wildlife of central and eastern Europe, ranging, for example, from 0.709 to 0.711 in Denmark[39] and from 0.711 to 0.712 in Estonia,[40] highlighting the contrasting ^{87}Sr/^{86}Sr values between Fennoscandia and continental Europe.[41,42] ^{87}Sr/^{86}Sr values have been used before for spatial tracking of animals[43,44] and humans,[45,46] and also for provenance studies.[47–50] Yet, few studies have combined information on δ^2H and ^{87}Sr/^{86}Sr values to infer the geographic origin of terrestrial wildlife. Here, we used a combination of δ^2H and ^{87}Sr/^{86}Sr values from fur keratin of Nathusius' pipistrelles captured on German offshore islands to discriminate between a continental European and a Fennoscandian summer origin of long-distance migratory individuals. Specifically, we tested if Nathusius' pipistrelles observed during spring and autumn migration on German offshore islands originated from Fennoscandia. We predicted that our western capturing sites (Fehmarn and Heligoland) should include more bats of Fennoscandian origin (higher ^{87}Sr/^{86}Sr values) than our eastern capturing site (Greifswalder Oie; Figure 1). This assumes that bats of Fennoscandian origin cross Denmark and the Baltic Sea along a northeast–southwest trajectory between Sweden and Germany. Alternatively, Nathusius' pipistrelles observed at the western capturing sites could originate from Baltorussian populations but make use of the Fennoscandian route to reach Germany via Denmark.

2 | METHODS

2.1 | Data collection

We captured 458 Nathusius' pipistrelles during the non-migratory period for δ^2H reference material, and 10 for ^{87}Sr/^{86}Sr reference material; and 59 during the migratory period with unknown origin. Bats were captured with mist nets and from bat boxes. δ^2H reference material bats were captured in several European sites (Table SM1, supporting information) and ^{87}Sr/^{86}Sr reference material bats were captured in Engure Lake, Latvia (57°09′44.3″N 23°13′11.0″E; Table SM2, supporting information). For the reference materials, we defined July 15th as the end of moulting period,[27] and thus included only fur samples of animals that were captured a few weeks before that date. However, we acknowledge that individual and geographic variation may cause deviations from this date.

The bats with an unknown origin were collected from two islands in the Baltic Sea, Greifswalder Oie (54°14′51.9″N 13°55′06.9″E) and Fehmarn (Wallnau 54°29′00.0″N 11°00′45.5″E), and one island in the North Sea, Heligoland (54°10′49.2″N 7°53′20.2″E; Figure 1; Table SM2, supporting information). Nathusius' pipistrelles do not establish breeding colonies on the islands of Greifswalder Oie and Heligoland, while they are assumed to breed on the island of Fehmarn, because of their presence on this island during the whole plant growth period. Sample collection was conducted in spring and autumn 2017 (May and September, respectively) and autumn 2018 (September), and samples were analysed for both isotope ratios. Spring and autumn captures were analysed separately. It is important to remember for bats captured during the spring migration period that predicted places of origin are related to the moulting area where bats remained during the summer of the previous year.

From each individual, we collected a small tuft of fur from the interscapular region. Afterwards, all bats were released at the site of capture. Fur samples were transferred to plastic vials and stored dry until further analysis. Capture and fur sample collection were conducted with the permission of local authorities, specifically permit 44.30-2017-177-Os, 60.5/Sr, VG-S-17-021, 60.5/Br and VG-19-010.

2.2 | Stable isotope analysis

We analysed samples for stable hydrogen isotope ratios (reference material and unknown origin samples) at the Stable Isotope Laboratory of the Leibniz Institute for Zoo and Wildlife Research, Berlin, Germany. Before analyses, samples were washed in 2:1 chloroform–methanol solution for 24 h to remove external contaminants. Afterwards, all samples, including keratin standards, were dried in an oven at 50°C for ten days to facilitate the equilibration of non-exchangeable hydrogen. We then loaded 0.3-mL silver capsules (IVA Analysetechnik e.K., Meerbusch, Germany) with 0.274 ± 0.01 mg of each sample, which were then transferred to an autosampler (Zero Blank Autosampler, Costech Analytical Technologies Inc., Firenze, Italy). In the autosampler, samples equilibrated for 1 h with chemically pure helium (70 mL/min; Linde, Leuna, Germany). Afterwards, they were pyrolysed at 1450°C in a Delta V Advantage isotope ratio mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) connected via a Finnigan ConFlo III interface (Thermo Fisher Scientific) to an HTO elemental analyser (Hekatech GmbH, Wegberg, Germany). We report values of stable hydrogen isotope ratios in relation to the international standard V-SMOW in the unit of per mille (‰) using the delta notation, δ^2H. During measurements, we included one USGS42 keratin standard (Tibetan Human Hair; US Geological Survey, Reston, VA, USA; −72.2 ± 0.9‰) and three laboratory keratin standards (powdered sheep hair from Sweden (Kstd.1, −111.7‰); powdered sheep hair from Spain (Kstd.2, −61.5‰); and powdered goat hair from Tanzania (Kstd.3, −26.4‰)) every 10 samples. We referenced our keratin standards to a previously established keratin standard of another laboratory and the USGS42 keratin standard.[28,51] The analytical precision based on the repeated measurements of stable hydrogen ratios in laboratory keratin standards was always better than 2‰ (one standard deviation of mean ratios).

Stable strontium isotope ratios ($^{87}Sr/^{86}Sr$) were measured in the TIMS laboratory at the Technical University Bergakademie Freiberg, Germany. The washed and dried samples (see previous paragraph; 0.5–5 mg) were loaded into clean Teflon beakers and the initial digestion was achieved with a mix of 16 M HNO_3 and 6 M HCl. After drying samples on a hotplate (80°C under clean conditions) they were taken up in 100 μL of suprapure H_2O_2 and dried again. The final dissolution step was a combined nitric acid plus hydrogen peroxide attack where the sample was taken up in 100 μL of 16 M HNO_3 + 100 μL of 35% H_2O_2 to decompose organic compounds.[52] The Sr separation procedure was based on the micro-Sr column chemistry method designed for samples with small amounts of Sr available.[53]

The microcolumns (200-μL) were made from shrinkable Teflon. The separation of Sr was achieved by Sr spec resin (Eichrom technologies LLC, Lisle, IL, USA) that was thoroughly precleaned with 6 M HCl, 0.5 M HNO_3 and H_2O.[53] All reagents had a blank contribution less than 1 pg Sr blank. With each batch of samples, one total procedural blank was determined. The average Sr total procedural blank was low (6.6 ± 3.3 pg; 2 standard deviations, $n = 4$). Samples were loaded together with TaF_5 activator[54] onto tungsten filaments that were pre-outgassed at 4.5 A for 240 min. The Sr isotope ratios were measured with a Phoenix TI mass spectrometer (Isotopx Ltd, Middlewich, UK) at the Isotope Laboratory of the Technical University Bergakademie Freiberg. The newly developed IsotopX ATONA Faraday cup detector amplifiers can precisely and accurately measure low ion currents.[55] The measurement signal of ^{87}Sr was usually >2 mV. The international Sr standard NBS 987 was measured at the same low intensities and yielded a $^{87}Sr/^{86}Sr$ ratio of 0.71024 ± 0.00010 (2 standard deviations, $n = 10$). The measurement errors of samples were usually within 0.00010, i.e. within two standard deviations.

2.3 | Geographic assignment based on keratin δ^2H values

We used the package IsoriX[56] to generate group maps based on δ^2H values that showed the likely summer origin of Nathusius' pipistrelles from each sampling location and season (Greifswalder Oie was sampled in spring and autumn). The approach uses the Global Network of Isotopes in Precipitation (GNIP) monthly data (May to July 1960 to 2018) from the International Atomic Energy Agency (IAEA, Vienna, Austria) aggregated by 4 × 4 cell group to generate a precipitation δ^2H isoscape for Europe with a 0.03° grid resolution. We only considered possible origin locations inside the breeding area of the species as defined by the IUCN records and modified by national bat experts of the UNEP/EUROBATS advisory committee.

We established the transfer function by regressing δ^2H values based on 458 δ^2H reference samples against mapped δ^2H values of mean monthly precipitation.[57] The transfer function defines how data from bats with unknown origin are related to the environment where fur was collected, providing the basis for a δ^2H isoscape with

predicted sample values. It also accounts for potential geospatial assignment errors. We compared the slope and the uncertainty around values of the previously established transfer function of common noctule bats[58] with our newly developed transfer function for Nathusius' pipistrelles using a t-test.

Using the δ^2H isoscape with predicted sample values, we assigned every individual a p-value for each of the 0.03 km grid cells to test the putative summer origin.[56] Any location with $p \leq 0.05$ was not considered to be a likely place of origin (i.e. the δ^2H value of the individual fell outside the 95% confidence interval of the possible origin location). Individuals were classified as long-distance migrants when the sampling location was not considered a possible origin location (i.e. $p \leq 0.05$). All other animals were considered regional bats. To simplify the description of the migratory origin of Nathusius' pipistrelles, we categorized individuals into groups based on their δ^2H values, covering ranges of 20‰, so-called isotopic bins, from −140‰ to −60‰. For each isotopic bin, we pooled the individual p-values using Fisher's combined probability test to generate a single map showing the possible origin locations for all bats of the same bin.[56]

2.4 | Geographic assignment based on keratin $^{87}Sr/^{86}Sr$ values

We developed additional maps to show the likely summer origin of Nathusius' pipistrelles from each sampling location and season based on $^{87}Sr/^{86}Sr$ values. We aggregated $^{87}Sr/^{86}Sr$ values from soil data provided in Bataille et al[42] by calculating mean $^{87}Sr/^{86}Sr$ values within 10 × 10 cell groups to return a 0.07° × 0.06° grid resolution. We used the aggregated $^{87}Sr/^{86}Sr$ data as a strontium isoscape for Europe. Aggregation was necessary to limit computational time. We used the $^{87}Sr/^{86}Sr$ values of individuals of the same isotopic δ^2H bin to generate maps showing possible origin locations inside the species distribution range. We used a Kolmogorov–Smirnov test to compare the probability distribution of $^{87}Sr/^{86}Sr$ in bats and each possible origin location.

Previous studies tested for a possible fractionation of strontium isotopes in consumer tissue samples and overall observed no significant deviation from local bedrock chemistry.[37,59–61] It has thus been generalized that strontium isotope ratios do not fractionate in relation to endogenous sources such as diet.[37] Yet, most previous studies investigated bone collagen and dentine in mammals, or chitin in insects.[37,48,62,63] In human hair, $^{87}Sr/^{86}Sr$ values seem to vary according to the relative contribution of endogenous (diet) and exogenous (ambient humidity, dust and other contaminants) sources.[46,64] We inferred from these findings that the $^{87}Sr/^{86}Sr$ values of wildlife fur might deviate from those of dietary sources if contaminants add a distinct strontium isotope signal to keratin. Given the paucity of data for strontium isotopes in wildlife species in general and bats in particular, we tested for a possible fractionation effect of strontium isotopes in bat fur. To this end, we calculated a mean $^{87}Sr/^{86}Sr$ value within a 100 km buffer zone around the $^{87}Sr/^{86}Sr$

reference material capturing site (i.e. Engure Lake, Latvia). We then calculated the mean difference between $^{87}Sr/^{86}Sr$ bedrock values and the $^{87}Sr/^{86}Sr$ values of the reference material bats. This mean difference was used as the trophic discrimination. We used this trophic discrimination factor to create alternative $^{87}Sr/^{86}Sr$ maps for the likely origin of Nathusius' pipistrelles captured at the three German offshore islands.

We also ran two-way analysis of variance models to test the influence of location and sex on δ^2H and $^{87}Sr/^{86}Sr$ values, followed by a *post hoc* Tukey's test. All analyses were conducted in R.[65]

3 | RESULTS

3.1 | Isoscapes

We established a hydrogen isoscape for the summer locations of Nathusius' pipistrelles at a 0.03 km grid resolution. Within the

Nathusius' pipistrelle breeding range, the highest δ^2H values of the isoscape were observed in central Italy and between the Black Sea and the Caspian Sea (Figure 2). We also observed relatively high δ^2H values for France and the UK. Lowest values were observed in northern Russia. The established $^{87}Sr/^{86}Sr$ isoscape (0.07 km grid resolution) showed average $^{87}Sr/^{86}Sr$ values of 0.7163 for the Fennoscandia breeding area and 0.7100 for the continental European breeding area. Peak $^{87}Sr/^{86}Sr$ values were observed in southeastern Sweden and lowest $^{87}Sr/^{86}Sr$ values in western Estonia (Figure 2). Relatively high $^{87}Sr/^{86}Sr$ values were also reported for western France, for parts of Ukraine, Czech Republic, the UK and the Caucasian mountains (Figure 2).

3.2 | Capture and stable isotope data

In total, we captured 59 Nathusius' pipistrelles at three places in northern Germany: one island in the North Sea (Heligoland: 9 males/2
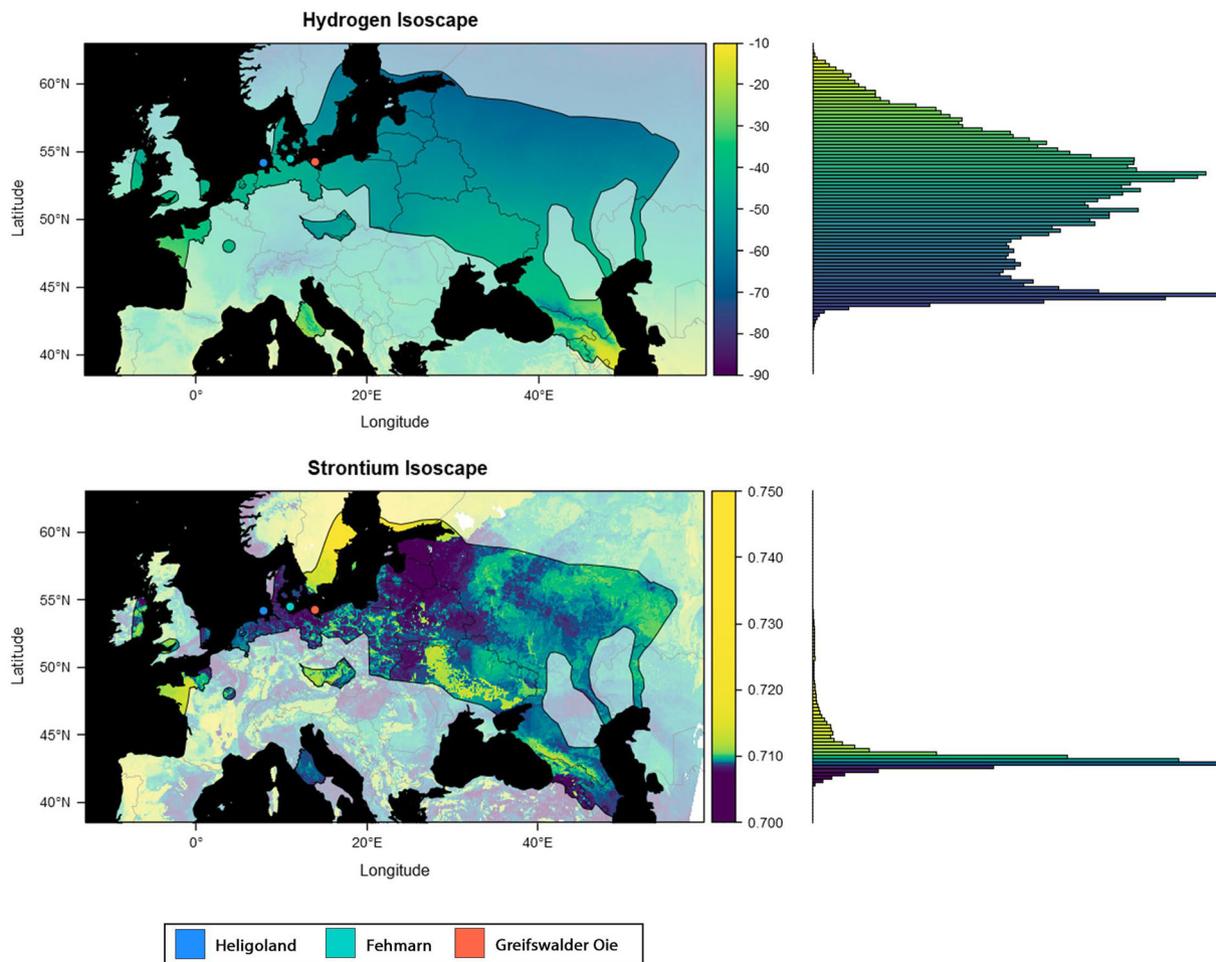
FIGURE 2 European isoscapes of stable hydrogen and strontium isotope ratios from precipitation and bioavailable strontium data, respectively. The highlighted polygon is the current breeding range of Nathusius' pipistrelles in Europe (IUCN report, adjusted in Eastern Europe based on pers. comm. Dekker J, Keribirou C, Kravchenko K). We used a non-linear colour pallet for the $^{87}Sr/^{86}Sr$ isoscape, following[42], because the distribution of $^{87}Sr/^{86}Sr$ values is heavily skewed. Dots represent Heligoland (blue), Fehmarn (green) and Greifswalder Oie (orange). We also report density plots for both isotope ratios

females) and two islands in the Baltic Sea (Greifswalder Oie: spring: 3 males/12 females and 3 unknown sex; autumn: 7 unknown sex; Fehmarn: 12 males/10 females and 1 unknown sex). The fur keratin of bats captured on Fehmarn was more than 10‰ enriched in deuterium in relation to protium than that of the fur of bats captured at other sites (mean (and standard deviation) values: Fehmarn: −102.8‰ (16.6); Greifswalder Oie spring: −111.3‰ (8.2); Greifswalder Oie autumn: −118.9‰ (5.3); Heligoland: −116.3‰ (11.8)). The δ^2H values did not differ for Nathusius' pipistrelles captured during autumn and spring on the island Greifswalder Oie (Table SM3, supporting information). The $^{87}Sr/^{86}Sr$ values varied by 0.0001 between the three islands, with fur from bats captured on Greifswalder Oie being most depleted and that from bats captured on Fehmarn most enriched in heavy to light strontium isotopes (Figure 3).

The δ^2H values of Nathusius' pipistrelles captured on the three islands were significantly different between locations ($F_{2,56} = 5.17$, $p = 0.0047$) but not between sexes ($F_{3,55} = 2.08$, $p = 0.11$). The $^{87}Sr/^{86}Sr$ values of fur keratin were significantly lower in females than in males ($F_{3,55} = 5.17$, $p = 0.0032$), but did not differ between locations ($F_{2,56} = 0.53$, $p = 0.59$). Average and standard deviations of δ^2H and $^{87}Sr/^{86}Sr$ values are reported for each location in Table SM3 (supporting information).

3.3 | Transfer function

Based on the δ^2H reference material, we established a transfer function for the relationship between δ^2H values in fur keratin of Nathusius' pipistrelles and those of mean annual precipitation water at the capture sites of bats (Figure 4). The transfer function for

Nathusius' pipistrelles reads: intercept = −83.96 ± 14.48, slope = 0.74 ± 0.31. The slope of the transfer function for the other European non-migratory bats[66] (intercept = −30.54 ± 5.27; slope = 0.92 ± 0.09) was significantly steeper than that of the Nathusius' pipistrelle transfer function ($t = 2.07$; df = 791; $p = 0.038$).

For the $^{87}Sr/^{86}Sr$ bat reference material, we calculated a trophic discrimination factor of 0.0028 ± 0.0002, based on the difference between the mean of the bioavailable $^{87}Sr/^{86}Sr$ values in the local ecosystem (Table SM3, supporting information) and the mean of the fur keratin $^{87}Sr/^{86}Sr$ values.

3.4 | Geographic assignment based on keratin stable isotope ratios

Nathusius' pipistrelles were assigned to three isotopic bins based on δ^2H values with possible locations from northeastern to southwestern Europe: range 1 (−140‰ to −121‰; northern range; Eastern Europe), range 2 (−120‰ to −101‰; central range; Central Europe) and range 3 (−100‰ to −85.4‰; southern range; Western Europe; Figures 5A–5C). The δ^2H values of fur keratin classified them as long-distance migrants ($n = 58$). One individual from Fehmarn was not assigned to any bin and considered an outlier ($\delta^2H = −66‰$), and therefore excluded from all further analyses. All the Nathusius' pipistrelles captured on Heligoland were assigned to ranges 1 and 2 (45% and 55%, respectively; Figure 5). The majority of Nathusius' pipistrelles captured in spring on Greifswalder Oie probably originated from range 2 (72%). Bats captured on the island of Fehmarn and in autumn on Greifswalder Oie were assigned to all three ranges at varying proportions (Fehmarn: 43%, 30%, 22%; Greifswalder Oie: 11%, 72%, 17%, respectively; Figures 5A–5C).
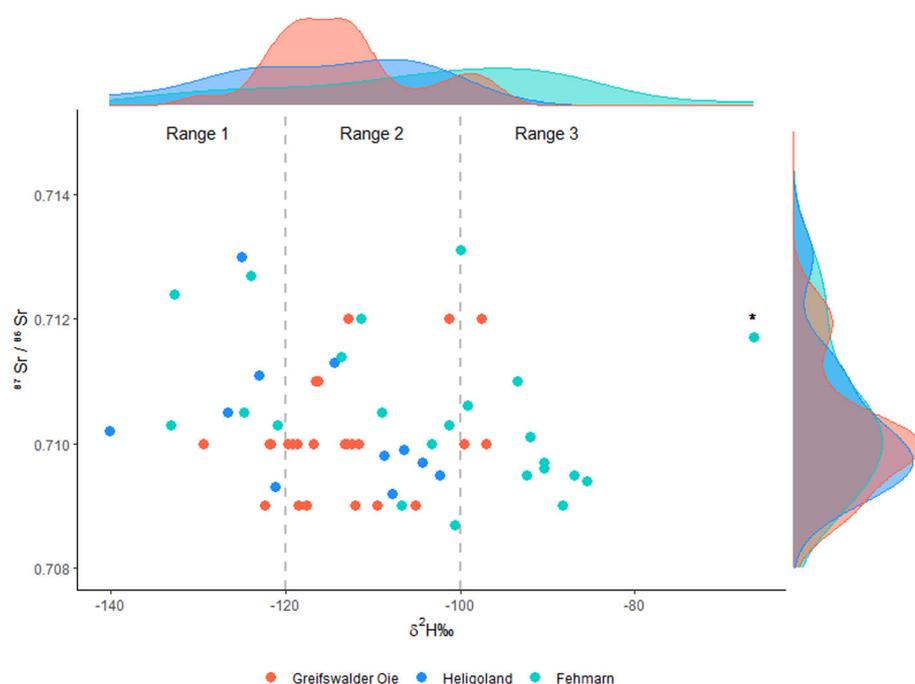
FIGURE 3 δ^2H (‰) and $^{87}Sr/^{86}Sr$ raw values of 59 Nathusius' pipistrelles captured during the migratory period on offshore islands in Germany. Ranges 1 to 3 represent the ranges in assignment maps. Note the outlier shown (*) was not used for further analysis

FIGURE 4 Relationship between the stable hydrogen isotopic ratios (δ^2H values) of fur keratin in 458 Nathusius' pipistrelles captured during the non-migration period in Europe and the isotopic ratios of monthly precipitation (δ^2H values) in their environment (red triangles). In addition, we plotted the same relationship for 335 bats of five non-migratory species ($n = 224$) and common noctules ($n = 111$) captured during their non-migration period for comparison ([67]; black dots). Solid lines represent the transfer functions for each dataset and dashed lines represent the confidence intervals
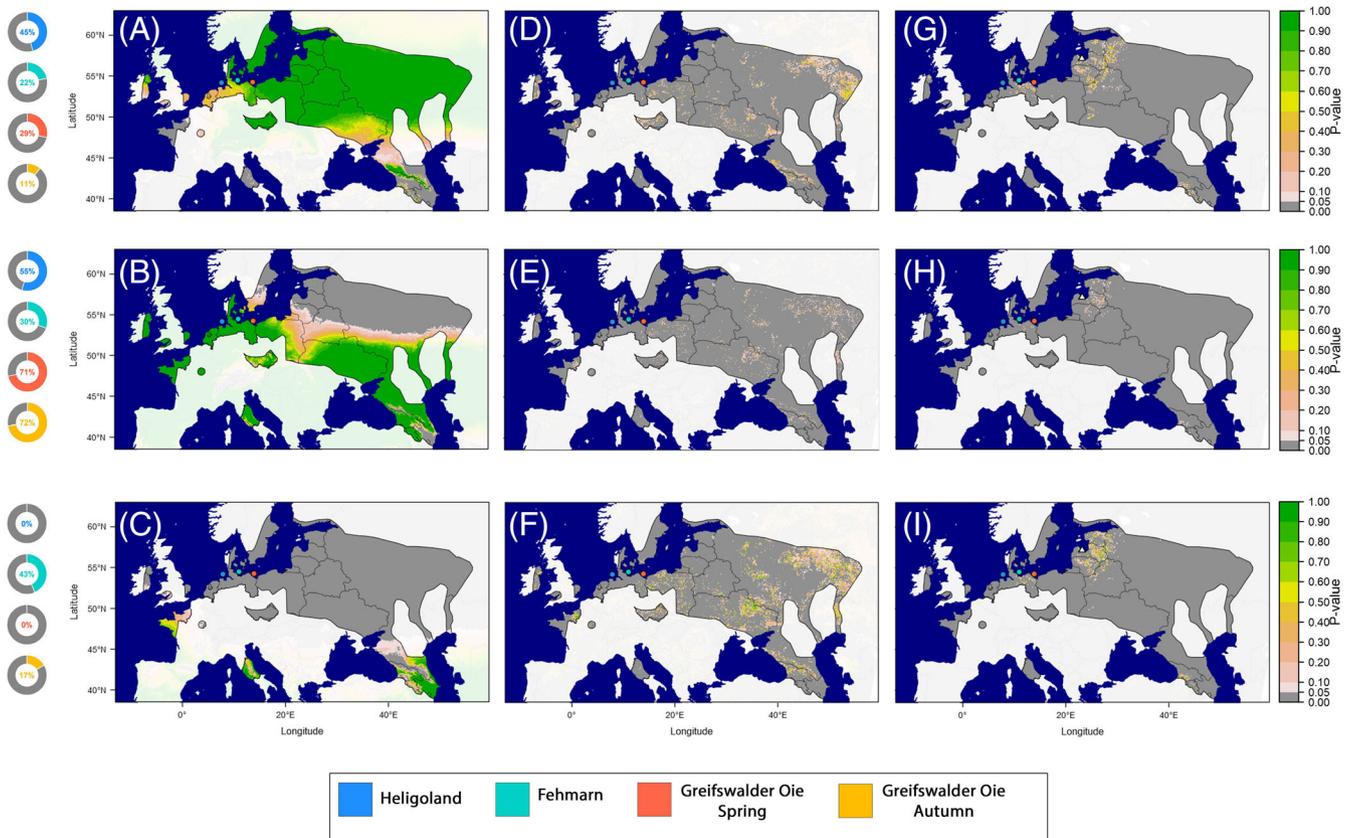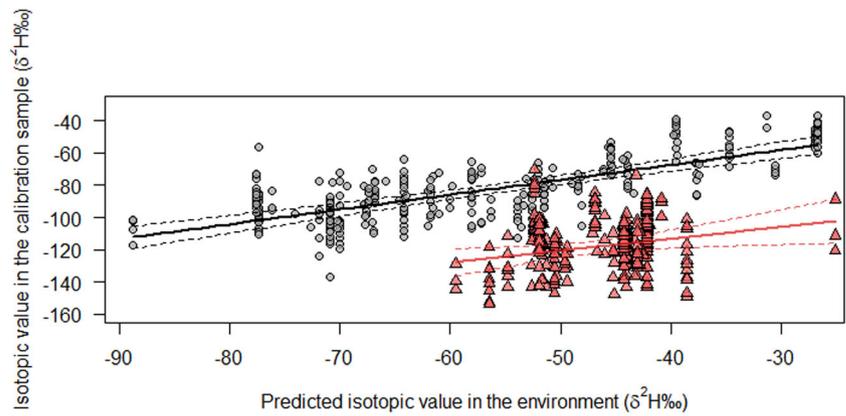
FIGURE 5 Maps depicting the likely summer origin of 58 Nathusius' pipistrelles captured on three offshore islands in Germany (Heligoland, Fehmarn, Greifswalder Oie). Geographic assignments were based on keratin δ^2H values (A, B, C), raw ^{87}Sr/^{86}Sr values (D, E, F) and ^{87}Sr/^{86}Sr values corrected for isotopic discrimination based on values from bats captured at Engure Lake, Latvia (white triangle; G, H, I). Bats were assigned to three isotopic bins derived from 20 ‰ ranges of precipitation δ^2H values, referring to a northern origin (A, D, G), central origin (B, E, H) and southern origin (C, F, I). P-values above 0.05 (indicated by a non-grey colour) are possible places of origin and p-values below 0.05 (indicated by grey) unlikely places of origin. The pie charts represent the proportion of the bats assigned to each of the possible origins captured in Heligoland (blue), Fehmarn (green) and Greifswalder Oie spring (orange) and autumn (yellow), respectively. The highlighted polygon is the current breeding range of Nathusius' pipistrelles in Europe modified from the IUCN distribution map and according to bat experts from the UNEP/EUROBATS advisory committee (see Figure 2 for details)

The ^{87}Sr/^{86}Sr values in fur keratin of bats (^{87}Sr/^{86}Sr keratin; Table SM2, supporting information) indicated that captured individuals were unlikely to have moulted in summer in Fennoscandia. The most likely places of summer origin for bats included multiple spots in continental Europe (Figures 5D–5F) with a concentration of possible origins in Poland, Russia and Ukraine. This pattern changed when assuming trophic discrimination of strontium isotopes as observed in the local population of Engure Lake, Latvia (^{87}Sr/^{86}Sr keratin$_{corr}$; Table SM3, supporting information; Figures 5G–5I). After correcting for trophic discrimination, the isoscape models suggested

likely places of summer origin in the Baltic countries for bats from the northern and central range (isotopic bins 1 and 2; Figures 5A, 5G, 5B and 5H). For bats of southern origin, probability maps of δ^2H and $^{87}Sr/^{86}Sr$ did not reach a consensus for the likely place of summer origin (Figures 5C and 5I). Similar to uncorrected $^{87}Sr/^{86}Sr$ values, the corrected values of Nathusius' pipistrelles were always lower than values reported for wildlife in Fennoscandia (0.737 in tooth enamel and 0.728 in bone material of wildlife species from Sweden[38]).

4 | DISCUSSION

Migration is an essential part of the annual life cycle of billions of animals globally. However, anthropogenic changes to the environment threaten many of these animals during their seasonal journeys. In Europe, migratory bats are frequently killed by wind turbines.[6,67] To prevent bat fatalities, we need to gain a more detailed understanding about the summer and wintering ranges of these bats, in combination with information on the corridors used for their seasonal journeys. Nathusius' pipistrelles hold the world record for long-distance migration with animals moving seasonally between the Baltic countries and southern France or northern Spain.[20] Previous studies suggested coastal and offshore migration for this bat species,[8,13,14,22,68,69] yet we lack data on the breeding origins of individuals observed at these coastal migratory corridors. Here, we established a dual-isotope approach to aid the probability maps built on hydrogen isoscapes only. This study is the first to use strontium isotope ratios in fur keratin to establish connectivity between the places where migratory Nathusius' pipistrelles were captured en route (three offshore islands in Germany) and the summer moulting places. Our data suggest possible movements of Nathusius' pipistrelles in eastern (origin in France and the British Isles) and western directions (origin in Russia and the Baltic countries). However, they do not support a large influx of Fennoscandian Nathusius' pipistrelles via Denmark to Germany which would have been expected for the western islands (Heligoland and Fehmarn). Consequently, this evidence refutes our hypothesis that Nathusius' pipistrelles captured on German offshore islands originate from either Fennoscandia or Baltorussia, depending on a western or eastern location of the islands, respectively.

Based on isotopic evidence, our study indicates that most Nathusius' pipistrelles move from the northeastern summer range to the three German offshore islands in the Baltic Sea (Greifswalder Oie and Fehmarn) and North Sea (Heligoland). Most captured bats were categorized as long-distance migrants from the Baltic countries, Poland, or Ukraine and Russia, with some individuals potentially originating from western Europe (France). Bats are known to occur in Fennoscandia[13,14,22]; however, we found no evidence of Fennoscandian origin in our data. This may be because bats of Fennoscandian origin make up only a small proportion of the migratory population and were missed by our limited sampling. Alternatively, Fennoscandian bats may use different pathways that do not include our study sites. Finally, some Nathusius' pipistrelles

observed during late summer in Fennoscandia could have originated from the Baltorussian region, which could occur if bats show northward movements before heading in a southwestern direction to the wintering ranges (Figure 1). In this case, bats observed in Fennoscandia would show an isotopic profile similar to that of bats originating in the Baltic countries.

Although our findings support a Baltorussian origin of most Nathusius' pipistrelles captured on German offshore islands, we cannot rule out the possibility that those bats are using two migratory corridors to reach the offshore islands: one from Baltorussia along the Polish coastline to western Europe and the other from Baltorussia, to Finland, Sweden and Denmark, then to Germany. The latter migratory route would involve offshore migration.

The lack of individuals with a Fennoscandian origin at our capture site is surprising since the island of Fehmarn is located inside the presumed migratory corridor of Fennoscandian bats.[8] Alternatively, bats of Fennoscandian origin may choose other migratory corridors that we missed with our limited field campaign. Isotopic evidence of bats captured on the island of Fehmarn suggested a broad area of origin across continental Europe. If we assume that the raw $^{87}Sr/^{86}Sr$ values in fur keratin are representative of local bedrock type, our probability maps indicated that some individuals at the Fehmarn sample site may have originated from western France (Normandy and Brittany) where a small breeding population exists.[70,71] However, we observed a difference between the $^{87}Sr/^{86}Sr$ values of fur keratin and those of the local ecosystem in bats captured during the moulting period in Latvia. This is surprising because stable isotopes of relatively heavy elements, such as strontium, are not expected to fractionate along the food chain.[37] Possibly, the assumption of strontium isotopes not undergoing trophic discrimination may be an artefact of previous studies focusing almost exclusively on teeth enamel or bone material of mammals[72] or chitin of insects[37] as a matrix for analysis. The $^{87}Sr/^{86}Sr$ values of these matrices may be dominated by dietary sources which could explain the close match with the environmental baseline. The $^{87}Sr/^{86}Sr$ values of keratin are known to carry both an internal dietary and an environmental signal.[73] These environmental signals stem from aerosols, particulates and environmental water.[74] They alter the $^{87}Sr/^{86}Sr$ values of hair and fur keratin,[64] yet their impact on geographic assignments may remain small,[64] particularly when the $^{87}Sr/^{86}Sr$ values of internal sources (diet) resemble those of external sources (e.g. meteoric water). We consider it unlikely that external sources contaminated the geospatial signal of $^{87}Sr/^{86}Sr$ values because bats usually do not expose their fur to precipitation, as this would impair their flight ability.[75] We suggest establishing $^{87}Sr/^{86}Sr$ data for other mammal species and other regions to verify if the trophic discrimination observed for fur keratin in this particular bat species is consistent for other wildlife species. If the corrected $^{87}Sr/^{86}Sr$ values in fur keratin of Nathusius' pipistrelles more accurately match local bedrock type, our data suggest a Baltic origin of our northern and central groups (isotopic bins 1 and 2). This is consistent with migration pathways established in banding studies (i.e. a northeastern and southwestern pathway). We did not find a consensus for derived hydrogen and strontium enriched probability

maps, since presumed places of origin in western Europe (France and British Isles) were inconsistent between the strontium data. These contradictory results require additional studies to ensure that accurate conclusions are reached with these data.

Our isotopic approach was impeded by the large variation in δ^2H values observed in non-migratory Nathusius' pipistrelles across Europe, which resulted in broad isotopic bins (i.e. most of central Europe was covered in range 2). Indeed, the transfer function established for Nathusius' pipistrelles deviated largely from the previously established transfer function of a sympatric aerial-hawking species, the common noctule bat.[66] This is probably caused by a larger contribution of food items of aquatic origin, such as chironomid flies, in the diet of Nathusius' pipistrelles than, for example, in the diet of common noctule bats and other bats.[12,76,77] A previous isotopic study with a focus on non-migratory European bat species showed that δ^2H values varied largely with the relative contribution of aquatic to terrestrial food items to the diet of bats.[78] By these Nathusius' pipistrelles consuming a mixture of insects of aquatic and terrestrial origin, the δ^2H values in their fur might become highly variable within local populations, which may explain why the transfer function of this species shows an offset and a larger variation of δ^2H values for a given location than the transfer function previously established for non-migratory bats.

It is noteworthy that we documented a likely origin of Nathusius' pipistrelles in various areas of Poland, Russia and Ukraine. This would imply that populations from these countries head northward before changing to the southwestern direction of their wintering sites. Although our isotopic evidence for this scenario is weak, acoustic recordings also suggest a northward movement of migratory Nathusius' pipistrelles from Baltic countries to Finland.[13] Migratory movements of Nathusius' pipistrelles may be more complex than previously assumed, especially when animals perform indirect migration. Isotopic assignments of animals may be insufficient to offer unambiguous insights into the exact migratory corridors used by bats when moving along or across the Baltic Sea. We suggest engaging in large-scale radio-tracking campaigns using miniaturized radio transmitters and automated receiver stations to unravel the exact locations of migratory corridors. At this point, our data do not argue against an influx of Nathusius' pipistrelles migrating from Sweden to Germany; however, these individuals may originate from Baltorussian populations.

ORCID

Cecilia Kruszynski https://orcid.org/0000-0001-5673-3236
Liam D. Bailey https://orcid.org/0000-0002-8226-9454
Alexandre Courtiol https://orcid.org/0000-0003-0637-2959

REFERENCES

1. Koh LP, Dunn RR, Sodhi NS, Colwell RK, Proctor HC, Smith VS. Species coextinctions and the biodiversity crisis. *Science*. 2004;305 (5690):1632-1634. https://doi.org/10.1126/science.1101101
2. O'Connor B, Bojinski S, Röösli C, Schaepman ME. Monitoring global changes in biodiversity and climate essential as ecological crisis intensifies. *Eco Inform*. 2020;55:101033. https://doi.org/10.1016/j.ecoinf.2019.101033
3. Henriques S, Böhm M, Collen B, et al. Accelerating the monitoring of global biodiversity: Revisiting the sampled approach to generating red list indices. *Conserv Lett*. 2020;13(3). https://doi.org/10.1111/conl.12703
4. de Camargo Barbosa KV, Rodewald AD, Ribeiro MC, Jahn AE. Noise level and water distance drive resident and migratory bird species richness within a neotropical megacity. *Landsc Urban Plan*. 2020;197(January):103769. https://doi.org/10.1016/j.landurbplan.2020.103769
5. Cardenas-Ortiz L, Bayly NJ, Kardynal KJ, Hobson KA. Defining catchment origins of a geographical bottleneck: Implications of population mixing and phenological overlap for the conservation of neotropical migratory birds. *Condor*. March 2020;122(2). https://doi.org/10.1093/condor/duaa004
6. Voigt CC, Lehnert LS, Petersons G, Adorf F, Bach L. Wildlife and renewable energy: German politics cross migratory bats. *Eur J Wildl Res*. 2015;61(2):213-219. https://doi.org/10.1007/s10344-015-0903-y
7. Dallimer M, Strange N. Why socio-political borders and boundaries matter in conservation. *Trends Ecol Evol*. 2015;30(3):132-139. https://doi.org/10.1016/j.tree.2014.12.004
8. Kurvits T, Nellermann C, Alfthan B, et al. *Living Planet: Connected Planet. Preventing the End of the World's Wildlife Migrations through Ecological Networks*; 2011.
9. Hutterer R, Ivanova T, Meyer-Cords C, Rodrigues LL. *Bat Migrations in Europe: A Review of Banding Data and Literature*; 2005. https://www.researchgate.net/publication/311443225_Bat_migration_in_europe_A_review_of_banding_data_and_literature. Accessed March 11, 2020.
10. Krauel JJ, McCracken GF. Recent advances in bat migration research. In: *Bat Evolution, Ecology, and Conservation*. New York, NY: Springer; 2013:293-313.
11. Popa-Lisseanu AG, Voigt CC. Bats on the move. *J Mammal*. 2009;90 (6):1283-1289. https://doi.org/10.1644/09-MAMM-S-130R2.1
12. Furmankiewicz J, Kucharska M. Migration of bats along a large river valley in southwestern Poland. *J Mammal*. 2009;90(6):1310-1317. https://doi.org/10.1644/09-mamm-s-099r1.1
13. Gaultier SP, Blomberg AS, Ijäs A, et al. Bats and wind farms: The role and importance of the Baltic Sea countries in the European context of power transition and biodiversity conservation. *Environ Sci Technol*. 2020;54(17):10385-10398. https://doi.org/10.1021/acs.est.0c00070

14. Ijäs A, Kahilainen A, Vasko VV, Lilley TM. Evidence of the migratory bat, *Pipistrellus nathusii*, aggregating to the coastlines in the northern Baltic Sea. *Acta Chiropterologica*. 2017;19(1):127. https://doi.org/10.3161/15081109acc2017.19.1.010

15. Lindecke O, Voigt CC, Pētersons G, Holland RA. Polarized skylight does not calibrate the compass system of a migratory bat. *Biol Lett*. 2015;11(9):20150525. https://doi.org/10.1098/rsbl.2015.0525

16. Ciechanowski M, Jakusz-Gostomska A, Żmihorski M. Empty in summer, crowded during migration? Structure of assemblage, distribution pattern and habitat use by bats (Chiroptera: Vespertilionidae) in a narrow, marine peninsula. *Mammal Res*. 2016; 61(1):45-55. https://doi.org/10.1007/s13364-015-0249-6

17. Rydell J, Bach L, Dubourg-Savage M-J, Green M, Rodrigues L, Hedenström A. Bat mortality at wind turbines in northwestern Europe. *Acta Chiropterologica*. 2010;12(2):261-274. https://doi.org/10.3161/150811010x537846

18. Arnett EB, Baerwald EF, Mathews F, et al. Impacts of wind energy development on bats: A global perspective. In: *Bats in the Anthropocene: Conservation of Bats in a Changing World*. Cham: Springer International; 2016:295-323.

19. Alcalde JT, Jiménez M, Brila I, Vintulis V, Voigt CC, Pētersons G. Transcontinental 2200 km migration of a Nathusius' pipistrelle (*Pipistrellus nathusii*) across Europe. *Mammalia*. 2020. https://doi.org/10.1515/mammalia-2020-0069

20. Pētersons G. Seasonal migrations of north-eastern populations of Nathusius' bat *Pipistrellus nathusii* (Chiroptera). *Myotis*. 2004;41–42 (November):29-56. https://www.researchgate.net/publication/313473217 Accessed March 11, 2020

21. Rydell J, Bach L, Bach P, et al. Phenology of migratory bat activity across the Baltic Sea and the south-eastern North Sea. *Acta Chiropterologica*. 2014;16(1):139-147. https://doi.org/10.3161/150811014x683354

22. Ahlén I, Baagøe HJ, Bach L. Behavior of Scandinavian bats during migration and foraging at sea. *J Mammal*. 2009;90(6):1318-1323. https://doi.org/10.1644/09-MAMM-S-223R.1

23. Hobson K, Wassenaar L. *Tracking Animal Migration with Stable Isotopes*. London: Elsevier; 2019. https://doi.org/10.1016/C2017-0-01125-4

24. Vander Zanden HB, Nelson DM, Wunder MB, Conkling TJ, Katzner T. Application of isoscapes to determine geographic origin of terrestrial wildlife for conservation and management. *Biol Conserv*. 2018;228: 268-280. https://doi.org/10.1016/j.biocon.2018.10.019

25. West JB, Bowen GJ, Dawson TE, Tu KP. *Isoscapes: Understanding Movement, Pattern, and Process on Earth Through Isotope Mapping*. The Netherlands: Springer; 2010.

26. Cryan PM, Bogan MA, Rye RO, Landis GP, Kester CL. Stable hydrogen isotope analysis of bat hair as evidence for seasonal molt and long-distance migration. *J Mammal*. 2004;85(5):995-1001. https://doi.org/10.1644/BRG-202

27. Voigt CC, Lindecke O, Schnborn S, Kramer-Schadt S, Lehmann D. Habitat use of migratory bats killed during autumn at wind turbines. *Ecol Appl*. 2016;26(3):771-783. https://doi.org/10.1890/15-0671/suppinfo

28. Voigt CC, Lehnert LS. Tracking of movements of terrestrial mammals using stable isotopes. *In: Tracking Animal Migration with Stable Isotopes*. 2nd ed. The Netherlands: Elsevier; 2019:117-135.

29. Bowen GJ. Isoscapes: Spatial pattern in isotopic biogeochemistry. *Annu Rev Earth Planet Sci*. 2010;38(1):161-187. https://doi.org/10.1146/annurev-earth-040809-152429

30. Popa-Lisseanu AG, Sörgel K, Luckner A, et al. A triple-isotope approach to predict the breeding origins of European bats. *PLoS ONE*. 2012;7(1):e30388. https://doi.org/10.1371/journal.pone.0030388

31. Hobson KA, Van Wilgenburg SL, Wesołowski T, et al. A multi-isotope (δ^2H, δ^{13}C, δ^{15}N) approach to establishing migratory connectivity in Palearctic-Afrotropical migrants: An example using wood warblers *Phylloscopus sibilatrix*. *Acta Ornithol*. 2014;49(1):57-69. https://doi.org/10.3161/000164514X682896

32. Wassenaar LI, Hobson KA. Stable-carbon and hydrogen isotope ratios reveal breeding origins of red-winged blackbirds. *Ecol Appl*. 2000;10 (3):911-916. https://doi.org/10.1890/1051-0761(2000)010[0911:SCAHIR]2.0.CO;2

33. Reudink MW, Kyle CJ, McKellar AE, et al. Linking isotopes and panmixia: High within-colony variation in feather δ^2H, δ^{13}C, and δ^{15}N across the range of the American white pelican. *PLoS ONE*. 2016;11 (3):1-14. https://doi.org/10.1371/journal.pone.0150810

34. Wunder MB, Kester CL, Knopf FL, Rye RO. A test of geographic assignment using isotope tracers in feathers of known origin. *Oecologia*. 2005;144(4):607-617. https://doi.org/10.1007/s00442-005-0071-y

35. Wunder MB. Determining geographic patterns of migration and dispersal using stable isotopes in keratins. *J Mammal*. 2012;93(2): 360-367. https://doi.org/10.1644/11-MAMM-S-182.1

36. Åberg G. The use of natural strontium isotopes as tracers in environmental studies. *Water Air Soil Pollut*. 1995;79(1–4):309-322. https://doi.org/10.1007/BF01100444

37. Flockhart DTT, Kyser TK, Chipley D, Miller NG, Norris DR. Experimental evidence shows no fractionation of strontium isotopes (^{87}Sr/^{86}Sr) among soil, plants, and herbivores: Implications for tracking wildlife and forensic science. *Isot Environ Health Stud*. 2015; 51(3):372-381. https://doi.org/10.1080/10256016.2015.1021345

38. Bäckström Y, Price TD. Social identity and mobility at a pre-industrial mining complex, Sweden. *J Archaeol Sci*. 2016;66:154-168. https://doi.org/10.1016/j.jas.2016.01.004

39. Price TD, Nielsen JN, Frei KM, Lynnerup N. Sebbersund: Isotopes and mobility in an 11th-12th c. AD Danish churchyard. *J Archaeol Sci*. 2012;39(12):3714-3720. https://doi.org/10.1016/j.jas.2012.06.015

40. Oras E, Lang V, Rannamäe E, et al. Tracing prehistoric migration: Isotope analysis of bronze and pre-Roman iron age coastal burials in Estonia. *Est J Archaeol*. 2016;20(1):3. https://doi.org/10.3176/arch.2016.1.01

41. Hoogewerff JA, Reimann C, Ueckermann H, et al. Bioavailable ^{87}Sr/^{86}Sr in European soils: A baseline for provenancing studies. *Sci Total Environ*. 2019;672(March):1033-1044. https://doi.org/10.1016/j.scitotenv.2019.03.387

42. Bataille CBE, Wooller MJ, Bowen GJ. Advances in global bioavailable strontium isoscapes. *Palaeogeogr Palaeoclimatol Palaeoecol*. 2020;555 (43):109849. https://doi.org/10.1016/j.palaeo.2020.109849

43. Koch PL, Heisinger J, Moss C, Carlson RW, Fogel ML, Behrensmeyer AK. Isotopic tracking of change in diet and habitat use in African elephants. *Science*. 1995;267(5202):1340-1343. https://doi.org/10.1126/science.267.5202.1340

44. Sellick MJ, Kyser TK, Wunder MB, Chipley D, Norris DR. Geographic variation of strontium and hydrogen isotopes in avian tissue: Implications for tracking migration and dispersal. *PLoS ONE*. 2009;4 (3):1-10. https://doi.org/10.1371/journal.pone.0004735

45. Font L, Van Der Peijl G, Van Wetten I, Vroon P, Van Der Wagt B, Davies G. Strontium and lead isotope ratios in human hair: Investigating a potential tool for determining recent human geographical movements. *J Anal At Spectrom*. 2012;27(5):719-732. https://doi.org/10.1039/c2ja10361c

46. Tipple BJ, Valenzuela LO, Ehleringer JR. Strontium isotope ratios of human hair record intra-city variations in tap water source. *Sci Rep*. 2018;8(1):1-10. https://doi.org/10.1038/s41598-018-21359-0

47. Bataille CP, Bowen GJ. Mapping ^{87}Sr/^{86}Sr variations in bedrock and water for large scale provenance studies. *Chem Geol*. 2012;304-305: 39-52. https://doi.org/10.1016/j.chemgeo.2012.01.028

48. Van Der Merwe NJ, Lee-Thorp JA, Thackeray JF, et al. Source-area determination of elephant ivory by isotopic analysis. *Nature*. 1990; 346(6286):744-746. https://doi.org/10.1038/346744a0

49. Voerkelius S, Lorenz GD, Rummel S, et al. Strontium isotopic signatures of natural mineral waters, the reference to a simple geological map and its potential for authentication of food. *Food Chem*. 2010;118(4):933-940. https://doi.org/10.1016/j.foodchem.2009.04.125

50. Vogel JC, Eglington B, Auret JM. Isotope fingerprints in elephant bone and ivory. *Nature*. 1990;346(6286):747-749. https://doi.org/10.1038/346747a0

51. Soto DX, Koehler G, Wassenaar LI, Hobson KA. Re-evaluation of the hydrogen stable isotopic composition of keratin calibration standards for wildlife and forensic science applications. *Rapid Commun Mass Spectrom*. 2017;(May):1193-1203. https://doi.org/10.1002/rcm.7893

52. Font L, Nowell GM, Graham Pearson D, Ottley CJ, Willis SG. Sr isotope analysis of bird feathers by TIMS: A tool to trace bird migration paths and breeding sites. *J Anal At Spectrom*. 2007;22(5):513-522. https://doi.org/10.1039/b616328a

53. Charlier BLA, Ginibre C, Morgan D, et al. Methods for the microsampling and high-precision analysis of strontium and rubidium isotopes at single crystal scale for petrological and geochronological applications. *Chem Geol*. 2006;232(3–4):114-133. https://doi.org/10.1016/j.chemgeo.2006.02.015

54. Birck JL. Precision KRbSr isotopic analysis: Application to RbSr chronology. *Chem Geol*. 1986;56(1–2):73-83. https://doi.org/10.1016/0009-2541(86)90111-7

55. Cox SE, Hemming SR, Tootell D. The Isotopx NGX and ATONA faraday amplifiers. *Geochronology*. 2020;2(2):231-243. https://doi.org/10.5194/gchron-2-231-2020

56. Courtiol A, Rousset F, Rohwäder M-S, et al. Isoscape computation and inference of spatial origins with mixed models using the R package IsoriX. In: *Tracking Animal Migration with Stable Isotopes*. 2nd ed. The Netherlands: Elsevier; 2019:207-236.

57. Bowen GJ, Wassenaar LI, Hobson KA. Global application of stable hydrogen and oxygen isotopes to wildlife forensics. *Oecologia*. 2005;143(3):337-348. https://doi.org/10.1007/s00442-004-1813-y

58. Lehnert LS, Kramer-Schadt S, Schönborn S, Lindecke O, Niermann I, Voigt CC. Wind farm facilities in Germany kill noctule bats from near and far. *PLoS ONE*. 2014;9(8):e103106. https://doi.org/10.1371/journal.pone.0103106

59. Blum JD, Taliaferro EH, Weisse MT, Holmes RT. Changes in Sr/Ca, Ba/Ca and ^{87}Sr/^{86}Sr ratios between trophic levels in two forest ecosystems in the northeastern USA. *Biogeochemistry*. 2000;49(1):87-101. https://doi.org/10.1023/A:1006390707989

60. Knudson KJ, Williams HM, Buikstra JE, Tomczak PD, Gordon GW, Anbar AD. Introducing $\delta^{88/86}$Sr analysis in archaeology: A demonstration of the utility of strontium isotope fractionation in paleodietary studies. *J Archaeol Sci*. 2010;37(9):2352-2364. https://doi.org/10.1016/j.jas.2010.04.009

61. Wassenaar LI. *Introduction to Conducting Stable Isotope Measurements for Animal Migration Studies*. 2nd ed. London: Elsevier; 2019:25-51. https://doi.org/10.1016/B978-0-12-814723-8.00002-7

62. Bentley RA. Strontium isotopes from the earth to the archaeological skeleton: A review. *J Archaeol Method Theory*. 2006;13(3):135-187. https://doi.org/10.1007/s10816-006-9009-x

63. Frei KM, Douglas Price T. Strontium isotopes and human mobility in prehistoric Denmark. *Archaeol Anthropol Sci*. 4(2):103-114. https://doi.org/10.1007/s12520-011-0087-7

64. Tipple BJ, Chau T, Chesson LA, Fernandez DP, Ehleringer JR. Isolation of strontium pools and isotope ratios in modern human hair. *Anal Chim Acta*. 2013;798:64-73. https://doi.org/10.1016/j.aca.2013.08.054

65. R Core Team. R: A language and environment for statistical computing security 2020. https://www.r-project.org/

66. Lehnert LS, Kramer-Schadt S, Teige T, et al. Variability and repeatability of noctule bat migration in Central Europe: Evidence for partial and differential migration. *Proc R Soc B Biol Sci*. 2018;285(1893):20182174. https://doi.org/10.1098/rspb.2018.2174

67. Voigt CC, Popa-Lisseanu AG, Niermann I, Kramer-Schadt S. The catchment area of wind farms for European bats: A plea for international regulations. *Biol Conserv*. 2012;153:80-86. https://doi.org/10.1016/j.biocon.2012.04.027

68. Ahlén I. Migratory behaviour of bats at south Swedish coasts. *Jnt J Mamm Biol*. 1997;62:375-380. http://www.biodiversitylibrary.org/ Accessed October 7, 2020

69. Lagerveld S, Poerink BJ, Haselager R, Verdaat H. Bats in Dutch offshore wind farms in autumn 2012. *Lutra*. 2014;57(2):61-69.

70. Rideau C. Densité et comportement reproducteur de la pipistrelle de Nathusius en Normandie. *Le Petit Lérot*. 2002;59:21-24.

71. Bellion P. Pipistrelle de Nathusius. In: F. S, ed. *Atlas Des Mammifères de Bretagne, Groupe Mammalogique Breton*. Locus Solus:2015.

72. Maurer AF, Galer SJG, Knipper C, et al. Bioavailable ^{87}Sr/^{86}Sr in different environmental samples: Effects of anthropogenic contamination and implications for isoscapes in past migration studies. *Sci Total Environ*. 2012;433:216-229. https://doi.org/10.1016/j.scitotenv.2012.06.046

73. Kempson IM, Lombi E. Hair analysis as a biomonitor for toxicology, disease and health status. *Chem Soc Rev*. 2011;40(7):3915-3940. https://doi.org/10.1039/c1cs15021a

74. Tipple BJ, Jameel Y, Chau TH, et al. Stable hydrogen and oxygen isotopes of tap water reveal structure of the San Francisco Bay Area's water system and adjustments during a major drought. *Water Res*. 2017;119:212-224. https://doi.org/10.1016/j.watres.2017.04.022

75. Voigt CC, Schneeberger K, Voigt-Heucke SL, Lewanzik D. Rain increases the energy cost of bat flight. *Biol Lett*. 2011;7(May):793-795. https://doi.org/10.1098/rsbl.2011.0313

76. Flaquer C, Puig-Montserrat X, Goiti U, Vidal F, Curcó A, Russo D. Habitat selection in Nathusius' pipistrelle (*Pipistrellus nathusii*): The importance of wetlands. *Acta Chiropterologica*. 2009;11(1):149-155. https://doi.org/10.3161/150811009x465767

77. Krüger F, Clare EL, Symondson WOC, Keišs O, Petersons G. Diet of the insectivorous bat *Pipistrellus nathusii* during autumn migration and summer residence. *Mol Ecol*. 2014;233672-3683(15):3672-3683. https://doi.org/10.1111/mec.12547

78. Voigt CC, Lehmann D, Greif S. Stable isotope ratios of hydrogen separate mammals of aquatic and terrestrial food webs. *Methods Ecol Evol*. 2015;6(11):1332-1340. https://doi.org/10.1111/2041-210X.12414

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.
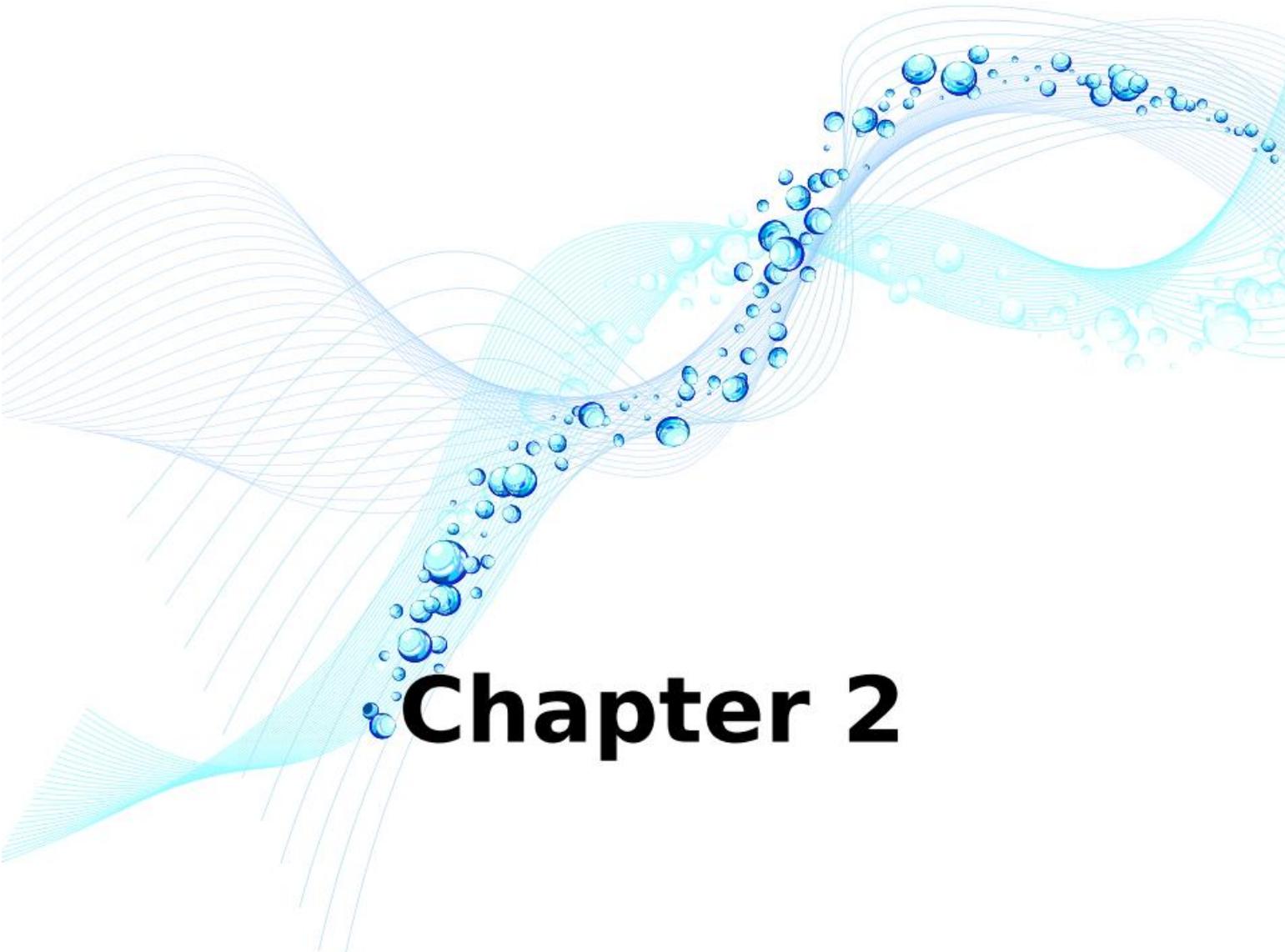
Chapter 2

High vulnerability of juvenile
Nathusius' pipistrelle bats
(*Pipistrellus nathusii*)
at wind turbines

HIGH VULNERABILITY OF JUVENILE NATHUSIUS' PIPISTRELLE BATS (*PIPISTRELLUS NATHUSII*) AT WIND TURBINES

ARTICLE

High vulnerability of juvenile Nathusius' pipistrelle bats (*Pipistrellus nathusii*) at wind turbines

Cecilia Kruszynski[1,2] | Liam D. Bailey[1] | Lothar Bach[3] | Petra Bach[3] |
Marcus Fritze[1] | Oliver Lindecke[1] | Tobias Teige[4] | Christian C. Voigt[1,2]

[1]Leibniz Institute for Zoo and Wildlife Research, Berlin, Germany

[2]Institute of Biology, Freie Universität Berlin, Berlin, Germany

[3]Bach Freilandforschung, zoologische Gutachten, Bremen, Germany

[4]Büro für faunistische Fachgutachten, Berlin, Germany

Correspondence
Cecilia Kruszynski
Email: ceciliak.assis@gmail.com

Handling Editor: Juan C. Corley

Abstract

Large numbers of bats are killed by wind turbines globally, yet the specific demographic consequences of wind turbine mortality are still unclear. In this study, we compared characteristics of Nathusius' pipistrelles (*Pipistrellus nathusii*) killed at wind turbines ($N = 119$) to those observed within the live population ($N = 524$) during the summer migration period in Germany. We used generalized linear mixed-effects modeling to identify demographic groups most vulnerable to wind turbine mortality, including sex (female or male), age (adult or juvenile), and geographic origin (regional or long-distance migrant; depicted by fur stable hydrogen isotope ratios). Juveniles contributed with a higher proportion of carcasses at wind turbines than expected given their frequency in the live population suggesting that juvenile bats may be particularly vulnerable to wind turbine mortality. This effect varied with wind turbine density. Specifically, at low wind turbine densities, representing mostly inland areas with water bodies and forests where Nathusius' pipistrelles breed, juveniles were found more often dead beneath turbines than expected based on their abundance in the live population. At high wind turbine densities, representing mostly coastal areas where Nathusius' pipistrelles migrate, adults and juveniles were equally vulnerable. We found no evidence of increased vulnerability to wind turbines in either sex, yet we observed a higher proportion of females than males among both carcasses and the live population, which may reflect a female bias in the live population most likely caused by females migrating from their northeastern breeding areas migrating into Germany. A high mortality of females is conservation concern for this migratory bat species because it affects the annual reproduction rate of populations. A distant origin did not influence the likelihood of getting killed at wind turbines. A disproportionately high vulnerability of juveniles to wind turbine mortality may reduce juvenile recruitment, which may limit the resilience of Nathusius' pipistrelles to environmental stressors such as climate change or habitat loss. Schemes to mitigate wind turbine mortality, such as elevated cut-in speeds, should be

implemented throughout Europe to prevent population declines of Nathusius' pipistrelles and other migratory bats.

INTRODUCTION

In an effort to reduce the negative effects of CO_2 emissions on the global climate, many countries have been promoting energy production from renewable sources, such as wind (IRENA, 2020). Although it is considered environmentally friendly, wind energy production may come at significant costs to biodiversity (Saidur et al., 2011; Schuster et al., 2015; Thaker et al., 2018). First, animal populations may suffer from habitat loss during wind turbine constructions in sensitive areas, such as forests or wetlands. Second, some taxa may be killed by operating turbines. Among birds, raptors, gulls, and some songbirds are regularly observed colliding with the rotating blades of wind turbines during daytime (e.g., Barrios & Rodríguez, 2004; Nazir et al., 2020; Smallwood & Bell, 2020). At night, bats are frequently killed by wind turbines either by blunt-force trauma when colliding with blades, or by barotrauma in the tailwind vortices of the spinning blades (Baerwald et al., 2008; Voigt et al., 2015b).

The exact number of bat fatalities at wind turbines is currently unknown. Estimates obtained from systematic environmental impact assessments that control for scavenger removal and searcher inefficiency suggest that a single turbine may lead to 2–30 bat fatalities per year if no mitigation scheme is implemented (Brinkmann et al., 2011; Korner-Nievergelt et al., 2013; Măntoiu et al., 2020). This may add up to hundreds of thousands of bats killed per year in countries with high wind energy production, such as Germany (Fritze et al., 2019; Voigt et al., 2015b) and the United States (Cryan & Barclay, 2009; Hayes, 2013). Migratory bats are most often found dead below wind turbines in the temperate zone (Arnett et al., 2016), suggesting that wind energy infrastructures may impede the connectivity between their summer and wintering habitats (Cryan & Barclay, 2009; Voigt et al., 2015b). Populations may be unable to compensate for the additional losses of individuals at wind turbines since bats have a low reproductive rate, with only one or two offspring per year (Garbino et al., 2021). Recent population trend analyses suggested that some species with high collision risk may be in decline (Frick et al., 2017; Zahn et al., 2014), yet population effects are difficult to monitor in bats because wind turbines can kill migratory bats that originate from both local and distant populations of unknown location (Lehnert et al., 2014; Voigt et al., 2012).

European Nathusius' pipistrelles (*Pipistrellus nathusii*) hold the record for long-distance bat migration, with one individual banded in Latvia and recaptured in Northern Spain, a 2,200-km air-line distance (Alcalde et al., 2021). Populations of Nathusius' pipistrelles undergo a seasonal migration from northeastern Europe to western and southern Europe (Hutterer et al., 2005; Meschede et al., 2017; Pētersons, 2004). During migration, bats can use flight corridors along coastlines that are also heavily used for wind energy production (Gaultier et al., 2020; Kruszynski et al., 2020; Kurvits et al., 2011). When migrating inland, Nathusius' pipistrelles have been observed to use larger water bodies, such as lakes or rivers, for migration and stopovers, preferably in forested areas (Furmankiewicz & Kucharska, 2009). Apart from coastal areas, migration of Nathusius' pipistrelles in Germany is not restricted to distinct migratory corridors but instead includes broad, large-scale migratory paths (Meschede et al., 2017). The species ranks second concerning fatality rates at wind turbines in Germany (Rydell et al., 2010). Multiyear recaptures demonstrate site fidelity at summer grounds, maternity roosts, and mating sites (Hutterer et al., 2005), suggesting that a significant proportion of the European population of Nathusius' pipistrelles crosses Germany twice each year. Consequently, Germany has an essential responsibility for protecting this and other migratory species due to the country's central location in the flyways of European migratory bats (Voigt et al., 2015b), and its obligations under national and international laws (EU Directive 92/32/CEE – Annexes II and IV; §44 and §7 of the German "Bundesnaturschutzgesetz"; Conservation of Migratory Species of Wild Animals; UNEP/EUROBATS agreement signed Bonn, 1979, and London, 1991).

While wind turbine bat mortality has been clearly documented, the long-term consequences of such mortality are less clear. Previous studies have attempted to identify demographic groups most at risk by sampling carcasses found beneath wind turbines (Baerwald and Barclay 2011, Lehnert et al., 2014, Chipps et al. 2020, Davy et al. 2021). Greater numbers of carcasses from one sex or age group may be evidence of differences in vulnerability to wind turbine mortality. For example, 72% of common noctule bats

(*Nyctalus noctula*) found dead below wind turbines in Germany were of regional origin, whereas 28% of bats were defined as long-distance migrants (Lehnert et al., 2014). However, without corresponding data from the live population at that point in time, it is unclear whether such results are due to unequal ratios of sex, age, and origin in the population or are truly driven by a higher vulnerability in certain demographic groups. To better assess the demographic consequences of wind turbine mortality, we expanded these previous approaches by comparing the sex (males/females), age (juveniles/adults), and origin (regional/distant) of Nathusius' pipistrelles found dead beneath wind turbines to bats within the live population.

Identifying demographic groups with higher wind turbine vulnerability will help us better understand the impact of wind turbine fatalities on Nathusius' pipistrelles populations. A higher vulnerability of females at wind turbines may lower reproduction in maternity colonies and, thus, the birth rate in source populations. Similarly, the higher mortality risk of juveniles compared to adults may impair juvenile recruitment into the adult population, with negative consequences for population growth and sizes. Additionally, juvenile bats are key for shifting the species' distribution range in response to climate change (Kravchenko et al., 2020). Thus, increased mortality of juvenile bats at wind turbines might constrain a species' ability to respond adequately to global warming. Understanding these potential negative consequences of wind turbines on bats is essential as the prevalence of wind energy will continue to increase.

We predicted that wind turbines would be more likely to kill individuals of distant origin, so-called long-distance migrants, than conspecifics of regional origin. During the migration period, Nathusius' pipistrelles are more likely to use corridors (e.g., on the sea coast) with a high density of wind turbines, increasing their vulnerability (Gaultier et al., 2020). Additionally, we expected females to have a higher vulnerability at wind turbines than males, based on the observed attraction of female bats towards wind turbines, that is, search for social partners or roosts for mating at wind turbines (Roeleke et al., 2016). Further, we expected juveniles to have a higher vulnerability at wind turbines than adult conspecifics because they are less experienced around wind turbines and they seem to exhibit a high exploratory behavior (Horn et al., 2008).

METHODS

Sample collection and study area

We used carcasses of Nathusius' pipistrelles from the central repository of Brandenburg's Federal Agency for Environment (Landesumweltamt, Potsdam, Germany), administered at the Vogelschutzwarte Buckow, and the Natural Science Collections of the University Halle-Wittenberg. Carcasses originated from surveys conducted at wind farms between August 2003 and September 2013. Carcasses were kept frozen from the day of collection until fur sampling. In total, we considered 119 Nathusius' pipistrelle carcasses as sufficiently fresh (without signs of decomposition from the field) to be included in our study. After thawing, we collected a small tuft of fur from the interscapular region of each carcass from which we estimated geographic origin using stable isotopes. We confirmed the species, age (juvenile or adult), and sex by visual inspection.

We obtained fur samples from 524 live Nathusius' pipistrelles that were encountered during routine population surveys conducted as part of official population monitoring schemes between August 2006 and September 2018. We only used samples included in the late summer/early autumn migratory season, ranging from about July 15 to September 31. In Nathusius' pipistrelles, molting occurs in the summer habitat prior to the migratory period (Voigt et al., 2016). Samples were obtained from bats encountered during daytime surveys of artificial roosts or mist netting. Surveying experts identified the species and noted the sex and age cohort. In the remainder of this manuscript, we refer to the survey type where we acquired the data from live animals as bat box data (BB) and to those from carcasses as wind turbine data (WT).

Sex was recorded in 408 of our samples (119 BB, 299 WT). Age was estimated by visually inspecting the bone density close to the epiphyseal junction of the finger bones. Reliable age estimates were obtained for 186 specimens (118 BB, 68 WT). The majority of samples from both survey types were obtained from eastern parts of Germany and from coastal areas of the North Sea where regional wind energy density is high (Figure 1). Dry fur samples were shipped in plastic vials to the stable isotope laboratory of the Leibniz Institute for Zoo and Wildlife Research (IZW) in Berlin.

Wind turbine density varies throughout Germany (Lütkehus et al., 2013), which may affect bat mortality. We used data from the renewable power plants in Open Power System Data (OPSD) for Germany to calculate wind turbine density around each sampling site (Figure 1; https://open-power-system-data.org/). We counted the number of wind turbines within a 10-km buffer around each sampling site, corresponding to the maximum travel distance of Nathusius' pipistrelles per night (Schorcht et al., 2002). We used data from all turbines commissioned between 1983 and 2018 that were still operating when a carcass was found. To illustrate wind energy density in Figure 1, we

used the two-dimensional kernel density estimation provided by the "ggplot2" package in R (Wickham, 2016).

Stable isotope analyses

In our study, we used an isotopic approach to identify the geographical origin of bats. This approach is based on two observations. First, stable hydrogen isotope ratios of precipitation water vary latitudinally across continents following rainfall patterns and ambient temperature (Bowen et al., 2005; Hobson, 1999). Second, body tissues or products, such as hair keratin, are composed of elements that animals consume as water and food. Specifically, stable isotope ratios of fur keratin correlate with those of surface water where molting occurs, usually the summer habitat of migratory bats (Cryan et al., 2004; Lehnert et al., 2018; Popa-Lisseanu et al., 2012; Voigt et al., 2016). Stable hydrogen isotope ratios in fur keratin have been used before to differentiate the origins of bat carcasses observed at wind turbines (Baerwald et al., 2014; Lehnert et al., 2014, 2018; Pylant et al., 2016).

We cleaned the fur from surface oils and contaminants using a 2:1 chloroform-methanol solution and then dried them in an oven for 24 h at 50°C. From each fur sample, we placed a subsample of 0.274 mg ($\pm$ 0.1 mg) in a silver foil capsule (IVA Analysetechnik e.K. Meerbusch, Germany). The methodological approach in analyzing stable hydrogen isotope ratios in the IZW laboratory has been described in full detail in Kruszynski et al. (2020). Briefly, fur samples were placed in the autosampler (Zero Blank autosampler, Costech Analytical Technologies, Milan, Italy) of the elemental analyzer (HT Elementaranalysator HEKAtech GmbH, Wegberg, Germany). Before combustion, samples were flushed in the autosampler for at least 1 h with chemically pure helium (Linde, Leuna, Germany). We used a Delta V Advantage isotope ratio mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) that was connected via an interface (Finnigan Conflo III, Thermo Fisher Scientific, Bremen, Germany) with the elemental analyzer. For every 10 samples, we included one USGS42 keratin standard (Tibetan Human Hair; U.S. Geological Survey) and three lab keratin standards (powdered sheep hair from Sweden [Kstd.1] and Spain [Kstd.2], and powdered goat hair from
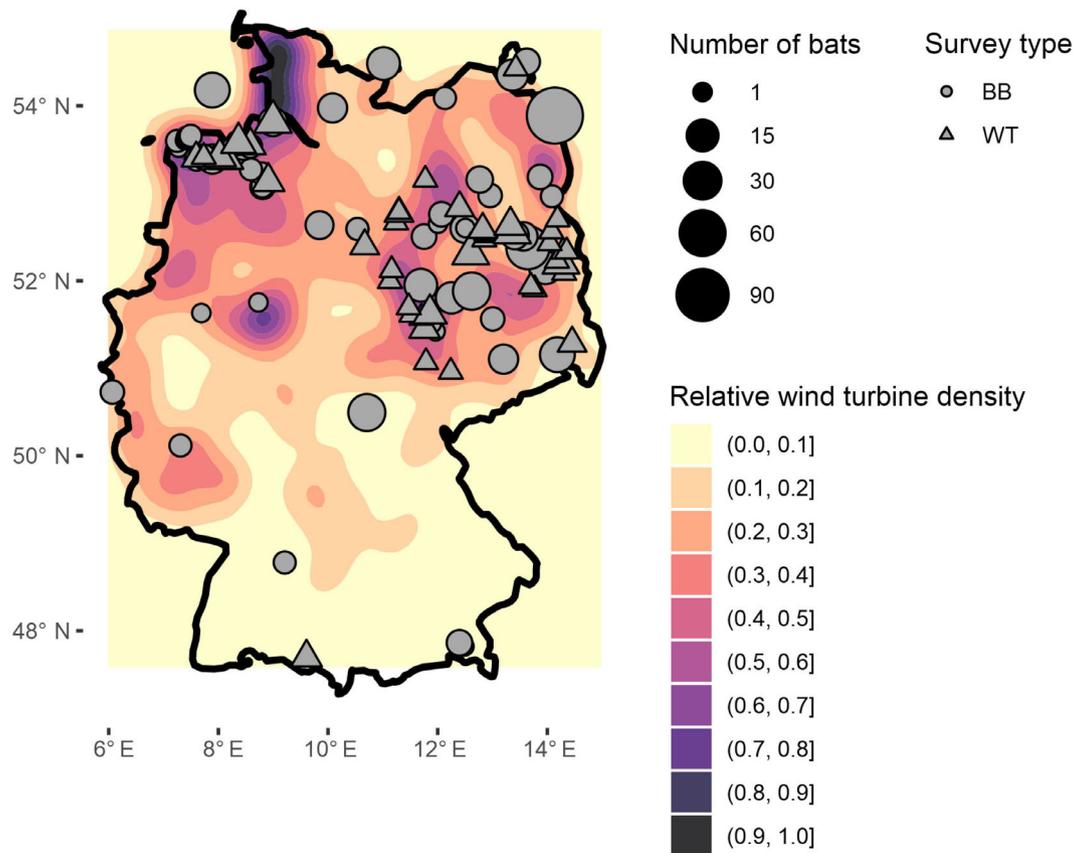
FIGURE 1 Sampling locations in Germany from which material was obtained in relation to wind turbine densities (in shades of yellow to black, indicating absence/low density and high density of wind turbines scaled from 0 to 1, respectively). Turbine density was calculated by counting the number of turbines within a 10-km buffer around the location where carcasses were found. The survey type from which data for Nathusius' pipistrelles originated is indicated by triangles for animals found dead below wind turbines (WT) and by circles for live animals from artificial daytime roosts, so-called bat boxes (BB). The sizes of triangles and circles indicate the number of samples collected per location

Tanzania [Kstd.3]). Stable hydrogen isotope ratios of samples were normalized to the stable isotope ratio of the non-exchangeable portion of hydrogen in standard material following Soto et al. (2017). All values are given in the δ notation as parts per mill (‰) deviation from the international standard V-SMOW. The δ^2H values of keratin standards equaled $-73.1\% \pm 0.5\%$ for USGS42, -167.3% for Kstd.1, -110.02% for Kstd.2, and -65.5% for Kstd.3. Analytical precision based on the repeated analyses of stable hydrogen ratios in laboratory keratin standards was always better than 1.3% (one standard deviation). Twenty-nine samples were used in another publication (Kruszynski et al., 2020) and they are referenced in Kruszynski et al. (2021).

Estimating migratory status based on keratin δ^2H values

We used the package IsoriX (Courtiol et al., 2019) to classify individuals as either long-distance or regional migrants. The approach uses the Global Network of Isotopes in Precipitation (GNIP) annual data (from 1960 to 2018) from the International Atomic Energy Agency (IAEA, Vienna, Austria). We used a transfer function for regressing δ^2H values based on 458 data points from Nathusius' pipistrelles during the non-migration period against mapped δ^2H values of mean annual precipitation (Kruszynski et al., 2020). With this function, we established a δ^2H isoscape map with predicted sample values. This isoscape map was then used to assign every individual a P value within each grid cell to test the putative origin (Courtiol et al., 2019). Individuals were classified as being long-distance migrants when the sampling location was not considered a possible origin location (i.e., $P \leq 0.05$). All other animals were considered as being of regional origin (Kruszynski et al., 2020). This is a conservative approach in the case of Nathusius' pipistrelles given the relative imprecision of the species' transfer function (Kruszynski et al., 2020), which limits the predictive power of the regression function when used in isoscape origin models. The probability maps of long-distance migrants were plotted separately for bats of the WT and BB population and males and females (Figure 2). The probability maps were calculated by overlaying P values > 0.5 of individuals in each aforementioned group.

Statistical models

Demographic differences in wind turbine mortality

We fitted generalized linear mixed effects logistic regression models for each of the three response variables: (1) probability to sample a female, (2) a juvenile, and
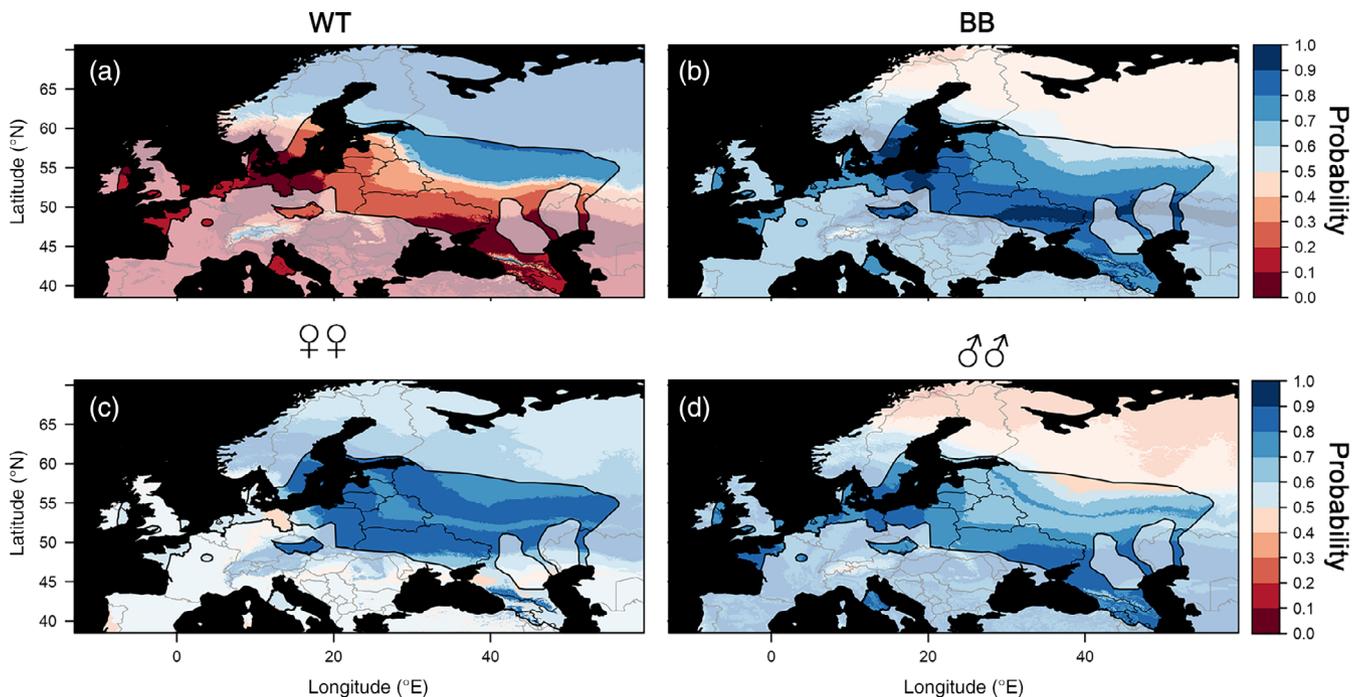
FIGURE 2 Probability maps for the likely origin of long-distance migrating Nathusius' pipistrelles found (a) dead below wind turbines ($n = 8$) or (b) live in bat boxes ($n = 49$), and (c) females ($n = 23$) and (d) males ($n = 33$). Probabilities were calculated by overlaying $P > 0.5$ of migratory bats in each category. The highlighted polygon represents the current breeding range of Nathusius' pipistrelles (Kruszynski et al., 2020)

(3) a long-distance migrant. For each response variable, we used a subset of our data that had complete information for the target response, that is, we excluded all data that did not have the information on the variable being tested. We use "y" in the model descriptions below to cover these three response variables. For each response variable, we compared three model structures using Akaike information criterion corrected for sample size (AICc) model selection. These models are as follows:

1. Model 1: No effect of survey type (WT/BB) on the response variable. Neither demographic group is particularly vulnerable to wind turbine mortality

$$Mod1 = y \sim 1 + (1|location).$$

2. Model 2: Effect of survey type (WT/BB) on the response variable. One demographic group is more vulnerable to wind turbine mortality

$$Mod2 = y \sim survey\ type + (1|location).$$

3. Model 3: Effect of survey type (WT/BB) on the response variable. One demographic group is more vulnerable to mortality. The effect of wind turbine survey type differs with wind turbine density. The variance in wind turbine density around bat boxes was set to zero, therefore, the interaction between BB and wind turbine density was excluded during the process of model fitting

$$Mod3 = y \sim survey\ type + survey\ type :$$
$$number\ of\ wind\ turbines + (1|location).$$

In order to account for the uncertainty in the models, we presented coefficients from each model individually and model-averaged coefficients using the MuMIn package in R (Barton, 2020).

Isotopic differences between demographic groups

We fitted a generalized linear mixed model to test the influence of sex (male/female), age (juvenile/adult), and the interaction between survey type (WT or BB) and origin (regional/distant) on δ^2H values, with sampling site (unique locations where bats were collected) as a random effect to account for potential spatial autocorrelation. We did not consider samples with unknown sex or age in this analysis.

All analyses were conducted in R (R Core Team, 2020).

RESULTS

Models

We obtained data from 119 carcasses (WT; 59 females, 50 males, 10 individuals of unknown sex) of Nathusius' pipistrelles found dead below wind turbines and from 524 live conspecifics of local populations (BB; 164, 135, 225) in Germany (Figure 1). For analysis of each response variable, we used a subset of data that had comprehensive information for the target response. Sample sizes for each model are reported in Table 1.

Model 3 (effect of survey type and interaction with wind turbine density) best explained variation in age patterns (Table 1). At low wind turbine densities, model 3 showed juvenile bats were found dead beneath wind turbines more often (19 out of 68; 27.9%) than expected based on their abundance in the live population (14 out of 118; 11.8%). The probability to encounter juveniles dead below wind turbines decreased with increasing wind turbine density (Table 2). Due to model selection uncertainty, we cannot definitively exclude alternative models 1 and 2 (ΔAICc <2), therefore we also presented model-averaged coefficients and standard errors (Table 2). Model average coefficients showed qualitatively similar results to our top model, although model-averaged confidence intervals overlapped with 0.

For sex and migratory status, Model 1 (intercept-only model) had the lowest AICc (Table 1), suggesting no effect of survey type or wind turbine density on the sex or migratory status of sampled individuals at wind turbines. Females were observed more often than males both beneath wind turbines and in the live population (54.6%; Table 2), while regional migrants were more common than long-distance migrants both in the carcass and live population (91.1%; Table 2).

Stable isotope data and general statistics

δ^2H values of male bats were higher ($-112.2\% \pm 19.1\%$) than those of females ($-115.0\% \pm 17.9\%$; $\beta_{SEX} = 5.27$, df = 165.6, $t = 2.34$, $P = 0.02$), yet the difference between sexes was small. We did not find any difference in δ^2H values between adults ($-113.9\% \pm 18.1\%$) and juveniles ($-118.6\% \pm 26.8\%$; $\beta_{AGE} = -0.83$, df = 178.5, $t = -0.25$, $P = 0.7$).

Eight out of 119 Nathusius' pipistrelle bats (6.7%) killed by wind turbines were classified as long-distance migrants, compared to 49 out of 524 (9.3%) of bats from the live population. Migrants of distant origin from the live population had higher δ^2H values ($-114.4\% \pm 27.1\%$) than those found dead below wind

TABLE 1 Model comparison to test the effects of the independent variables (age, sex, and migratory status) for the mortality risk Nathusius' pipistrelles at wind turbines

Variable, N (BB/WT), model	Parameter[a]	AICc	ΔAICc	Weight
Age, 118/68				
3	survey type + survey type:nrwt + (1|location)	159.8	0	0.507
1	1 + (1|location)	161	1.21	0.277
2	survey type + (1|location)	161.5	1.71	0.216
Sex, 299/109				
1	1 + (1|location)	574.2	0	0.604
2	survey type + (1|location)	575.8	1.6	0.271
3	survey type + survey type:nrwt + (1|location)	577.4	3.16	0.124
Status, 524/119				
1	1 + (1|location)	158.9	0	0.551
2	survey type + (1|location)	160.1	1.17	0.306
3	survey type + survey type:nrwt + (1|location)	161.6	2.71	0.142

Notes: In addition to the AICc values (Akaike information criterion corrected for sample size), sample sizes (N), Akaike weights, and ΔAICc (the difference in AICc between this model and the best model) are also presented. Model 3 presents an interaction between survey type and wind turbine density, however, the variance around bat boxes was set to zero, therefore, this effect at the bat boxes was excluded during model fitting.

[a]Variables are survey type, wind turbine (WT) or bat boxes (BB); location, sampling sites; nrwt, number of wind turbines inside a 10-km buffer around the sampling site.

turbines ($-169.7\% \pm 48\%$; $\beta_{SAMPLE\_LOCATION} = -39.13$, df = 91.53, $t = -4.08$, $P < 0.001$). δ^2H values of regional Nathusius' pipistrelles were higher than conspecifics of distant origin for both BB and WT populations (regional BB, $-111.6\% \pm 12.6\%$; regional WT, $-117.7\% \pm 14.9\%$; $\beta_{STATUS} = 24.42$, df = 84.48, $t = 3.34$, $P = 0.001$), although this effect was greater in bats from the WT than from those of the BB population ($\beta_{STATUS:\ SAMPLE\_LOCATION} = 33.9$, df = 129.25, $t = 3.4$, $P < 0.001$, Appendix S1: Figure S1).

Long-distance migrants from the WT population were assigned to more northeastern areas in Europe, such as Russia and Finland (Figure 2a), than long-distance migrants from the live population (Figure 2b). Within long-distance migrants, females had the most likely origin in eastern and central Europe (Figure 2c) whereas males had most likely originated from western areas in Europe (Figure 2d).

DISCUSSION

Wind energy production has been endorsed worldwide as a CO_2-free renewable energy source; however, wind energy production comes at high ecological costs since turbines kill large numbers of bats and birds when no mitigation measures are practiced (Cryan & Barclay, 2009; Hayes, 2013; Nazir et al., 2020; Smallwood & Bell, 2020; Voigt et al., 2015b). To improve our understanding of how wind turbines might cause

population declines (Frick et al., 2020; Zahn et al., 2014), we studied the European long-distance migratory bat species Nathusius' pipistrelles (*Pipistrellus nathusii*). We assessed whether individual characteristics (sex, age, migratory origin) affected vulnerability to wind turbine mortality, that is, whether certain individuals are killed more than would be predicted based on their relative proportion in the live population. To this end, we compared demographic and isotopic data of live Nathusius' pipistrelles encountered during mist-netting and surveys of artificial daytime roosts (so-called bat boxes, also referred to as bats from the live population) with data from conspecifics found dead beneath wind turbines, during the late summer/early autumn migration season. Model selection suggested juveniles were more likely to die at wind turbines than expected given their abundance in the live population, yet this pattern varied with wind turbine density. An elevated fatality rate of juvenile bats was most apparent where wind turbine density was low. At high wind turbine densities, juvenile Nathusius' pipistrelles were as likely to collide with wind turbines as adult bats. The underlying reason for this pattern remains unclear. There was no evidence of increased vulnerability of either sex. However, we observed a higher proportion of females than males in the population, which could be derived from females migrating south from maternity colonies in northeastern Europe. Finally, geographic origin (regional/distant) did not explain the likelihood of getting killed at wind turbines.

TABLE 2 Parameter estimates, standard errors (SE), and 95% confidence intervals (CI) of the models for Nathusius' pipistrelles

Variable, model, and parameter[a]	Estimate	Conditional SE	t	Lower 95% CI	Upper 95% CI
Age					
1					
Intercept	−1.57	0.45	−3.44	−2.8	−0.70
2					
Intercept	−2.23	0.72	−3.06	−4.13	−0.93
Survey type (wt)	1.06	0.84	1.26	−0.48	3.12
3					
Intercept	−2.23	0.73	−3.05	−4.38	−0.95
Survey type (wt)	1.74	0.93	1.86	0.07	3.89
Survey type = "wt":nrwt	−0.04	0.02	−1.63	−1.14	−0.006
Average					
Intercept	2.05			−3.48	−0.61
Survey type (wt)	1.11			−0.35	3.44
Survey type = "wt":nrwt	−0.02			−0.09	0.009
Sex					
1					
Intercept	0.2	0.15	1.32	−0.11	0.48
2					
Intercept	0.13	0.17	0.76	−0.2	0.48
Survey type (wt)	0.20	0.32	0.65	−0.42	0.8
3					
Intercept	0.13	0.17	0.77	−0.22	0.48
Survey type (wt)	0.05	0.39	0.12	−0.73	0.84
Survey type = "wt":nrwt	0.006	0.009	0.68	−0.01	0.02
Average					
Intercept	0.17			−0.14	0.49
Survey type (wt)	0.06			−0.53	0.85
Survey type = "wt":nrwt	0.0008			−0.01	0.02
Status					
1					
Intercept	−7.38	2.02	−3.65	−26.0	−4.87
2					
Intercept	−8.06	2.4	−3.34	−29.42	−5.11
Survey type (wt)	1.23	1.37	0.89	−2.54	6.26
3					
Intercept	−8.41	2.74	−3.06	−29.94	−5.23
Survey type (wt)	1.9	1.75	1.08	−2.71	13.73
Survey type = "wt":nrwt	−0.03	0.05	−0.63	−37.43	0.09
Average					
Intercept	−7.73			−12.24	−3.22
Survey type (wt)	0.65			−1.56	4.46
Survey type = "wt":nrwt	−0.004			−0.13	0.06

Note: The interaction between survey type and wind turbine density is not considering the variance around bat boxes because it was set to 0, therefore, this effect at the bat boxes was excluded during model fitting.

[a]Variable abbreviations are survey type (wt), survey type on WT; nrwt, number of wind turbines inside a 10-km buffer around the sampling site.

Most of the sampled carcasses used for this study were collected in high wind turbine density areas along the German North Sea coastline (Figure 1), that is, within the coastal migration corridors of Nathusius' pipistrelles (Kurvits et al., 2011, Gaultier et al., 2020, Kruszynski et al., 2020,). Additionally, we received carcass data from another area with high wind turbine density close to the river Oder (at the border between Germany and Poland), which is also known to function as a migration corridor for Nathusius' pipistrelles (Furmankiewicz & Kucharska, 2009). Within these migration corridors, our analysis suggested that the relative risk for Nathusius' pipistrelles to collide with wind turbines was independent of age. In these corridors, adults and juveniles are flying at speeds of about 25 km/h (Troxell et al., 2019), most likely during continuous flights of several hours. As both sexes and all age groups migrate in the same manner, we would expect similar vulnerability for all migrating individuals. Outside migration corridors, predominantly in northeastern Germany where wind turbines occur at lower densities (Figure 1), the risk for Nathusius' pipistrelles appeared to be unevenly distributed. The region is dominated by large forests and several larger lakes areas. In this region, juvenile Nathusius' pipistrelles were more likely to die at wind turbines than are adult conspecifics. Outside the migration corridors, adult Nathusius' pipistrelles may pause migration at stopover sites where they court and mate (Voigt-Heucke et al., 2016). Yet, juveniles may still be attracted to the tall structures of wind turbines because of an increased exploratory behavior (Horn et al., 2008) and a lack of experience in responding to environmental cues, such as the Earth's magnetic field for orientation, during the first southward journey when their navigation system has not yet fully developed (Lindecke et al. 2019). Without behavioral data from bats in areas of both high and low wind turbine density, it is difficult to provide a mechanistic explanation for our results with certainty. Future studies are needed to address in more detail why juvenile Nathusius' pipistrelles appear to be more vulnerable at wind turbines at low densities, and it would be important to understand whether wind turbines present a similarly high risk for juveniles of other migratory bat species.

The sex ratio of Nathusius' pipistrelles populations is known to vary across Europe during the migratory season (Boshamer & Bekker, 2008; Flaquer et al., 2009; Gukasova et al., 2011; Pētersons, 2004). Our results from both the live population and the carcasses from wind turbines suggested a female-biased sex ratio in Germany. Germany is a major pathway and stopover site for bats migrating southward, and we expect many of the sampled females would be moving from northern maternity colonies (Alcalde et al., 2021; Hutterer et al., 2005; Kurvits et al., 2011). Wind turbine mortality in Germany during the migratory season may therefore be particularly problematic as it could include higher numbers of deaths for reproductively active females. A female sex ratio bias has also been reported in other species with northern maternity colonies (Nyctalus noctula; Lehnert et al., 2014), suggesting that the risks posed by wind turbines in Germany may extend beyond Nathusius' pipistrelles and should therefore be a focus for future mitigation actions.

Based on the results of our study, we perceive three possible consequences of wind turbine mortality for Nathusius' pipistrelles source populations. First, mortality of females at wind turbines during the migratory season could lead to declining numbers of maternity colonies and may lead to the eventual extirpation of local populations (Frick et al., 2017; Zahn et al., 2014). Second, a disproportionately higher vulnerability of juvenile Nathusius' pipistrelles at wind turbines may affect the recruitment of juveniles, which may lead to population declines. Third, the elevated mortality risk of juvenile bats might reduce the dispersal of juveniles to novel breeding or wintering areas. Adult Nathusius' pipistrelles show high site fidelity in their wintering and summer areas (Lehnert et al., 2018), making dispersal of juveniles a key driver of northward range shifts in response to climate change (Kravchenko et al., 2020). The so-called "generational shift" that is responsible for the northward range expansion of bats might be impaired when juveniles are killed by wind turbines during their first journeys, and thus limiting the ability of the species to adequately respond to a warming climate.

Our ability to distinguish between regional and long-distance migrants, and to identify differing vulnerability in either group, may have been impeded by the weak isotopic transfer function available for Nathusius' pipistrelles. This function aims to regress δ^2H values of the likely place of molt based on δ^2H values in fur keratin of focal animals. The transfer function is relatively imprecise for Nathusius' pipistrelles compared to transfer functions of other species such as common noctule bats (Lehnert et al., 2018). The imprecision yields large areas of more than 1000 km across latitude and longitude as likely places of origin for this species (Kruszynski et al., 2020). This imprecision may be a result of Nathusius' pipistrelles foraging between limnic and terrestrial food webs (Voigt et al., 2015a), and because of an age effect on δ^2H values. δ^2H values of juvenile bats were shown to be lower than those of corresponding mothers, most likely because of the consumption of deuterium-depleted fat-rich milk by juveniles (Kravchenko et al., 2019). This further complicates the geographic assignment of juveniles based on stable isotopes (Kravchenko et al., 2019).

This is one of the first studies to account for demographic characteristics in the live population when studying bat wind turbine mortality. Without this comparison,

it is impossible to disentangle whether demographic data from carcasses represent true differences in vulnerability or simply biased demographic ratios within the studied population. However, our analysis relies on observational data collected with inconsistent effort and thus our results should be seen only as a first step towards understanding the uneven impact of wind turbines on bats. Systematic data collection that includes both consistent effort sampling below wind turbines and live population surveys within close proximity to wind turbine sites would provide us with a more robust test of the results presented here. Such systematic effort should also focus on areas with different levels of wind turbine densities to better understand how juvenile mortality may vary with turbine density.

In this study, we demonstrated that the threat of wind turbines can be uneven across bat populations. The higher vulnerability of juveniles to wind turbine mortality may lead to population declines of migratory species and to an impaired ability of populations to respond adequately to warming global temperatures. This calls for more robust mitigation measures, such as curtailment speeds (Arnett et al., 2011), to reduce the number of fatalities of bats at wind turbines. Furthermore, wind turbine construction should be limited in sensitive areas for bats, such as forested areas with large water bodies. Understanding and mitigating the effects of wind turbine mortality on bats will be an important conservation challenge for the future as countries transition to renewable energy.

ACKNOWLEDGMENTS

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Supplementary data (Kruszynski et al., 2021) is provided in Zenodo: https://doi.org/10.5281/zenodo.4557292.

REFERENCES

Alcalde, J.T., M. Jimenez, I. Brila, V. Vintulis, C.C. Voigt, and G. Petersons. 2021. "Trans-Continental 2,200 km Migration of a Nathusius Pipistrelle (*Pipistrellus Nathusii*)." *Mammalia* 85: 161–3.

Arnett, E.B., E.F. Baerwald, F. Mathews, L. Rodrigues, A. Rodríguez-Durán, J. Rydell, R. Villegas-Patraca, and C.C. Voigt. 2016. "Impacts of Wind Energy Development on Bats: A Global Perspective." In *Bats in the Anthropocene: Conservation of Bats in a Changing World*, edited by C. Voigt and T. Kingston, 295–323. Cham, Switzerland: Springer International Publishing.

Arnett, E.B., M.M. Huso, M.R. Schirmacher, and J.P. Hayes. 2011. "Altering Turbine Speed Reduces Bat Mortality at Wind-Energy Facilities." *Frontiers in Ecology and the Environment* 9: 209–14.

Baerwald, E.F., G.H. D'Amours, B.J. Klug, and R.M.R. Barclay. 2008. "Barotrauma Is a Significant Cause of Bat Fatalities at Wind Turbines." *Current Biology* 18: 695–6.

Baerwald, E.F., and R.M.R. Barclay. 2011. "Patterns of Activity and Fatality of Migratory Bats at a Wind Energy Facility in Alberta, Canada." *Journal of Wildlife Management* 75: 1103–14.

Baerwald, E.F., W.P. Patterson, and R.M.R.R. Barclay. 2014. "Origins and Migratory Patterns of Bats Killed by Wind Turbines in Southern Alberta: Evidence from Stable Isotopes." *Ecosphere* 5: 1–17.

Barrios, L., and A. Rodríguez. 2004. "Behavioural and Environmental Correlates of Soaring-Bird Mortality at On-Shore Wind Turbines." *Journal of Applied Ecology* 41: 72–81.

Barton, K. 2020. "MuMIn: Multi-Model Inference." R package version 1.43.17. https://CRAN.R-project.org/package=MuMIn

Boshamer, J.P.C., and J.P. Bekker. 2008. "Nathusius' Pipistrelles (*Pipistrellus nathusii*) and Other Species of Bats on Offshore Platforms in the Dutch Sector of the North Sea." *Lutra* 51: 17–36.

Bowen, G.J., L. Chesson, K. Nielson, T.E. Cerling, and J.R. Ehleringer. 2005. "Treatment Methods for the Determination of D2H and D18O of Hair Keratin by Continuous-Flow Isotope-Ratio Mass Spectrometry." *Rapid Communications in Mass Spectrometry* 19: 2371–8.

Brinkmann, R., O. Behr, F. Korner-Nievergelt, J. Mages, I. Niermann, and M. Reich. 2011. *Entwicklung von Methoden zur Untersuchung und Reduktion des Kollisionsrisi kos von Fledermäusen an Onshore-Windenergieanlagen*. Göttingen: Cuvillier Verlag.

Chipps, A.S., A.M. Hale, S.P. Weaver, and D.A. Williams. 2020. "Genetic Approaches are Necessary to Accurately Understand Bat-Wind Turbine Impacts." *Diversity* 12: 1–11.

Courtiol, A., F. Rousset, M.-S. Rohwäder, D.X. Soto, L.S. Lehnert, C.C. Voigt, K.A. Hobson, L.I. Wassenaar, and S. Kramer-Schadt. 2019. "Isoscape Computation and Inference of Spatial Origins with Mixed Models using the R Package IsoriX." In *Tracking Animal Migration with Stable Isotopes*, edited by Keith A. Hobson and Leonard I. Wassenaar, 207–36. Academic Press.

Cryan, P.M., and R.M.R. Barclay. 2009. "Causes of Bat Fatalities at Wind Turbines: Hypotheses and Predictions." *Journal of Mammalogy* 90: 1330–40.

Cryan, P.M., M.A. Bogan, R.O. Rye, G.P. Landis, and C.L. Kester. 2004. "Stable Hydrogen Isotope Analysis of Bat Hair as Evidence for Seasonal Molt and Long-Distance Migration." *Journal of Mammalogy* 85: 995–1001.

Davy, C.M., K. Squires, and J.R. Zimmerling. 2021. "Estimation of Spatiotemporal Trends in Bat abundance from Mortality Data Collected at Wind Turbines." *Conservation Biology* 35: 227–38.

Flaquer, C., X. Puig-Montserrat, U. Goiti, F. Vidal, A. Curcó, and D. Russo. 2009. "Habitat Selection in Nathusius' Pipistrelle (*Pipistrellus Nathusii*): The Importance of Wetlands." *Acta Chiropterologica* 11: 149–55.

Frick, W.F., E.F. Baerwald, J.F. Pollock, R.M.R. Barclay, J.A. Szymanski, T.J. Weller, A.L. Russell, S.C. Loeb, R.A. Medellin, and L.P. McGuire. 2017. "Fatalities at Wind Turbines May Threaten Population Viability of a Migratory Bat." *Biological Conservation* 209: 172–7.

Frick, W.F., T. Kingston, and J. Flanders. 2020. "A Review of the Major Threats and Challenges to Global Bat Conservation." *Annals of the New York Academy of Sciences* 1469: 5–25.

Fritze, M., L.S. Lehnert, O. Heim, O. Lindecke, and M. Roeleke. 2019. "Fledermausschutz im Schatten der Windenergie." *Naturschutz und Landschaftsplanung* 51: 20–7.

Furmankiewicz, J., and M. Kucharska. 2009. "Migration of Bats along a Large River Valley in Southwestern Poland." *Journal of Mammalogy* 90: 1310–7.

Garbino, G.S.T., A. Feijó, R. Beltrão-Mendes, and P.A. Da Rocha. 2021. "Evolution of Litter Size in Bats and its Influence on Longevity and Roosting Ecology." *Biological Journal of the Linnean Society* 132: 676–84.

Gaultier, S.P., A.S. Blomberg, A. Ijäs, V. Vasko, E.J. Vesterinen, J.E. Brommer, and T.M. Lilley. 2020. "Bats and Wind Farms: The Role and Importance of the Baltic Sea Countries in the European Context of Power Transition and Biodiversity Conservation." *Environmental Science and Technology* 54: 10385–98.

Gukasova, A., A. Vlaschenko, G. Kosenkov, and K. Kravchenko. 2011. "Fauna and Structure of Bat (Chiroptera) Assemblage of the National Park 'Smolensk Lakeland', Western Russia." *Acta Zoologica Lituanica* 21: 173–80.

Hayes, M.A. 2013. "Bats Killed in Large Numbers at United States Wind Energy Facilities." *Bioscience* 63: 975–9.

Hobson, K.A. 1999. "Tracing Origins and Migration of Wildlife using Stable Isotopes: A Review." *Oecologia* 120: 314–26.

Horn, J.W., E.B. Arnett, and T.H. Kunz. 2008. "Behavioral Responses of Bats to Operating Wind Turbines." *Journal of Wildlife Management* 72: 123–32.

Hutterer, R., T. Ivanova, C. Meyer-Cords, and L.L. Rodrigues. 2005. *Bat migrations in Europe a Review of Banding Data and Literature.* Federal Agency for Nature Conservation.

IRENA. 2020. *Reaching Zero with Renewables: Eliminating CO_2 Emissions from Industry and Transport in Line with the 1.5°C Climate Goal.* Placeholder TextInternational Renewable Energy Agency, Abu Dhabi.

Korner-Nievergelt, F., R. Brinkmann, I. Niermann, and O. Behr. 2013. "Estimating Bat and Bird Mortality Occurring at Wind Energy Turbines from Covariates and Carcass Searches using Mixture Models." *PLoS ONE* 8: e67997.

Kravchenko, K.A., L.S. Lehnert, A.S. Vlaschenko, and C.C. Voigt. 2019. "Multiple Isotope Tracers in Fur Keratin Discriminate between Mothers and Offspring." *Rapid Communications in Mass Spectrometry* 33: 907–13.

Kravchenko, K.A., A.S. Vlaschenko, L.S. Lehnert, A. Courtiol, and C.C. Voigt. 2020. "Generational Shift in the Migratory Common Noctule Bat: First-Year Males Lead the Way to Hibernacula at Higher Latitudes." *Biology Letters* 16: 20200351.

Kruszynski, C., L. Bailey, L. Bach, P. Bach, M. Fritze, O. Lindecke, T. Teige, and C.C. Voigt. 2021. "High Vulnerability of Juvenile Nathusius' Pipistrelle Bats (*Pipistrellus nathusii*) at Wind Turbines [data set] in Ecological Applications." *Zenodo.* https://doi.org/10.5281/zenodo.4557292

Kruszynski, C., L. Bailey, A. Courtiol, L. Bach, P. Bach, M. Göttsche, M. Göttsche, et al. 2020. "Identifying Migratory Pathways of Nathusius' Pipistrelles (*Pipistrellus nathusii*) using Stable Hydrogen and Strontium Isotopes." *Rapid Communications in Mass Spectrometry* 35: e9031.

Kurvits, T., C. Nellermann, B. Alfthan, A. Kuhl, P. Prokosch, M. Virtue, and J.F. Skaalvik. 2011. *Living Planet: Connected Planet - Preventing the End of the World's Wildlife Migrations through Ecological Networks.* Norway: United Nations Environment Programme.

Lehnert, L.S., S. Kramer-Schadt, S. Schönborn, O. Lindecke, I. Niermann, and C.C. Voigt. 2014. "Wind Farm Facilities in Germany Kill Noctule Bats from Near and Far." *PLoS ONE* 9: e103106.

Lehnert, L.S., S. Kramer-Schadt, T. Teige, U. Hoffmeister, A. Popa-Lisseanu, F. Bontadina, M. Ciechanowski, et al. 2018. "Variability and Repeatability of Noctule Bat Migration in Central Europe: Evidence for Partial and Differential Migration." *Proceedings of the Royal Society B* 285: 20182174.

Lindecke, O., A. Elksne, R.A. Holland, G. Pētersons, and C.C. Voigt. 2019. "Orientation and Flight Behaviour Identify the Soprano Pipistrelle as a Migratory Bat Species at the Baltic Sea Coast." *Journal of Zoology* 308: 56–65.

Lütkehus, I., H. Salecker, K. Adlunger, T. Klaus, C. Vollmer, C. Alsleben, R. Spiekermann, A. Bauerdorff, J. Günther, G. Schütze, and S. Bofinger. 2013. *Potenzial der Windenergie an Land.* Dessau-Roßlau: Umweltbundesamt.

Măntoiu, D.Ş, K. Kravchenko, L.S. Lehnert, A. Vlaschenko, O.T. Moldovan, I.C. Mirea, R.C. Stanciu, et al. 2020. "Wildlife and Infrastructure: Impact of Wind Turbines on Bats in the Black Sea Coast Region." *European Journal of Wildlife Research* 66: 44.

Meschede, A., W. Schorcht, I. Karst, M. Biedermann, D. Fuchs, and F. Bontadina. 2017. *Wanderrouten der Fledermäuse.* Bonn - Bad Godesberg: BfN-Skripten.

Nazir, M.S., N. Ali, M. Bilal, and H.M.N. Iqbal. 2020. "Potential Environmental Impacts of Wind Energy Development: A Global Perspective." *Current Opinion in Environmental Science and Health* 13: 85–90.

Pētersons, G. 2004. "Seasonal Migrations of North-Eastern Populations of Nathusius' Bat *Pipistrellus nathusii* (Chiroptera)." *Myotis* 41–42: 29–56.

Popa-Lisseanu, A.G., K. Sörgel, A. Luckner, L.I. Wassenaar, C. Ibáñez, S. Kramer-Schadt, M. Ciechanowski, et al. 2012. "A Triple-Isotope Approach to Predict the Breeding Origins of European Bats." *PLoS One* 7: e30388.

Pylant, C.L., D.M. Nelson, M.C. Fitzpatrick, J.E. Gates, and S.R. Keller. 2016. "Geographic Origins and Population Genetics of Bats Killed at Wind-Energy Facilities." *Ecological Applications* 26: 1381–95.

R Core Team. 2020. *R: A Language and Environment for Statistical Computing.* Vienna, Austria: R Foundation for Statistical Computing. www.R-project.org

Roeleke, M., T. Blohm, S. Kramer-Schadt, Y. Yovel, and C.C. Voigt. 2016. "Habitat Use of Bats in Relation to Wind Turbines Revealed by GPS Tracking." *Scientific Reports* 6: 28961.

Rydell, J., L. Bach, M.-J. Dubourg-Savage, M. Green, L. Rodrigues, and A. Hedenström. 2010. "Bat Mortality at Wind Turbines in Northwestern Europe." *Acta Chiropterologica* 12: 261–74.

Saidur, R., N.A. Rahim, M.R. Islam, and K.H. Solangi. 2011. "Environmental Impact of Wind Energy." *Renewable and Sustainable Energy Reviews* 15(5): 2423–30.

Schorcht, W., C. Tress, M. Biedermann, R. Koch, and J. Tress. 2002. "Zur Ressourcennutzung von Rauhautfledermäusen (*Pipistrellus Nathusii*) in Mecklenburg." *Schriftenreihe für Landschaftspflege und Naturschutz* 71: 191–212.

Schuster, E., L. Bulling, and J. Köppel. 2015. "Consolidating the State of Knowledge: A Synoptical Review of Wind Energy's Wildlife Effects." *Environmental Management* 56(2): 300–31.

Smallwood, K.S., and D.A. Bell. 2020. "Effects of Wind Turbine Curtailment on Bird and Bat Fatalities." *Journal of Wildlife Management* 84: 685–96.

Soto, D.X., G. Koehler, L.I. Wassenaar, and K.A. Hobson. 2017. "Re-evaluation of the Hydrogen Stable Isotopic Composition of Keratin Calibration Standards for Wildlife and Forensic Science Applications." *Rapid Communications in Mass Spectrometry* 31: 1193–203.

Thaker, M., A. Zambre, and H. Bhosale. 2018. "Wind Farms Have Cascading Impacts on Ecosystems across Trophic Levels." *Nature Ecology and Evolution* 2: 1854–8.

Troxell, S.A., M.W. Holderied, G. Pētersons, and C.C. Voigt. 2019. "Nathusius' Bats Optimize Long-Distance Migration by Flying at Maximum Range Speed." *Journal of Experimental Biology* 222: 1–7.

Voigt, C.C., D. Lehmann, and S. Greif. 2015a. "Stable Isotope Ratios of Hydrogen Separate Mammals of Aquatic and Terrestrial Food Webs." *Methods in Ecology and Evolution* 6: 1332–40.

Voigt, C.C., L.S. Lehnert, G. Petersons, F. Adorf, and L. Bach. 2015b. "Wildlife and Renewable Energy: German Politics Cross Migratory Bats." *European Journal of Wildlife Research* 61: 213–9.

Voigt, C.C., O. Lindecke, S. Schnborn, S. Kramer-Schadt, and D. Lehmann. 2016. "Habitat Use of Migratory Bats Killed during Autumn at Wind Turbines." *Ecological Applications* 26: 771–83.

Voigt, C.C., A.G. Popa-Lisseanu, I. Niermann, and S. Kramer-Schadt. 2012. "The Catchment Area of Wind Farms for European Bats: A Plea for International Regulations." *Biological Conservation* 153: 80–6.

Voigt-Heucke, S.L., S. Zimmer, and S. Kipper. 2016. "Does Interspecific Eavesdropping Promote Aerial Aggregations in European Pipistrelle Bats during Autumn?" *Ethology* 122: 745–57.

Wickham, H. 2016. *ggplot2: Elegant Graphics for Data Analysis*. New York: Springer-Verlag.

Zahn, A., A. Lustig, and M. Hammer. 2014. *Potenzielle Auswirkungen von Windenergieanlagen Auf Fledermauspopulationen Zusammenfassung*. 36(1): 21–35. Page ANLIEGEN NATUR.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

> **How to cite this article:** Kruszynski, Cecilia, Liam D. Bailey, Lothar Bach, Petra Bach, Marcus Fritze, Oliver Lindecke, Tobias Teige, and Christian C. Voigt. 2022. "High Vulnerability of Juvenile Nathusius' Pipistrelle Bats (*Pipistrellus nathusii*) at Wind Turbines." *Ecological Applications* 32(2): e2513. https://doi.org/10.1002/eap.2513

GENERAL DISCUSSION

4. Mind the gap – Bats, climate change, and wind turbines

Given the growing impacts of anthropogenic changes and global climate change, it is essential to gain greater insight into migratory pathways and fine-scale movements of migratory species. This has been done using onboard devices or permanent markers, which require recapture of individuals, can be expensive, and restricted to larger migratory species. Using state-of-the-art stable isotope analysis in fur samples collected from populations of migratory bats, I was able to investigate the origins of individuals discovered at different sites along their migratory route and differentiate potential threats to regional and migratory populations in the path of wind turbines.

In **chapter 1**, while checking that the stable isotope analysis method can be applied to identify the migration patterns of Nathusius' pipistrelles, I could distinguish between two probable pathways they use to move from their summer breeding area to their wintering areas. First, I established isoscapes for two isotopes used in this study (H and Sr) with a suitable grid resolution (0.3 and 0.7 km, respectively). δ^2H analysis suggested all bats have a long-distance origin. Then, I established three isotopic bins (range 1: east Europe; range 2: central Europe; range 3: west Europe) in order to present the results of the 58 individuals (one individual was considered an outlier, and its results were not presented). The bats from different islands were assigned to different bins, suggesting weak connectivity within the population of this species. Although δ^2H analysis suggested a possible Fennoscandian origin, ^{87}Sr/^{86}Sr analysis excluded this possibility. Instead, the data suggest that the individuals captured migrating along the German coastline had a continental European summer origin. The data did not support an influx of Fennoscandian bats in Germany, however, I could not exclude the possibility that the

migratory corridor starts in Baltorussia and ends in Germany passing through Finland, Sweden, and Denmark. Although their movement first northward and then south is unusual and the isotopic evidence for this is weak, acoustic recordings show their presence during summer in northern countries (Gaultier et al. 2020). Fennoscandian bats might use different pathways that were not included in our sampling efforts. Moreover, I found possible movements from the east (Baltorussia) and west (Normandy and Brittany) directions where the species have breeding populations.

In this chapter, I also emphasise the importance of distinguishing the fractionation of isotopes as it can hamper the interpretation of the data. The Sr fractionation found between the bat samples and the environment could be a feature that only happens in bats' fur or could also occur in other mammals. In order to verify the accuracy of the data, more studies should focus on reporting ^{87}Sr/^{86}Sr values for different tissues, animals, and regions. In the case of hydrogen, the fractionation was established with the sedentary Nathusius' pipistrelles data, but the resulting transfer function was not as solid as for other bats (e.g., five European bat species (Lehnert et al. 2018)). For Nathusius' pipistrelles, the transfer function shows an unclear relationship with precipitation data probably influenced by their diet, which is composed of both terrestrial and aquatic insects (Krüger et al. 2014). A recent study found that emerging insects might contribute to the diet of insectivorous bats regardless of the distance to streams (Recalde et al. 2021), which could be a reason we find this unclear pattern between bat fur and environmental data. I concluded that the migratory pathways and patterns of Nathusius' pipistrelles are more complex than previously assumed, thus, more efforts are needed to disentangle their movements between summer and winter grounds and their feeding behaviour.

In **chapter 2**, I used stable hydrogen isotopes in fur keratin to determine the migratory status of Nathusius' pipistrelle carcasses and living conspecifics to evaluate the vulnerability of long-distance and regional groups to wind turbines. The models showed evidence of an unequal vulnerability of age to wind turbines. Specifically, at low wind turbine density, juveniles were found dead beneath turbines significantly more often than expected based on their abundance in the living population. Extensive local exploratory behaviour is energetically costly, as is migratory flight (Currie et al. 2020). While adults from both sexes spend time in mating territories, juveniles may spend time exploring and constantly foraging *en route*. Therefore, the vulnerability of juveniles to wind turbines may be higher than for adults. It is not uncommon for juveniles to be disproportionately impacted by wind turbines. Other studies have also shown this pattern in golden eagles (*Aquila chrysaetos*) (Hunt et al. 2017) as well as other soaring bird species (Barrios and Rodríguez 2004). However, when wind turbines are installed at high density, I found equal vulnerability of both ages, which has also been shown in black kites (*Milvus migrans*) (Santos et al. 2021). As both sexes and all age groups of *P. nathusii* migrate in the same manner, we would expect similar vulnerability for all migrating individuals in high wind turbine areas. In areas with a low density of wind turbines, mainly in north-eastern Germany, there are more forested areas and lakes where *P. nathusii* roost and feed. Recent findings showed that diverse habitats with a mixture of open vegetated areas, watercourses, and broadleaf forests were the most important land features for a diverse bat fauna, along with high connectivity via tree cover and linear landscape elements (Skog 2021). Adults can use those areas as stopover sites, where they court and mate. However, the juveniles might be attracted to wind turbines as they have less experience with dangerous situations. In addition, the juveniles' strong exploratory behaviour might bring them in search of roosts in the vacant areas in an attempt to establish territories because they

could mistake wind turbines for tall trees (Arnett et al. 2008). Yet, a single underlying reason for the pattern found between low and high density of wind turbines remains unclear.

Wind turbine vulnerability did not differ between sexes or migratory status. The findings showed a female- and regional-biased trend in both survey types (carcasses and living). The small quota of long-distance migrants was mostly from north-eastern Europe; long-distance females and males differed in their origin. The first possibly originated from north-eastern Europe and the latter from west Europe. This contributes to the hypothesis that there are more females present in the area because they are coming from northeastern maternity colonies. However, more studies should focus on understanding this sex-skewed phenomenon that occurs in Germany during the migration season, and the reproductive biology of this species because such information could aid in making informed conservation strategies (Cryan et al. 2012). Moreover, the findings emphasise the importance of Germany as a stopover site and a major migratory pathway for long-distance migratory bats. The increased number of regional bats could be due to a high site fidelity also found in adults of other species of bats (Muñoz-Romo et al. 2008; Lehnert et al. 2014). My approach to using carcasses as well as living conspecifics was essential to remove the biases of unequal group abundances in the living population and to obtain reliable results for the population trends. Future studies still need to address in more detail the demographics of the European bat populations, including why juveniles appear to be more vulnerable at wind turbines at low densities; if they are particularly vulnerable to sensory traps due to a lack of experience; and whether migration and exploratory flights force them to spend more time at stopover sites or refuelling mid-air. In addition, it would be interesting to understand whether this pattern is also present in juveniles of other migratory bat species.

5. One isotope cannot rule them all – Future directions in stable isotope analysis

The more studies report on the trophic discrimination factors and fractionation values between tissues, the more we understand that there is no magic number that unites all species, such as 42[1]. Although there is a need to reduce and substitute animal studies *in vivo*, we still require animal experimentation and invasive sampling in order to develop a strong foundation to understand the dynamics of isotopes within and between species. One way to mitigate the experiments with living animals is using carcasses from environmental impact assessments of wind farms and other human constructions. The use of carcasses to study isotopic routing enables a unique insight because we can determine the isotopes in different tissues from the same individual without having to remove a living specimen from nature (Fisk et al. 2001; Măntoiu et al. 2020). Different tissues may vary their isotopic turnover rate and trophic discrimination factors (Fry 2006; Martínez del Rio et al. 2009). However, we still lack a systematisation of carcasses collection (e.g., which tissues to collect, at which places in the body), and standardisation of the metadata collected (e.g., Darwin core standards). Standardisation between species is still a challenge.

The type of tissues collected tends to vary depending on the research question, available equipment, etc, which makes the comparison within species and between species even more difficult. Through collaboration between researchers and professionals in other areas, we can create a global standard method for animal collection. For example, in biodiversity hotspots, the collection of animals that serves as voucher specimens (i.e., preserved specimens that serve as a verifiable and permanent record of wildlife) should include not only tissues for molecular analysis but also for other approaches, such as samples for isotopic analysis. Samples for isotopic analysis are easily stored when the material is metabolically inert (e.g.,

---

[1] Reference to the "Answer to the Ultimate Question of Life, the Universe, and Everything", in The Hitchhiker's Guide to the Galaxy by Douglas Adams.

keratinous tissue) or if metabolically active, by freezing (Wassenaar et al. 2015). In environmental impact assessment surveys, carcasses without visible signs of decay should be continuously collected and their organs separated and stored. This allows for the extraction of tissues from species that are not easily found in other surveys, are endangered, or are unknown to certain areas, without the need to remove the individual from the population. Moreover, this information allows a clearer overview of the relationship between and within tissues and species. In the last few years, the work of surveying and monitoring environmental impacts has substantially increased the scientific collections with materials from areas that have rarely or never been sampled, as well as other landscapes that have completely disappeared, such as those that constitute the reservoirs of large dams. These specimens and the tissues from individuals collected and/or found dead are evidence of the diversity still in the process of being described. Determining guidelines for an appropriate destination of biological materials combined with public policies can further hone the information that can be then organised by environmental departments and supervised by governmental agencies. Thus, there is a need to combine efforts to collect and store tissues whenever possible to address those gaps in knowledge. In addition, the standardisation method should include a simple and easy way to write comprehensive metadata associated with the samples collected. Recently, the use of big datasets has been considered vital to understanding global ecological patterns (e.g., Masson-Delmotte et al. (2021)). However, it is not uncommon to find errors and contradictions in the data. Therefore, a standard method to collect and write data as well as make the data publicly available should be considered in the near future.

Stable isotope analysis, however, has some limitations. Notably, the information obtained from a tissue sample will always reflect the place where it incorporated the isotopic signatures. Thus, the places used between the origin and the destination remain largely unknown using this method. Additionally, the advantage provided by only capturing the individuals once is

hampered by the cost of this method. On one hand, the lack of environmental data in certain locations can be an issue for isotopic geographical assignments, increasing the uncertainty in the isoscapes. Although models have improved over the past decades to account for this issue and the performance of laboratories worldwide has improved (Wassenaar et al. 2021), acquiring data on missing areas can help make isoscapes more detailed. On the other hand, the Global North concentrates most of the data, which can bias where most studies are performed. I believe that with the combination of methods such as the use of multiple isotopes (**chapter 1**), compound-specific isotope analysis of amino acids (reviewed in Whiteman et al. (2019)), and an online database for isotopes (e.g., Project Isobank), we could assign more detailed origins.

Finally, I have shown that the transfer function and fractionation of the tissues can influence the accuracy of the results obtained. Researchers have attempted to develop models to account for discrimination factors according to phylogenetics ("SIDER" R package (Healy et al. 2018)). However, it still needs actual isotopic values to increase the input of the models. Furthermore, the interaction of isotopes and tissues remains unclear. For example, only a few studies compared tissues' relationship to water (either drinking water or precipitation). Rodriguez Curras et al. (2018) measured the proportion of drinking water in tissues of laboratory mice (*Mus musculus*), showing that the overall contribution of water to muscle and liver was less than 20%. Hobson and Robbins (2009) evaluated the relationship of different tissues of nomadic Sedge Wren (*Cistothorus platensis*) to precipitation and found a weak relationship between liver and claw. Wolf et al. (2013) did not find a direct relationship between muscle and the drinking water of Japanese Quail (*Cortunix japonica*) but muscle tissues were depleted in relation to drinking water. Lastly, Lübcker et al. (2021) recommend caution in using moulted hair to address the diet and movement of animals after evaluating C

and N in moulted hair and the outermost epidermis of elephant seals (*Mirounga leonina*). Future studies focusing on the relationship between isotopes and tissues are required.

6. Shed light in the dark – Conservation efforts for migratory animals

The loss of migratory species is not inevitable. For example, the application of the dynamic conservation strategy for migratory species can improve the connectivity of habitats and protect migratory individuals (Reynolds et al. 2017). The dynamic conservation strategy aims to reduce threats to migratory species while providing opportunities to enhance the resilience of populations and their habitats (Zhao et al. 2021). It implements a set of conservation actions across the full annual cycle of migratory species. These actions include, but are not limited to, the protection and maintenance of key habitats, the creation of favourable conditions at migratory routes and stopover sites, and the mitigation of threats at source areas (Reynolds et al. 2017). The near-term ecological forecasting and the potential advantages of dynamic mitigation strategies have been shown to have successful results when applied to the conservation of birds in the U.S. (Horton et al. 2021). In addition, dynamic conservation management should be undertaken to support the long-term viability of migratory species. Considering the high temporal and spatial scales at which these actions are required, it is essential to involve a broad range of stakeholders at all levels of governance (e.g., local, national, regional, and international) in the planning, implementation, and evaluation of these actions.

With the recent development of offshore wind turbines, an urgent need for models that can map sites with the least impact on biodiversity is needed (May et al. 2021). A recent study modelled the impact of wind turbines in *L. cirineus* in the U.S. and Canada, and the simulations of population trends suggested past substantial population declines (Friedenberg

and Frick 2021). However, in order to achieve fatality minimisation, turbine curtailment may be sufficient to manage risks. Therefore, improvements to statistical methods such as species distribution models (e.g., McGowan et al. (2021)), occupancy modelling (e.g., Froidevaux et al. (2020)), hierarchical Bayesian models (e.g., Davy et al. (2021)), and data integration will further develop the analysis of historic trends in bat populations; harnessing the rapidly increasing number of data not only to track population trends in time and space but also to understand their drivers.

Several issues can be simultaneously tackled to protect migratory species. First, the effects of climate change on migratory behaviour should be continuously documented. This information can be used to identify the most vulnerable species and the habitats they use. In addition, the loss of migratory species may be prevented through the application of different policy managements that more effectively protect migratory animals (Voigt et al. 2018a). Such an option is the creation of protected areas that will prevent the degradation of important stopover sites along migratory routes. These areas can also be used to protect the habitats of species that are experiencing habitat loss, thus preventing further losses in migratory behaviour. Another option is to create corridors to connect fragmented habitats, as these can help migratory species maintain migratory behaviour (Zhao et al. 2021). These corridors can also be used to connect habitats to which species are shifting, thus giving them access to the habitats that are best suited to their needs. In addition, after identifying the corridors, they should be legislated as forbidden areas for new enterprises that can disrupt migratory pathways. Finally, education and citizen science (Fournier et al. 2017; Gili et al. 2020; Rodhouse et al. 2021) can be used to educate people about the importance of migratory species, the effects of climate change on them, and the steps that can be taken to prevent further losses of migratory species, helping them understand the importance of protecting migratory species.

REFERENCES FOR GENERAL INTRODUCTION AND DISCUSSION

Albarede F (2011) Isotope. In: Encyclopedia of Astrobiology. Springer Berlin Heidelberg, Berlin, Heidelberg, pp 860–860

Alcalde JT, Jiménez M, Brila I, et al (2021) Transcontinental 2200 km migration of a Nathusius' pipistrelle (Pipistrellus nathusii) across Europe. Mammalia 85:161–163. https://doi.org/10.1515/mammalia-2020-0069

Alerstam T, Bäckman J (2018) Ecology of animal migration. Curr. Biol. 28:R968–R972

Aristotle A History of Animals. Book VIII.

Arnett EB, Brown WK, Erickson WP, et al (2008) Patterns of Bat Fatalities at Wind Energy Facilities in North America. J Wildl Manage 72:61–78. https://doi.org/10.2193/2007-221

Baerwald EF, Barclay RMR (2011) Patterns of activity and fatality of migratory bats at a wind energy facility in Alberta, Canada. J Wildl Manage 75:1103–1114. https://doi.org/10.1002/jwmg.147

Baerwald EF, D'Amours GH, Klug BJ, Barclay RMR (2008) Barotrauma is a significant cause of bat fatalities at wind turbines. Curr Biol 18:R695–R696. https://doi.org/10.1016/j.cub.2008.06.029

Baerwald EF, Patterson WP, Barclay RMRR (2014) Origins and migratory patterns of bats killed by wind turbines in southern Alberta: Evidence from stable isotopes. Ecosphere 5:1–17. https://doi.org/10.1890/ES13-00380.1

Barbosa KV de C, Rodewald AD, Ribeiro MC, Jahn AE (2020) Noise level and water distance drive resident and migratory bird species richness within a Neotropical megacity. Landsc

Urban Plan 197:103769. https://doi.org/10.1016/j.landurbplan.2020.103769

Barrios L, Rodríguez A (2004) Behavioural and environmental correlates of soaring-bird
mortality at on-shore wind turbines. J Appl Ecol 41:72–81.
https://doi.org/10.1111/j.1365-2664.2004.00876.x

Bas Y, Kerbiriou C, Roemer C, Julien J (2020) Bat population trends

Bataille, Crowley BE, Wooller MJ, Bowen GJ (2020) Advances in global bioavailable
strontium isoscapes. Palaeogeogr Palaeoclimatol Palaeoecol 555:43.
https://doi.org/10.1016/j.palaeo.2020.109849

Bauer S, Hoye BJ (2014) Migratory animals couple biodiversity and ecosystem functioning
worldwide. Science (80-.). 344

Bauer S, Klaassen M (2013) Mechanistic models of animal migration behaviour - their
diversity, structure and use. J. Anim. Ecol. 82:498–508

Bearhop S, Waldron S, Votier SC, Furness RW (2002) Factors that influence assimilation
rates and fractionation of nitrogen and carbon stable isotopes in avian blood and feathers.
Physiol Biochem Zool 75:451–8. https://doi.org/10.1086/342800

Beebee TJC (1995) Amphibian breeding and climate. Nature 374:219–220.
https://doi.org/10.1038/374219a0

Bentley RA (2006) Strontium isotopes from the earth to the archaeological skeleton: A review.
J Archaeol Method Theory 13:135–187. https://doi.org/10.1007/s10816-006-9009-x

Berger J (2004) The last mile: How to sustain long-distance migration in mammals. Conserv.
Biol. 18:320–331

Blomberg AS, Vasko V, Salonen S, et al (2021) First record of a Nathusius' pipistrelle

(Pipistrellus nathusii) overwintering at a latitude above 60°N. Mammalia 85:74–78. https://doi.org/10.1515/mammalia-2020-0019

Bond AL, Diamond AW (2011) Recent Bayesian stable-isotope mixing models are highly sensitive to variation in discrimination factors. Ecol Appl 21:1017–1023. https://doi.org/10.1890/09-2409.1

Bowlin MS, Bisson I-A, Shamoun-Baranes J, et al (2010) Grand Challenges in Migration Biology. Integr Comp Biol 50:261–279. https://doi.org/10.1093/icb/icq013

Brinkmann R, Behr O, Korner-Nievergelt F, et al (2011) Entwicklung von Methoden zur Untersuchung und Reduktion des Kollisionsrisi kos von Fledermäusen an Onshore-Windenergieanlagen. Göttingen

Burgin CJ, Colella JP, Kahn PL, Upham NS (2018) How many species of mammals are there? J Mammal 99:1–14. https://doi.org/10.1093/jmammal/gyx147

Caizergues A, Van Wilgenburg SL, Hobson KA (2016) Unraveling migratory connectivity of two European diving ducks: a stable isotope approach. Eur J Wildl Res 62:701–711. https://doi.org/10.1007/s10344-016-1048-3

Cardenas-Ortiz L, Bayly NJ, Kardynal KJ, Hobson KA (2020) Defining catchment origins of a geographical bottleneck: Implications of population mixing and phenological overlap for the conservation of Neotropical migratory birds. Condor 122:. https://doi.org/10.1093/condor/duaa004

Caut S, Angulo E, Courchamp F (2008a) Discrimination factors ($\Delta 15 N$ and $\Delta 13 C$) in an omnivorous consumer: effect of diet isotopic ratio. Funct Ecol 22:255–263. https://doi.org/10.1111/j.1365-2435.2007.01360.x

Caut S, Angulo E, Courchamp F (2008b) Caution on isotopic model use for analyses of

consumer diet. Can J Zool 86:438–445. https://doi.org/10.1139/Z08-012

Cazzolla Gatti R, Callaghan T, Velichevskaya A, et al (2019) Accelerating upward treeline shift in the Altai Mountains under last-century climate change. Sci Rep 9:7678. https://doi.org/10.1038/s41598-019-44188-1

Cerling TE, Ayliffe LK, Dearing MD, et al (2007) Determining biological tissue turnover using stable isotopes: the reaction progress variable. Oecologia 151:175–189. https://doi.org/10.1007/s00442-006-0571-4

Chamberlain CP, Blum JD, Holmes RT, et al (1997) The use of isotope tracers for identifying populations of migratory birds. Oecologia 109:132–141. https://doi.org/10.1007/s004420050067

Chapman JW, Reynolds DR, Wilson K (2015) Long-range seasonal migration in insects: Mechanisms, evolutionary drivers and ecological consequences. Ecol. Lett. 18:287–302

Ciechanowski M, Zając T, Zielińska A, Dunajski R (2010) Seasonal activity patterns of seven vespertilionid bat species in Polish lowlands. Acta Theriol (Warsz) 55:301–314. https://doi.org/10.4098/j.at.0001-7051.093.2009

Clementz MT (2012) New insight from old bones: stable isotope analysis of fossil mammals. J Mammal 93:368–380. https://doi.org/10.1644/11-MAMM-S-179.1

Coulson T, Catchpole EA, Albon SD, et al (2001) Age, Sex, Density, Winter Weather, and Population Crashes in Soay Sheep. Science (80-) 292:1528–1531. https://doi.org/10.1126/science.292.5521.1528

Coulter DP, Bowen GJ, Höök TO (2017) Influence of diet and ambient water on hydrogen and oxygen stable isotope ratios in fish tissue: patterns within and among tissues and relationships with growth rates. Hydrobiologia 1–11. https://doi.org/10.1007/s10750-017-

3200-9

Courtiol A, Rousset F, Rohwäder M-S, et al (2019) Isoscape Computation and Inference of Spatial Origins With Mixed Models Using the R package IsoriX. In: Tracking Animal Migration with Stable Isotopes. pp 207–236

Cryan PM, Bogan MA, Rye RO, et al (2004) Stable Hydrogen Isotope Analysis of Bat Hair as Evidence for Seasonal Molt and Long-Distance Migration. J Mammal 85:995–1001. https://doi.org/10.1644/BRG-202

Cryan PM, Brown AC (2007) Migration of bats past a remote island offers clues toward the problem of bat fatalities at wind turbines. Biol Conserv 139:1–11. https://doi.org/10.1016/j.biocon.2007.05.019

Cryan PM, Stricker CA, Wunder MB (2014) Continental-scale, seasonal movements of a heterothermic migratory tree bat. Ecol Appl 24:602–616. https://doi.org/10.1890/13-0752.1

Cryan PM, Stricker CA, Wunder MB (2012) Evidence of cryptic individual specialization in an opportunistic insectivorous bat. J Mammal 93:381–389. https://doi.org/10.1644/11-MAMM-S-162.1

Currie SE, Boonman A, Troxell S, et al (2020) Echolocation at high intensity imposes metabolic costs on flying bats. Nat Ecol Evol 4:1174–1177. https://doi.org/10.1038/s41559-020-1249-8

Dallimer M, Strange N (2015) Why socio-political borders and boundaries matter in conservation. Trends Ecol. Evol. 30:132–139

Davis RW (2008) Bio-logging as a Method for Understanding Natural Systems. In: International Conference on Informatics Education and Research for Knowledge-

Circulating Society (icks 2008). IEEE, pp 12–17

Davy CM, Ford AT, Fraser KC (2017) Aeroconservation for the Fragmented Skies. Conserv Lett 10:773–780. https://doi.org/10.1111/conl.12347

Davy CM, Squires K, Zimmerling JR (2021) Estimation of spatiotemporal trends in bat abundance from mortality data collected at wind turbines. Conserv Biol 35:227–238. https://doi.org/10.1111/cobi.13554

Dietz R, Outridge PM, Hobson KA (2009) Anthropogenic contributions to mercury levels in present-day Arctic animals—A review. Sci Total Environ 407:6120–6131. https://doi.org/10.1016/j.scitotenv.2009.08.036

Dingle H (2014) Migration: the biology of life on the move. Oxford University Press, USA.

Dingle H (2006) Animal migration: Is there a common migratory syndrome? J. Ornithol. 147:212–220

Dingle H, Drake VA (2007) What is migration? Bioscience 57:113–121. https://doi.org/10.1641/B570206

Dunn PO, Winkler DW (1999) Climate change has affected the breeding date of tree swallows throughout North America. Proc R Soc London Ser B Biol Sci 266:2487–2490. https://doi.org/10.1098/rspb.1999.0950

Dzal Y, Hooton LA, Clare EL, Fenton MB (2009) Bat Activity and Genetic Diversity at Long Point, Ontario, an Important Bird Stopover Site. Acta Chiropterologica 11:307–315. https://doi.org/10.3161/150811009X485549

Ewert DN, Hall KR, Wunderle JM, et al (2012) Duration and rate of spring migration of Kirtland's Warblers. Wilson J Ornithol 124:9–14. https://doi.org/10.1676/11-073.1

Fisk AT, Hobson KA, Norstrom RJ (2001) Influence of Chemical and Biological Factors on Trophic Transfer of Persistent Organic Pollutants in the Northwater Polynya Marine Food Web. Environ Sci Technol 35:732–738. https://doi.org/10.1021/es001459w

Flack A, Fiedler W, Blas J, et al (2016) Costs of migratory decisions: A comparison across eight white stork populations. Sci Adv 2:e1500931. https://doi.org/10.1126/sciadv.1500931

Flaquer C, Puig-Montserrat X, Goiti U, et al (2009) Habitat Selection in Nathusius' Pipistrelle (Pipistrellus nathusii): The Importance of Wetlands. Acta Chiropterologica 11:149–155. https://doi.org/10.3161/150811009X465767

Fleming TH (2019) Bat Migration. In: Encyclopedia of Animal Behavior. Elsevier, pp 605–610

Fleming TH, Eby P, Kunz TH, Fenton MB (2003) Ecology of bat migration. In: Bat Ecology. pp 164–65

Fleming TH, Nunez RA, Sternberg L da SL (1993) Seasonal changes in the diets of migrant and non-migrant nectarivorous bats as revealed by carbon stable isotope analysis. Oecologia 94:72–75. https://doi.org/10.1007/BF00317304

Font L, Van Der Peijl G, Van Wetten I, et al (2012) Strontium and lead isotope ratios in human hair: Investigating a potential tool for determining recent human geographical movements. J Anal At Spectrom 27:719–732. https://doi.org/10.1039/c2ja10361c

Fournier AM V., Sullivan AR, Bump JK, et al (2017) Combining citizen science species distribution models and stable isotopes reveals migratory connectivity in the secretive <scp>V</scp> irginia rail. J Appl Ecol 54:618–627. https://doi.org/10.1111/1365-2664.12723

Fraser EE, Longstaffe FJ, Fenton MB (2013) Moulting matters: the importance of understanding moulting cycles in bats when using fur for endogenous marker analysis. Can J Zool 91:533–544. https://doi.org/10.1139/cjz-2013-0072

Fraser EE, McGuire LP, Eger JL, et al (2012) Evidence of Latitudinal Migration in Tri-colored Bats, Perimyotis subflavus. PLoS One 7:e31419. https://doi.org/10.1371/journal.pone.0031419

Frei KM, Frei R, Mannering U, et al (2009) Provenance of Ancient Textiles-A Pilot Study Evaluating the Strontium Isotope System in Wool. Archaeometry 51:252–276. https://doi.org/10.1111/j.1475-4754.2008.00396.x

Frick WF, Baerwald EF, Pollock JF, et al (2017) Fatalities at wind turbines may threaten population viability of a migratory bat. Biol Conserv 209:172–177. https://doi.org/10.1016/j.biocon.2017.02.023

Friedenberg NA, Frick WF (2021) Assessing fatality minimization for hoary bats amid continued wind energy development. Biol Conserv 262:109309. https://doi.org/10.1016/j.biocon.2021.109309

Fritze M, Lehnert LS, Heim O, et al (2019) Fledermausschutz im Schatten der Windenergie. Naturschutz und Landschaftsplan 51:20–27

Froidevaux JSP, Boughey KL, Hawkins CL, et al (2020) Evaluating survey methods for bat roost detection in ecological impact assessment. Anim Conserv 23:597–606. https://doi.org/10.1111/acv.12574

Fry B (2006) Stable Isotope Ecology. Springer New York, New York, NY

Furmankiewicz J, Kucharska M (2009) Migration of Bats along a Large River Valley in Southwestern Poland. J Mammal 90:1310–1317. https://doi.org/10.1644/09-MAMM-S-

099R1.1

Garbino GST, Feijo A, Beltrao-Mendes R, Da Rocha PA (2021) Evolution of litter size in bats and its influence on longevity and roosting ecology. Biol J Linn Soc 132:676–684. https://doi.org/10.1093/biolinnean/blaa203

Gaultier SP, Blomberg AS, Ijäs A, et al (2020) Bats and Wind Farms: The Role and Importance of the Baltic Sea Countries in the European Context of Power Transition and Biodiversity Conservation. Environ Sci Technol 54:10385–10398. https://doi.org/10.1021/acs.est.0c00070

Gauthreaux SA (2019) Bird migration. In: Encyclopedia of Animal Behavior. pp 622–632

Gili F, Newson SE, Gillings S, et al (2020) Bats in urbanising landscapes: habitat selection and recommendations for a sustainable future. Biol Conserv 241:108343. https://doi.org/10.1016/j.biocon.2019.108343

Gillis EA, Green DJ, Middleton HA, Morrissey CA (2008) Life history correlates of alternative migratory strategies in American Dippers. Ecology 89:1687–1695

Gilpin J (1786) Observations on the annual passage of herrings. Trans Am Philos Soc 2:236–239

Godlevska L V. (2015) Northward expansion of the winter range of Nyctalus noctula (Chiroptera: Vespertilionidae) in Eastern Europe. Mammalia 79:315–324. https://doi.org/10.1515/mammalia-2013-0178

Gonçalves F, Magioli M, Bovendorp RS, et al (2020) Prey Choice of Introduced Species by the Common Vampire Bat (Desmodus rotundus) on an Atlantic Forest Land-Bridge Island. Acta Chiropterologica 22:167. https://doi.org/10.3161/15081109ACC2020.22.1.015

Gresh T, Lichatowich J, Schoonmaker P (2000) An Estimation of Historic and Current Levels of Salmon Production in the Northeast Pacific Ecosystem: Evidence of a Nutrient Deficit in the Freshwater Systems of the Pacific Northwest. Fisheries 25:15–21. https://doi.org/10.1577/1548-8446(2000)025<0015:aeohac>2.0.co;2

Hartman G, Danin A (2010) Isotopic values of plants in relation to water availability in the Eastern Mediterranean region. Oecologia 162:837–852. https://doi.org/10.1007/s00442-009-1514-7

Hayes MA (2013) Bats Killed in Large Numbers at United States Wind Energy Facilities. Bioscience 63:975–979. https://doi.org/10.1525/bio.2013.63.12.10

Hays GC (2000) The implications of variable remigration intervals for the assessment of population size in marine turtles. J Theor Biol 206:221–227. https://doi.org/10.1006/jtbi.2000.2116

Healy K, Guillerme T, Kelly SBAA, et al (2018) SIDER: an R package for predicting trophic discrimination factors of consumers based on their ecology and phylogenetic relatedness. Ecography (Cop) 41:1393–1400. https://doi.org/10.1111/ecog.03371

Hedenström A (2009) Optimal Migration Strategies in Bats. J Mammal 90:1298–1309. https://doi.org/10.1644/09-MAMM-S-075R2.1

Hoare B (2009) Animal Migration: Remarkable Journeys in the Wild. United States: University of California Press.

Hobson K a., Robbins MB (2009) Origins of Late-Breeding Nomadic Sedge Wrens in North America: Limitations and Potential of Hydrogen-Isotope Analyses of Soft Tissue. Condor 111:188–192. https://doi.org/10.1525/cond.2009.080001

Hobson K, Wassenaar L (2019) Tracking Animal Migration with Stable Isotopes. Elsevier

Hobson KA (2003) Making Migratory Connections with Stable Isotopes. In: Avian Migration. Springer, Berlin, Heidelberg, pp 379–391

Hobson KA (2008) Applying Isotopic Methods to Tracking Animal Movements. In: Terrestrial Ecology. pp 45–78

Hobson KA (1999) Tracing origins and migration of wildlife using stable isotopes: a review. Oecologia 120:314–326. https://doi.org/10.1007/s004420050865

Hobson KA (2005) Using stable isotopes to trace long-distance dispersal in birds and other taxa. Divers Distrib 11:157–164. https://doi.org/10.1111/j.1366-9516.2005.00149.x

Hobson KA (2019) Application of Isotopic Methods to Tracking Animal Movements. In: Tracking Animal Migration with Stable Isotopes, Second Edi. Elsevier, pp 85–115

Hobson KA, Norris DR, Kardynal KJ, Yohannes E (2019) Animal Migration. In: Tracking Animal Migration with Stable Isotopes. Elsevier, pp 1–23

Hoogewerff JA, Reimann C, Ueckermann H, et al (2019) Bioavailable 87Sr/86Sr in European soils: A baseline for provenancing studies. Sci Total Environ 672:1033–1044. https://doi.org/10.1016/j.scitotenv.2019.03.387

Horn JW, Arnett EB, Kunz TH (2008) Behavioral Responses of Bats to Operating Wind Turbines. J Wildl Manage 72:123–132. https://doi.org/10.2193/2006-465

Horton KG, Van Doren BM, Albers HJ, et al (2021) Near-term ecological forecasting for dynamic aeroconservation of migratory birds. Conserv Biol cobi.13740. https://doi.org/10.1111/cobi.13740

Hunt WG, Wiens JD, Law PR, et al (2017) Quantifying the demographic cost of humanrelated mortality to a raptor population. PLoS One 12:e0172232.

https://doi.org/10.1371/journal.pone.0172232

Hutterer R, Ivanova T, Meyer-Cords C, Rodrigues LL (2005) Bat Migrations in Europe A Review of Banding Data and Literature

Ijäs A, Kahilainen A, Vasko V V., Lilley TM (2017) Evidence of the Migratory Bat, Pipistrellus nathusii, Aggregating to the Coastlines in the Northern Baltic Sea. Acta Chiropterologica 19:127. https://doi.org/10.3161/15081109ACC2017.19.1.010

IRENA (2020) Reaching zero with renewables: Eliminating CO2 emissions from industry and transport in line with the 1.5^0C climate goal. 216

Jahelková H, Horáček I (2011) Mating System of a Migratory Bat, Nathusius' Pipistrelle (Pipistrellus nathusii): Different Male Strategies. Acta Chiropterologica 13:123–137. https://doi.org/10.3161/150811011X578679

Jenni L, Kéry M (2003) Timing of autumn bird migration under climate change: Advances in long-distance migrants, delays in short-distance migrants. Proc R Soc B Biol Sci 270:1467–1471. https://doi.org/10.1098/rspb.2003.2394

Jepsen S, Schweitzer DF, Young B, Sears N (2015) Conservation Status and Ecology of the Monarch Butterfly in the United States. Xerces Soc Intvertebrate Conserv

Joly K, Klein DR, Verbyla DL, et al (2011) Linkages between large-scale climate patterns and the dynamics of Arctic caribou populations. Ecography (Cop) 34:345–352. https://doi.org/10.1111/j.1600-0587.2010.06377.x

Keller S, Valls M, Hidalgo M, Quetglas A (2014) Influence of environmental parameters on the life-history and population dynamics of cuttlefish Sepia officinalis in the western Mediterranean. Estuar Coast Shelf Sci 145:31–40. https://doi.org/10.1016/j.ecss.2014.04.016

Kirsanow K, Tuross N (2011) Oxygen and hydrogen isotopes in rodent tissues: Impact of diet, water and ontogeny. Palaeogeogr Palaeoclimatol Palaeoecol 310:9–16. https://doi.org/10.1016/j.palaeo.2011.03.022

Koch PL, Heisinger J, Moss C, et al (1995) Isotopic tracking of change in diet and habitat use in African elephants. Science (80-) 267:1340–1343. https://doi.org/10.1126/science.267.5202.1340

Korner-Nievergelt F, Brinkmann R, Niermann I, Behr O (2013) Estimating Bat and Bird Mortality Occurring at Wind Energy Turbines from Covariates and Carcass Searches Using Mixture Models. PLoS One 8:e67997. https://doi.org/10.1371/journal.pone.0067997

Krauel JJ, Mccracken GF (2013) Bat Evolution, Ecology, and Conservation. Springer New York, New York, NY

Krauel JJ, McCracken GF (2013) Recent Advances in Bat Migration Research. In: Bat Evolution, Ecology, and Conservation. Springer New York, New York, NY, pp 293–313

Kravchenko KA, Vlaschenko AS, Lehnert LS, et al (2020) Generational shift in the migratory common noctule bat: first-year males lead the way to hibernacula at higher latitudes. Biol Lett 16:20200351. https://doi.org/10.1098/rsbl.2020.0351

Kravchenko KA, Vlaschenko AS, Voigt CC (2015) Stable hydrogene isotope analysis in study of changes in migration status of *N . noctula* in Eastern Europe

Krüger F, Clare EL, Symondson WOCC, et al (2014) Diet of the insectivorous bat *Pipistrellus nathusii* during autumn migration and summer residence. Mol Ecol 23:3672–3683. https://doi.org/10.1111/mec.12547

Kunz TH, Fenton MB (eds) (2005) Bat ecology. University of Chicago Press.

Kurvits T, Nellermann C, Alfthan B, et al (2011) Living planet: Connected planet - Preventing the end of the world's wildlife migrations through ecological networks

Lehnert LS, Kramer-Schadt S, Schönborn S, et al (2014) Wind Farm Facilities in Germany Kill Noctule Bats from Near and Far. PLoS One 9:e103106. https://doi.org/10.1371/journal.pone.0103106

Lehnert LS, Kramer-Schadt S, Teige T, et al (2018) Variability and repeatability of noctule bat migration in Central Europe: evidence for partial and differential migration. Proc R Soc B Biol Sci 285:20182174. https://doi.org/10.1098/rspb.2018.2174

Lehodey P, Alheit J, Barange M, et al (2006) Climate variability, fish, and fisheries. J. Clim. 19:5009–5030

Limpens HJG, Schulte R (2000) Biologie und Schutz gefährdeter wandernder mitteleuropäischer Fledermausarten am Beispiel von Rauhhautfledermäusen (Pipistrellus nathusii) und Teichfledermäusen (Myotis dasycneme). Nyctalus (NF) 7:317–327

Lindecke O, Elksne A, Holland RA, et al (2019) Orientation and flight behaviour identify the Soprano pipistrelle as a migratory bat species at the Baltic Sea coast. J Zool 308:56–65. https://doi.org/10.1111/jzo.12654

Lohmann KJ, Hester JT, Lohmann CMF (1999) Long-distance navigation in sea turtles. Ethol Ecol Evol 11:1–23. https://doi.org/10.1080/08927014.1999.9522838

Lohmann KJ, Lohmann CMF, Putman NF (2007) Magnetic maps in animals: Nature's GPS. J. Exp. Biol. 210:3697–3705

Lübcker N, Newsome S, Bester M, de Bruyn P (2021) Validating the use of bulk tissue stable isotope and amino acid δ15N values measured in molted hair and epidermis of elephant seals to assess temporal foraging niche specialization. Mar Ecol Prog Ser 673:229–243.

https://doi.org/10.3354/meps13772

Măntoiu DȘ, Kravchenko K, Lehnert LS, et al (2020) Wildlife and infrastructure: impact of wind turbines on bats in the Black Sea coast region. Eur J Wildl Res 66:44. https://doi.org/10.1007/s10344-020-01378-x

Marra PP, Cohen E, Harrison AL, et al (2019) Migratory connectivity. In: Encyclopedia of Animal Behavior. pp 643–654

Martínez del Rio C, Wolf N, Carleton S a., Gannes LZ (2009) Isotopic ecology ten years after a call for more laboratory experiments. Biol Rev 84:91–111. https://doi.org/10.1111/j.1469-185X.2008.00064.x

Martínez del Rio C, Carleton S a. (2012) How fast and how faithful: the dynamics of isotopic incorporation into animal tissues: Fig. 1. J Mammal 93:353–359. https://doi.org/10.1644/11-MAMM-S-165.1

Mas M, Flaquer C, Rebelo H, López-Baucells A (2021) Bats and wetlands: synthesising gaps in current knowledge and future opportunities for conservation. Mamm. Rev. 51:369–384

Masson-Delmotte V, Zhai P, Pirani A, et al (2021) IPCC, 2021: Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press

May R, Jackson CR, Middel H, et al (2021) Life-cycle impacts of wind energy development on bird diversity in Norway. Environ Impact Assess Rev 90:106635. https://doi.org/10.1016/j.eiar.2021.106635

McCracken GF, Wilkinson GS (2000) Bat Mating Systems. In: Reproductive Biology of Bats. Academic Press, pp 321–362

McCutchan JH, Lewis WM, Kendall C, McGrath CC (2003) Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos 102:378–390. https://doi.org/10.1034/j.1600-0706.2003.12098.x

McGowan NE, Roche N, Aughney T, et al (2021) Testing consistency of modelled predictions of the impact of climate change on bats. Clim Chang Ecol 2:100011. https://doi.org/10.1016/j.ecochg.2021.100011

McGuire LP (2012) Physiological Ecology of Bat Migration. Electronic Thesis and Dissertation Repository. 431.

McGuire LP, Guglielmo CG, Mackenzie SA, Taylor PD (2012) Migratory stopover in the long-distance migrant silver-haired bat, Lasionycteris noctivagans. J Anim Ecol 81:377–385. https://doi.org/10.1111/j.1365-2656.2011.01912.x

McGuire LP, Jonasson KA, Guglielmo CG (2014) Bats on a Budget: Torpor-Assisted Migration Saves Time and Energy. PLoS One 9:e115724. https://doi.org/10.1371/journal.pone.0115724

Meehan T, Giermakowski JT, Cryan P (2004) GIS-based model of stable hydrogen isotope ratios in North American growing-season precipitation for use in animal movement studies. Isotopes Environ Health Stud 40:291–300. https://doi.org/10.1080/10256010410001731404

Milano S, Frahnert S, Hallau A, et al (2021) Isotope record tracks changes in historical wintering ranges of a passerine in sub-Saharan Africa. Glob Chang Biol 27:5460–5468. https://doi.org/10.1111/gcb.15794

Mirón M. LL, Herrera M. LG, Ramírez P. N, Hobson K a (2006) Effect of diet quality on carbon and nitrogen turnover and isotopic discrimination in blood of a New World

nectarivorous bat. J Exp Biol 209:541–548. https://doi.org/10.1242/jeb.02016

Moore M V., Zakova P, Shaeffer KA (1998) Potential effects of Canada geese and climate change on phosphorus inputs to suburban lakes of the Northeastern U.S.A. Lake Reserv Manag 14:52–59. https://doi.org/10.1080/07438149809354109

Muñoz-Romo M, Herrera EA, Kunz TH (2008) Roosting behavior and group stability of the big fruit-eating bat Artibeus lituratus (Chiroptera: Phyllostomidae). Mamm Biol 73:214–221. https://doi.org/10.1016/j.mambio.2007.05.013

O'Connor B, Bojinski S, Röösli C, Schaepman ME (2020) Monitoring global changes in biodiversity and climate essential as ecological crisis intensifies. Ecol Inform 55:101033. https://doi.org/10.1016/j.ecoinf.2019.101033

Oelze VM, Koch JK, Kupke K, et al (2012) Multi-isotopic analysis reveals individual mobility and diet at the early iron age monumental tumulus of magdalenenberg, germany. Am J Phys Anthropol 148:406–421. https://doi.org/10.1002/ajpa.22063

Orr TJ, Zuk M (2013) Does delayed fertilization facilitate sperm competition in bats? Behav Ecol Sociobiol 67:1903–1913. https://doi.org/10.1007/s00265-013-1598-2

Ossa G, Kramer-Schadt S, Peel AJ, et al (2012) The Movement Ecology of the Straw-Colored Fruit Bat, Eidolon helvum, in Sub-Saharan Africa Assessed by Stable Isotope Ratios. PLoS One 7:e45729. https://doi.org/10.1371/journal.pone.0045729

Parmesan C, Yohe G (2003) A globally coherent fingerprint of climate change impacts across natural systems. Nature 421:37–42. https://doi.org/10.1038/nature01286

Paunović M, Juste J (2016) Pipistrellus nathusii. In: IUCN Red List Threat. Species 2016

Peake S, McKinley RS (1998) A re-evaluation of swimming performance in juvenile

salmonids relative to downstream migration. Can J Fish Aquat Sci 55:682–687. https://doi.org/10.1139/f97-264

Pearson RM, van de Merwe JP, Connolly RM (2020) Global oxygen isoscapes for barnacle shells: Application for tracing movement in oceans. Sci Total Environ 705:135782. https://doi.org/10.1016/j.scitotenv.2019.135782

Pfeiffer B, Mayer F (2013) Spermatogenesis, sperm storage and reproductive timing in bats. J Zool 289:77–85. https://doi.org/10.1111/j.1469-7998.2012.00970.x

Pietsch SJ, Hobson KA, Wassenaar LI, Tütken T (2011) Tracking Cats: Problems with Placing Feline Carnivores on $\delta 18O$, δD Isoscapes. PLoS One 6:e24601. https://doi.org/10.1371/journal.pone.0024601

Pinti D (2011) Delta, Isotopic. In: Encyclopedia of Astrobiology. Springer Berlin Heidelberg, Berlin, Heidelberg, pp 418–418

Popa-Lisseanu AG, Sörgel K, Luckner A, et al (2012) A Triple-Isotope Approach to Predict the Breeding Origins of European Bats. PLoS One 7:e30388. https://doi.org/10.1371/journal.pone.0030388

Popa-Lisseanu AG, Voigt CC (2009) Bats on the Move. J Mammal 90:1283–1289. https://doi.org/10.1644/09-MAMM-S-130R2.1

Post DM (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83:703–718

Pylant CL, Nelson DM, Fitzpatrick MC, et al (2016) Geographic origins and population genetics of bats killed at wind-energy facilities. Ecol Appl 26:1381–1395. https://doi.org/10.1890/15-0541

Racey PA, Entwistle AC (2003) Conservation Ecology. Plenum, New York. In: Kunz TH, Fenton MB (eds) Bat Ecology. Plenum, New York, pp 680–743

Recalde FC, Breviglieri CPB, Kersch-Becker MF, Romero GQ (2021) Contribution of emergent aquatic insects to the trophic variation of tropical birds and bats. Food Webs 29:e00209. https://doi.org/10.1016/j.fooweb.2021.e00209

Reich MS, Flockhart DTT, Norris DR, et al (2021) Continuous-surface geographic assignment of migratory animals using strontium isotopes: A case study with monarch butterflies. Methods Ecol Evol. https://doi.org/10.1111/2041-210X.13707

Reppert SM, de Roode JC (2018) Demystifying Monarch Butterfly Migration. Curr. Biol. 28:R1009–R1022

Reudink MW, Kyle CJ, McKellar AE, et al (2016) Linking Isotopes and Panmixia: High Within-Colony Variation in Feather δ2H, δ13C, and δ15N across the Range of the American White Pelican. PLoS One 11:e0150810. https://doi.org/10.1371/journal.pone.0150810

Rex K, Czaczkes BI, Michener R, et al (2010) Specialization and omnivory in diverse mammalian assemblages. Écoscience 17:37–46. https://doi.org/10.2980/17-1-3294

Reynolds MD, Sullivan BL, Hallstein E, et al (2017) Dynamic conservation for migratory species. Sci Adv 3:e1700707. https://doi.org/10.1126/sciadv.1700707

Richter H V., Cumming GS (2008) First application of satellite telemetry to track African straw-coloured fruit bat migration. J Zool 275:172–176. https://doi.org/10.1111/j.1469-7998.2008.00425.x

Robinson RA, Crick HQP, Learmonth JA, et al (2009) Travelling through a warming world: Climate change and migratory species. Endanger. Species Res. 7:87–99

Rodhouse TJ, Rose S, Hawkins T, Rodriguez RM (2021) Audible bats provide opportunities for citizen scientists. Conserv Sci Pract 3:e435. https://doi.org/10.1111/csp2.435

Rodriguez Curras M, Fogel ML, Newsome SD (2018) Assimilation and discrimination of hydrogen isotopes in a terrestrial mammal. Oecologia 188:381–393. https://doi.org/10.1007/s00442-018-4221-4

Roeleke M, Blohm T, Kramer-Schadt S, et al (2016) Habitat use of bats in relation to wind turbines revealed by GPS tracking. Sci Rep 6:28961. https://doi.org/10.1038/srep28961

Rozhok A (2008) Orientation and navigation in vertebrates

Russ JM, Racey PA (2007) Species-specificity and individual variation in the song of male Nathusius' pipistrelles (Pipistrellus nathusii). Behav Ecol Sociobiol 61:669–677. https://doi.org/10.1007/s00265-006-0295-9

Rydell J, Bach L, Dubourg-Savage M-J, et al (2010) Bat Mortality at Wind Turbines in Northwestern Europe. Acta Chiropterologica 12:261–274. https://doi.org/10.3161/150811010X537846

Sachanowicz K, Ciechanowski M, Tryjanowski P, Kosicki JZ (2019) Wintering range of Pipistrellus nathusii (Chiroptera) in Central Europe: has the species extended to the north-east using urban heat islands? Mammalia 83:260–271. https://doi.org/10.1515/mammalia-2018-0014

Saidur R, Rahim NA, Islam MR, Solangi KH (2011) Environmental impact of wind energy. Renew. Sustain. Energy Rev. 15:2423–2430

Santos CD, Ferraz R, Munõz AR, et al (2021) Black kites of different age and sex show similar avoidance responses to wind turbines during migration. R Soc Open Sci 8:. https://doi.org/10.1098/rsos.201933

Schuster E, Bulling L, Köppel J (2015) Consolidating the State of Knowledge: A Synoptical Review of Wind Energy's Wildlife Effects. Environ Manage 56:300–331. https://doi.org/10.1007/s00267-015-0501-5

Sellick MJ, Kyser TK, Wunder MB, et al (2009) Geographic Variation of Strontium and Hydrogen Isotopes in Avian Tissue: Implications for Tracking Migration and Dispersal. PLoS One 4:e4735. https://doi.org/10.1371/journal.pone.0004735

Shaw AK (2016) Drivers of animal migration and implications in changing environments. Evol Ecol 30:991–1007. https://doi.org/10.1007/s10682-016-9860-5

Siemers BM, Greif S, Borissov I, et al (2011) Divergent trophic levels in two cryptic sibling bat species. Oecologia 166:69–78. https://doi.org/10.1007/s00442-011-1940-1

Skog NA (2021) Bats in Urban Sweden : A multiple regression analysis of bats' relationship to urbanization

Smotherman M, Knörnschild M, Smarsh G, Bohn K (2016) The origins and diversity of bat songs. J. Comp. Physiol. A Neuroethol. Sensory, Neural, Behav. Physiol. 202:535–554

Srygley RB, Dudley R, Oliveira EG, et al (2010) El Niño and dry season rainfall influence hostplant phenology and an annual butterfly migration from Neotropical wet to dry forests. Glob Chang Biol 16:936–945. https://doi.org/10.1111/j.1365-2486.2009.01986.x

Storm-Suke A, Wassenaar LI, Nol E, Norris DR (2012) The influence of metabolic rate on the contribution of stable-hydrogen and oxygen isotopes in drinking water to quail blood plasma and feathers. Funct Ecol 26:1111–1119. https://doi.org/10.1111/j.1365-2435.2012.02014.x

Studds CE, Marra PP (2011) Rainfall-induced changes in food availability modify the spring departure programme of a migratory bird. Proc R Soc B Biol Sci 278:3437–3443.

https://doi.org/10.1098/rspb.2011.0332

Šuba J, Petersons G, Rydell J (2013) Fly-and-Forage Strategy in the Bat Pipistrellus nathusii During Autumn Migration. Acta Chiropterologica 14:379. https://doi.org/10.3161/150811012x661693

Sullivan AR, Bump JK, Kruger LA, Peterson RO (2012) Bat-cave catchment areas: using stable isotopes (δD) to determine the probable origins of hibernating bats. Ecol Appl 22:1428–1434. https://doi.org/10.1890/11-1438.1

Taylor PD, Mackenzie SA, Thurber BG, et al (2011) Landscape movements of migratory birds and bats reveal an expanded scale of stopover. PLoS One 6:. https://doi.org/10.1371/journal.pone.0027054

Thaker M, Zambre A, Bhosale H (2018) Wind farms have cascading impacts on ecosystems across trophic levels. Nat Ecol Evol 2:1854–1858. https://doi.org/10.1038/s41559-018-0707-z

Thaxter CB, Buchanan GM, Carr J, et al (2017) Bird and bat species' global vulnerability to collision mortality at wind farms revealed through a trait-based assessment. Proc R Soc B Biol Sci 284:. https://doi.org/10.1098/rspb.2017.0829

Tieszen LL, Boutton TW, Tesdahl KG, Slade NA (1983) Fractionation and turnover of stable carbon isotopes in animal tissues: Implications for ?13C analysis of diet. Oecologia 57:32–37. https://doi.org/10.1007/BF00379558

Tipple BJ, Chau T, Chesson LA, et al (2013) Isolation of strontium pools and isotope ratios in modern human hair. Anal Chim Acta 798:64–73. https://doi.org/10.1016/j.aca.2013.08.054

Troxell SA, Holderied MW, Pētersons G, Voigt CC (2019) Nathusius' bats optimize long-

distance migration by flying at maximum range speed. J Exp Biol 222:1–7. https://doi.org/10.1242/jeb.176396

Usaid (2009) Adapting to coastal climate change : a guidebook for development planners. Nature 148

Van Buskirk J, Mulvihill RS, Leberman RC (2009) Variable shifts in spring and autumn migration phenology in North American songbirds associated with climate change. Glob Chang Biol 15:760–771. https://doi.org/10.1111/j.1365-2486.2008.01751.x

Vanderklift MA, Ponsard S (2003) Sources of variation in consumer-diet ?15N enrichment: a meta-analysis. Oecologia 136:169–182. https://doi.org/10.1007/s00442-003-1270-z

Vautour G, Poirier A, Widory D (2015) Tracking mobility using human hair: What can we learn from lead and strontium isotopes? Sci Justice 55:63–71. https://doi.org/10.1016/j.scijus.2014.10.001

Voerkelius S, Lorenz GD, Rummel S, et al (2010) Strontium isotopic signatures of natural mineral waters, the reference to a simple geological map and its potential for authentication of food. Food Chem 118:933–940. https://doi.org/10.1016/j.foodchem.2009.04.125

Vogel JC, Eglington B, Auret JM (1990) Isotope fingerprints in elephant bone and ivory. Nature 346:747–749. https://doi.org/10.1038/346747a0

Voigt C, Sörgel K, Šuba J, et al (2012a) The insectivorous bat Pipistrellus nathusii uses a mixed-fuel strategy to power autumn migration. Proc R Soc B Biol Sci 279:3772–3778. https://doi.org/10.1098/rspb.2012.0902

Voigt CC, Azam C, Dekker J, et al (2018a) Guidelines for consideration of bats in lighting projects. EUROBATS Publication Series No. 8

Voigt CC, Currie SE, Fritze M, et al (2018b) Conservation Strategies for Bats Flying at High Altitudes. Bioscience 68:427–435. https://doi.org/10.1093/biosci/biy040

Voigt CC, Helbig-Bonitz M, Kramer-Schadt S, Kalko EK V (2014) The third dimension of bat migration: evidence for elevational movements of Miniopterus natalensis along the slopes of Mount Kilimanjaro. Oecologia 174:751–764. https://doi.org/10.1007/s00442-013-2819-0

Voigt CC, Lehnert LS (2019) Tracking of Movements of Terrestrial Mammals Using Stable Isotopes. In: Tracking Animal Migration with Stable Isotopes, Second Edi. Elsevier, pp 117–135

Voigt CC, Lehnert LS, Petersons G, et al (2015) Wildlife and renewable energy: German politics cross migratory bats. Eur J Wildl Res 61:213–219. https://doi.org/10.1007/s10344-015-0903-y

Voigt CC, Lindecke O, Schnborn S, et al (2016) Habitat use of migratory bats killed during autumn at wind turbines. Ecol Appl 26:771–783. https://doi.org/10.1890/15-0671/suppinfo

Voigt CC, Matt F, Michener R, Kunz TH (2003) Low turnover rates of carbon isotopes in tissues of two nectar-feeding bat species. J Exp Biol 206:1419–1427. https://doi.org/10.1242/jeb.00274

Voigt CC, Popa-Lisseanu AG, Niermann I, Kramer-Schadt S (2012b) The catchment area of wind farms for European bats: A plea for international regulations. Biol Conserv 153:80–86. https://doi.org/10.1016/j.biocon.2012.04.027

Voigt CC, Schneeberger K, Luckner A (2013) Ecological and dietary correlates of stable hydrogen isotope ratios in fur and body water of syntopic tropical bats. Ecology 94:346–

355. https://doi.org/10.1890/12-1187.1

VOIGT CC, SPEAKMAN JR (2007) Nectar-feeding bats fuel their high metabolism directly with exogenous carbohydrates. Funct Ecol 21:913–921. https://doi.org/10.1111/j.1365-2435.2007.01321.x

Voigt CC, Voigt-Heucke SL, Kretzschmar AS (2012c) Isotopic evidence for seed transfer from successional areas into forests by short-tailed fruit bats (Carollia spp.; Phyllostomidae). J Trop Ecol 28:181–186. https://doi.org/10.1017/S0266467411000666

Walton LR, Cluff HD, Paquet PC, Ramsay MA (2001) Movement patterns of barren-ground wolves in the Central Canadian Arctic. J Mammal 82:867–876. https://doi.org/10.1644/1545-1542(2001)082<0867:MPOBGW>2.0.CO;2

Wassenaar LI (2018) Chapter 2 - Introduction to Conducting Stable Isotope Measurements for Animal Migration Studies. In: Tracking Animal Migration with Stable Isotopes. Academic Press, pp 25–51

Wassenaar LI, Hobson K a (2000) Improved method for determining the stable-hydrogen isotopic composition (delta D) of complex organic materials of environmental interest. Environ Sci Technol 34:2354–2360

Wassenaar LI, Hobson KA, Sisti L (2015) An online temperature-controlled vacuum-equilibration preparation system for the measurement of δ^2H values of non-exchangeable-H and of $\delta^{18}O$ values in organic materials by isotope-ratio mass spectrometry. Rapid Commun Mass Spectrom 29:397–407. https://doi.org/10.1002/rcm.7118

Wassenaar LI, Terzer-Wassmuth S, Douence C (2021) Progress and challenges in dual- and triple-isotope (δ 18 O, δ 2 H, Δ 17 O) analyses of environmental waters: an international

assessment of laboratory performance. Rapid Commun Mass Spectrom. https://doi.org/10.1002/rcm.9193

Weller TJ, Castle KT, Liechti F, et al (2016) First Direct Evidence of Long-distance Seasonal Movements and Hibernation in a Migratory Bat. Sci Rep 6:34585. https://doi.org/10.1038/srep34585

Whiteman JP, Smith EAE, Besser AC, Newsome SD (2019) A guide to using compound-specific stable isotope analysis to study the fates of molecules in organisms and ecosystems. Diversity 11:. https://doi.org/10.3390/d11010008

Wiederholt R, López-Hoffman L, Cline J, et al (2013) Moving across the border: Modeling migratory bat populations. Ecosphere 4:1–16. https://doi.org/10.1890/ES13-00023.1

Wieringa JG, Nagel J, Nelson DM, et al (2020) Using trace elements to identify the geographic origin of migratory bats. PeerJ 8:e10082. https://doi.org/10.7717/peerj.10082

Wikelski M, Kays RW, Kasdin NJ, et al (2007) Going wild: What a global small-animal tracking system could do for experimental biologists. J. Exp. Biol. 210:181–186

Wilcove DS, Wikelski M (2008) Going, going, gone: Is animal migration disappearing? PLoS Biol. 6:1361–1364

Winder VL, Allison TD, Diffendorfer JE, et al (2019) The ecological impacts of the siting and operation of wind energy in the United States. Issues Ecol 23

Wolf N, Newsome SD, Fogel ML, del Rio CM (2013) The relationship between drinking water and the hydrogen and oxygen stable isotope values of tissues in Japanese Quail (Cortunix japonica). Auk 130:323–330. https://doi.org/10.1525/auk.2013.12075

WWF (2020) Living Planet Report 2020 - Bending the curve of biodiversity loss

Zahn A, Lustig A, Hammer M (2014) Potenzielle Auswirkungen von Windenergieanlagen auf Fledermauspopulationen Potential effects of wind energy plants on bat populations Zusammenfassung

Zhao H, Liu D, Li F, et al (2021) Incorporating spatio-temporal connectivity for prioritized conservation of individual habitat patches in a dynamic landscape. Ecol Indic 124:107414. https://doi.org/10.1016/j.ecolind.2021.107414

Zimmerling JR, Francis CM (2016) Bat mortality due to wind turbines in Canada. J Wildl Manage 80:1360–1369. https://doi.org/10.1002/jwmg.21128

Zuluaga S, Speziale KL, Lambertucci SA (2022) Flying wildlife may mask the loss of ecological functions due to terrestrial habitat fragmentation. Sci. Total Environ. 803:150034

APPENDICES

Appendix Chapter 1

Appendix 1 - 1: Table SM 1: Reference material used in the δ^2H transfer function of the models. Location and measured stable hydrogen isotopic ratios in fur keratin of 458 *Pipistrellus nathusii* bats captured within the breeding range. (*Online access via https://doi.org/10.1002/rcm.9031*)

species	sex	age	date	location	lat	long	d2H	month
P. nathusii	NA	NA	7/1/2019	Baja, Hungary	46.18342	18.95382	-101.00	7
P. nathusii	NA	NA	7/1/2019	Baja, Hungary	46.18342	18.95382	-137.36	7
P. nathusii	NA	NA	7/1/2019	Baja, Hungary	46.18342	18.95382	-121.31	7
P. nathusii	NA	NA	7/1/2019	Baja, Hungary	46.18342	18.95382	-149.20	7
P. nathusii	NA	NA	7/1/2019	Baja, Hungary	46.18342	18.95382	-126.51	7
P. nathusii	NA	NA	7/1/2019	Baja, Hungary	46.18342	18.95382	-138.26	7
P. nathusii	NA	NA	7/1/2019	Baja, Hungary	46.18342	18.95382	-106.88	7
P. nathusii	NA	NA	7/1/2019	Baja, Hungary	46.18342	18.95382	-132.66	7
P. nathusii	NA	NA	7/1/2019	Baja, Hungary	46.18342	18.95382	-128.70	7
P. nathusii	NA	NA	7/1/2019	Baja, Hungary	46.18342	18.95382	-146.95	7
P. nathusii	NA	NA	7/1/2019	Baja, Hungary	46.18342	18.95382	-105.36	7
P. nathusii	NA	NA	7/1/2019	Baja, Hungary	46.18342	18.95382	-116.78	7
P. nathusii	NA	NA	7/1/2019	Versoix, Switzerland	46.28357	6.16592	-123.50	7
P. nathusii	NA	NA	7/1/2019	Taldykino, Oryol Oblast, Russia	52.5	35	-108.28	7
P. nathusii	NA	NA	7/1/2019	Taldykino, Oryol Oblast, Russia	52.5	35	-108.62	7
P. nathusii	NA	NA	7/1/2019	Taldykino, Oryol Oblast, Russia	52.5	35	-102.85	7
P. nathusii	NA	NA	7/1/2019	Taldykino, Oryol Oblast, Russia	52.5	35	-108.37	7
P. nathusii	NA	NA	7/1/2019	Taldykino, Oryol Oblast, Russia	52.5	35	-109.97	7
P. nathusii	NA	NA	7/1/2019	Taldykino, Oryol Oblast, Russia	52.5	35	-102.28	7
P. nathusii	NA	NA	7/1/2019	Taldykino, Oryol Oblast, Russia	52.5	35	-105.32	7
P. nathusii	NA	NA	7/1/2019	Taldykino, Oryol Oblast,	52.5	35	-106.99	7

Appendix 1 - 2: Table SM2: Location, migratory status, measured stable hydrogen and strontium isotopic ratios in fur keratin of 59 *Pipistrellus nathusii* bats captured in Fehmarn, Greifswalder Oie, and Heligoland (North of Germany). (*Online access via https://doi.org/10.1002/rcm.9031*)

species	sex	age	location	date	d2H	d87sr	long	lat	migratory_status	ranges
Pipistrellus nathusii	Female	adult	Helgoland	9/21/2017	-125	0.713	7.888944	54.18033	long-distance migrant	1
Pipistrellus nathusii	Male	adult	Helgoland	9/22/2017	-126.7	0.7105	7.888944	54.18033	long-distance migrant	1
Pipistrellus nathusii	Male	adult	Helgoland	9/22/2017	-121.2	0.7093	7.888944	54.18033	long-distance migrant	1
Pipistrellus nathusii	Male	adult	Helgoland	9/23/2017	-123.1	0.7111	7.888944	54.18033	long-distance migrant	1
Pipistrellus nathusii	Male	adult	Helgoland	9/23/2017	-140.2	0.7102	7.888944	54.18033	long-distance migrant	1
Pipistrellus nathusii	Female	adult	Wallnau Fehmarn	9/21/2017	-132.71	0.7124	11.01263	54.48333	long-distance migrant	1
Pipistrellus nathusii	Female	adult	Wallnau Fehmarn	9/21/2017	-133.15	0.7103	11.01263	54.48333	long-distance migrant	1
Pipistrellus nathusii	Female	adult	Wallnau Fehmarn	9/18/2018	-124.06	0.7127	11.01263	54.48333	long-distance migrant	1
Pipistrellus nathusii	Female	adult	Wallnau Fehmarn	9/18/2018	-120.93	0.7103	11.01263	54.48333	long-distance migrant	1
Pipistrellus nathusii	Female	adult	Wallnau Fehmarn	9/20/2018	-124.83	0.7105	11.01263	54.48333	long-distance migrant	1
Pipistrellus nathusii		NA	Greifswalder Oie	5/13/2017	-121.7	0.71	13.91857	54.24775	long-distance migrant	1
Pipistrellus nathusii		NA	Greifswalder Oie	5/14/2017	-122.4	0.709	13.91857	54.24775	long-distance migrant	1
Pipistrellus nathusii		NA	Greifswalder Oie	9/19/2017	-121.84	0.71	13.91857	54.24775	long-distance migrant	1
Pipistrellus nathusii		NA	Greifswalder Oie	9/19/2017	-129.48	0.71	13.91857	54.24775	long-distance migrant	1

Appendix 1 - 3: Table SM3: Mean δ^2H and ^{87}Sr/^{86}Sr values (± one standard deviation) of fur keratin in Nathusius' pipistrelles captured on the islands of Heligoland, Greifswalder Oie (both seasons), Fehmarn (all northern Germany) and during the pre-migration season close to Engure Lake (Latvia). We report raw ^{87}Sr/^{86}Sr values of fur (keratin), ^{87}Sr/^{86}Sr values corrected for trophic discrimination (keratin$_{corr}$) and bioavailable ^{87}Sr/^{86}Sr values (bioavailable) calculated for a 100 km buffer around the capture site. Ranges refer to isotopic bins of δ^2H values (see more details in the text). (*Online access via https://doi.org/10.1002/rcm.9031*)

location	n	season	range	keratin δ2H (‰)	keratin ^{87}Sr/^{86}Sr	keratin$_{corr}$ ^{87}Sr/^{86}Sr	bioavailable ^{87}Sr/^{86}Sr
Engure Lake	10	summer	NA	-100.96 ± 3.8	0.7112 ± 0.0002	0.7141 ± 0.0002	0.7088
Fehmarn	5	autumn	1	-127.14 ± 5.48	0.7112 ± 0.0012	0.7141 ± 0.0012	0.7086
Fehmarn	7	autumn	2	-106.57 ± 5.05	0.7102 ± 0.0011	0.7131 ± 0.0011	0.7086
Fehmarn	10	autumn	3	-91.84 ± 4.77	0.7101 ± 0.0011	0.713 ± 0.0011	0.7086
Greifswalder Oie	2	spring	1	-122.05 ± 0.49	0.7095 ± 0.0007	0.7123 ± 0.0007	0.7085
Greifswalder Oie	13	spring	2	-112.8 ± 5.41	0.71 ± 0.0011	0.7129 ± 0.0011	0.7085
Greifswalder Oie	3	spring	3	-98.03 ± 1.37	0.7106 ± 0.0011	0.7135 ± 0.0011	0.7085
Greifswalder Oie	2	autumn	1	-125.66 ± 5.4	0.71 ± 0	0.7128 ± 0	0.7085
Greifswalder Oie	5	autumn	2	-116.3 ± 2.05	0.7104 ± 0.0005	0.7132 ± 0.0005	0.7085
Heligoland	5	autumn	1	-127.24 ± 7.53	0.7108 ± 0.0013	0.7137 ± 0.0013	0.7088
Heligoland	6	autumn	2	-107.33 ± 4.17	0.7099 ± 0.0007	0.7127 ± 0.0007	0.7088

Appendix Chapter 2

Appendix 2 - 1: Table SM1: Raw stable hydrogen isotopic data of Nathusius' pipistrelles collected in Germany. (*Online access via DOI: 10.5281/zenodo.4557292*)

date	id	species	sex	age	forearm	mass	location	lat	long	d2h	long distant migrant_status	ref
8/19/2003	68	Pipistrellus nathusii	F	adult			Nauen	52.587	12.821	-103.733	regional migrant	
8/17/2004	61	Pipistrellus nathusii	F	adult			Wernitz	52.5489	12.883	-120.206	regional migrant	
8/17/2004	262	Pipistrellus nathusii	M	juvenile			Tremmen	52.522	12.892	-194.53	long distant migrant	
8/30/2004	211	Pipistrellus nathusii	F	juvenile			Nauen	52.587	12.821	-126.841	regional migrant	
8/30/2004	239	Pipistrellus nathusii	F	adult			Bliesdorf	52.702	14.186	-116.798	regional migrant	
9/20/2004	257	Pipistrellus nathusii	F	juvenile			Karstädt	53.155	11.774	-186.708	long distant migrant	
9/11/2005	267	Pipistrellus nathusii	M	adult			Ausleben	52.122	11.161	-146.941	regional migrant	
8/4/2006	DE-HAL040806-Pna01	Pipistrellus nathusii	M				Sachsen	51.10454	13.20174	-97.4846	regional migrant	
8/4/2006	DE-HAL040806-Pna01	Pipistrellus nathusii	M					51.28597	14.45252	-97.4846	regional migrant	
8/18/2006	243	Pipistrellus nathusii	F	juvenile			Domnitz	51.623	11.859	-129.067	regional migrant	
8/20/2006	DE-HAL200806-Pna01	Pipistrellus nathusii	F				Sachsen	51.10454	13.20174	-126.349	regional migrant	
8/20/2006	DE-HAL200806-Pna03	Pipistrellus nathusii	F				Sachsen	51.10454	13.20174	-130.469	regional migrant	
8/20/2006	DE-HAL200806-Pna02	Pipistrellus nathusii	M				Sachsen	51.10454	13.20174	-113.597	regional migrant	
8/20/2006	DE-HAL200806-Pna01	Pipistrellus nathusii	F					51.28597	14.45252	-126.349	regional migrant	

Appendix 2 - 2: Figure S1: Hydrogen isotopic ratios of long-distance and regional Nathusius' pipistrelle bats from carcasses (WT) and live population (BB) in Germany during the migratory period.
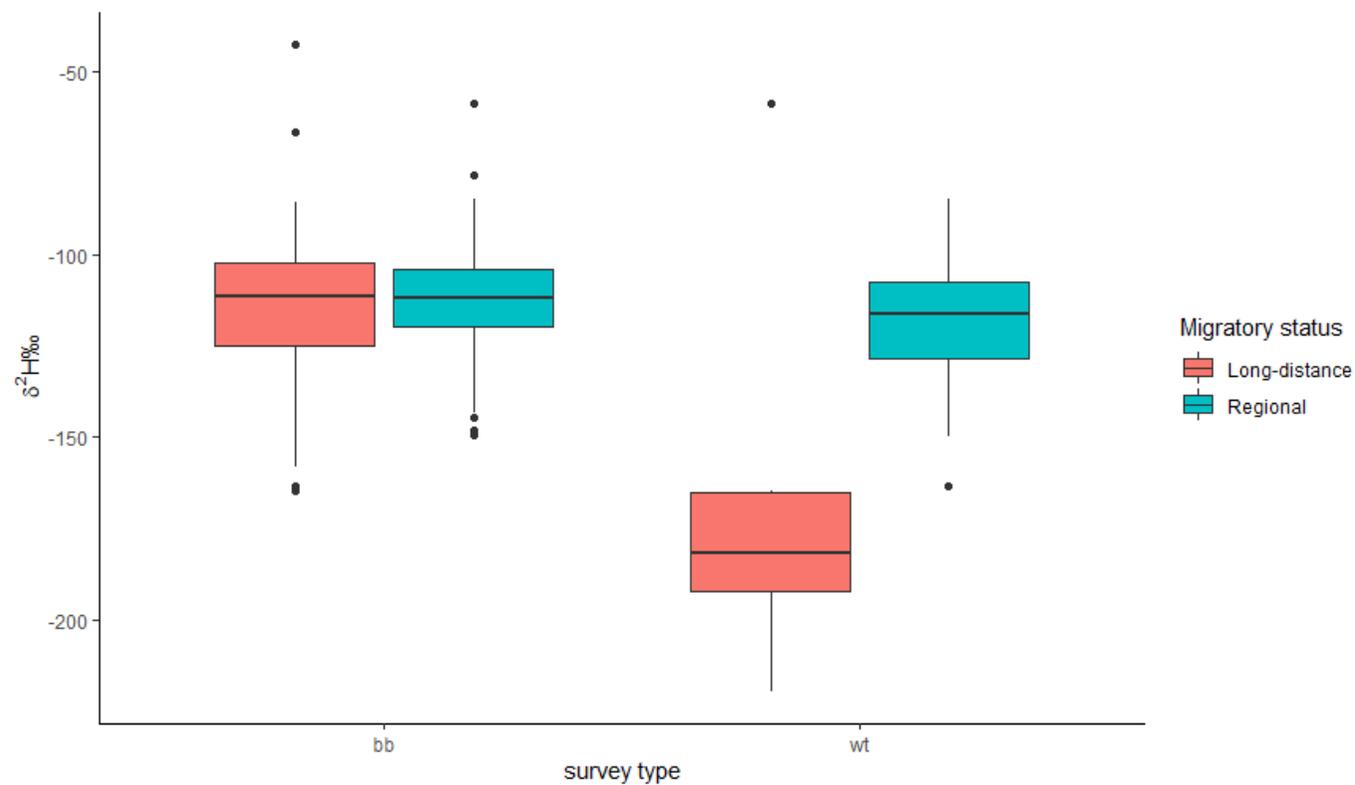
DECLARATION OF AUTHORSHIP

I hereby declare that I prepared this thesis independently under the guidance of my supervisor. All direct or indirect sources used are given as references. All contributions of co-authors are acknowledged.

Berlin, 09 June 2022

_____

Cecilia Kruszynski