Received: 20 October 2020 | Revised: 14 December 2020 | Accepted: 17 December 2020

DOI: 10.1002/rcm.9031

RESEARCH ARTICLE

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

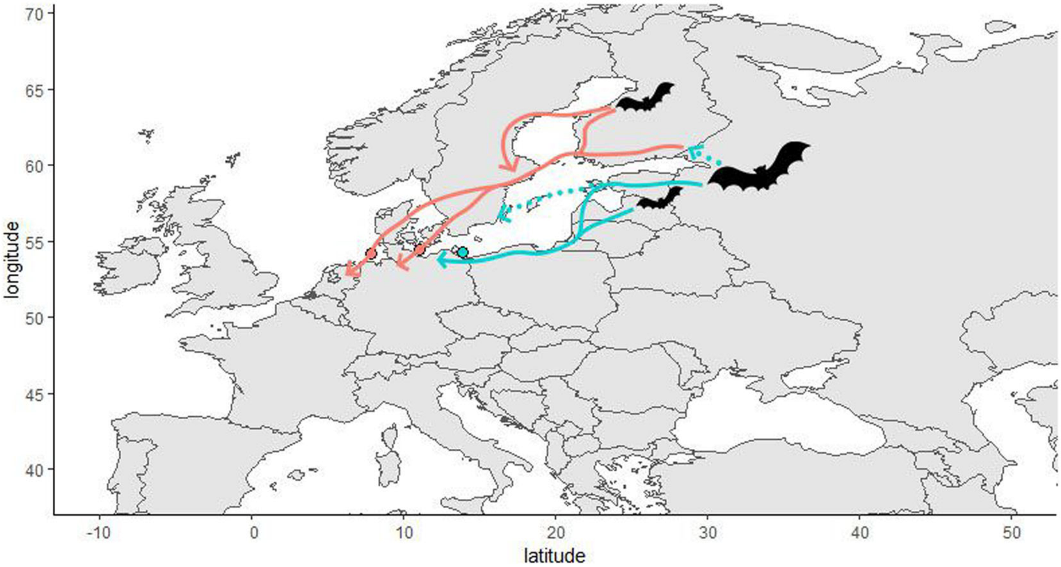

FIGURE 1 Map of potential migratory pathways of Nathusius' pipistrelles along the coast of the Baltic Sea. Blue lines highlight the pathway from Baltorussian populations to Poland and Germany. Red lines highlight the pathways of Fennoscandian populations via Denmark to Germany. Dashed blue lines represent 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

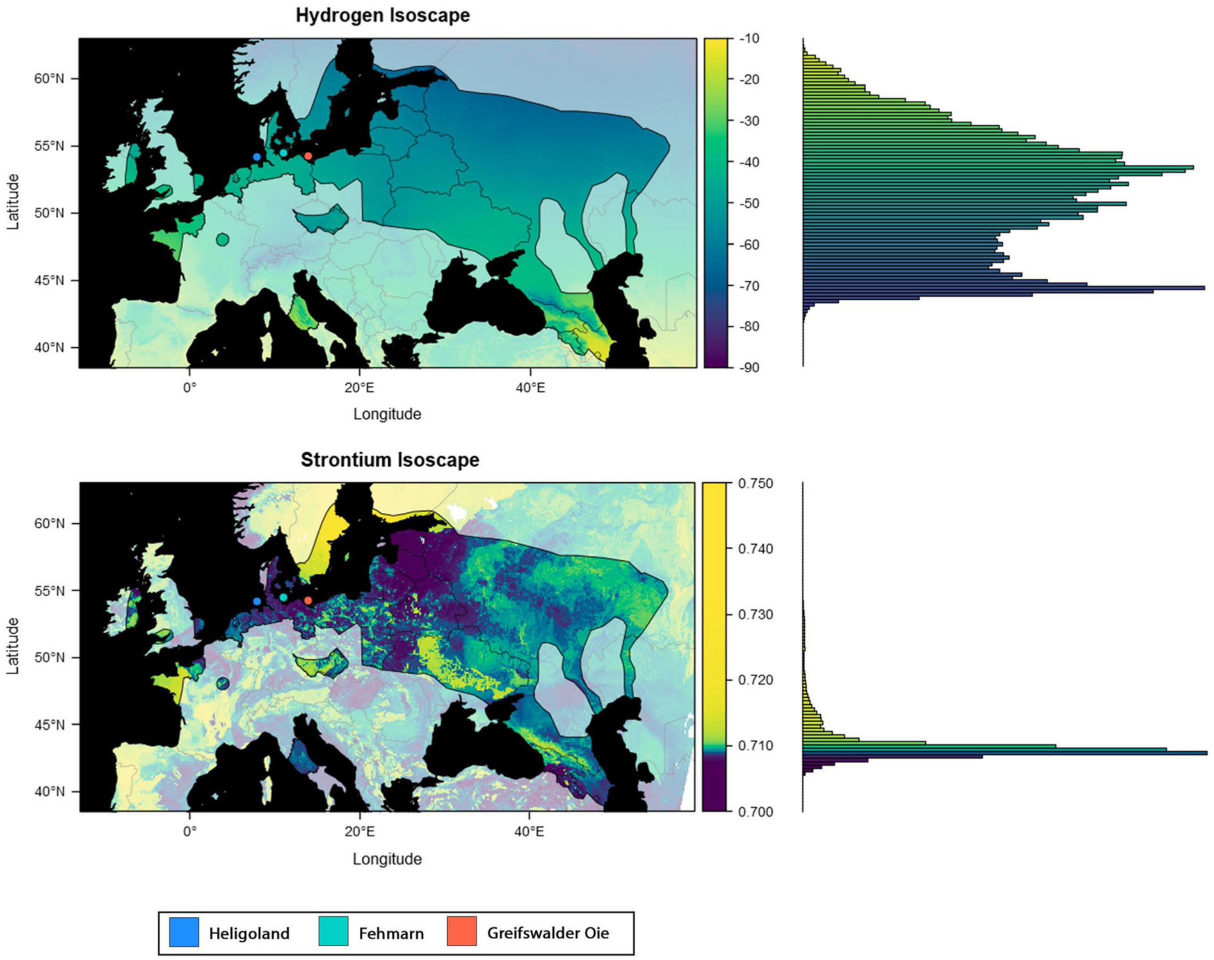

FIGURE 2 European isoscapes of stable hydrogen and strontium isotope ratios from precipitation and bioavailable strontium data, respectively. The highlighted polygon is the current breeding range of Nathusius' pipistrelles in Europe (IUCN report, adjusted in Eastern Europe based on pers. comm. Dekker 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 (δ^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).

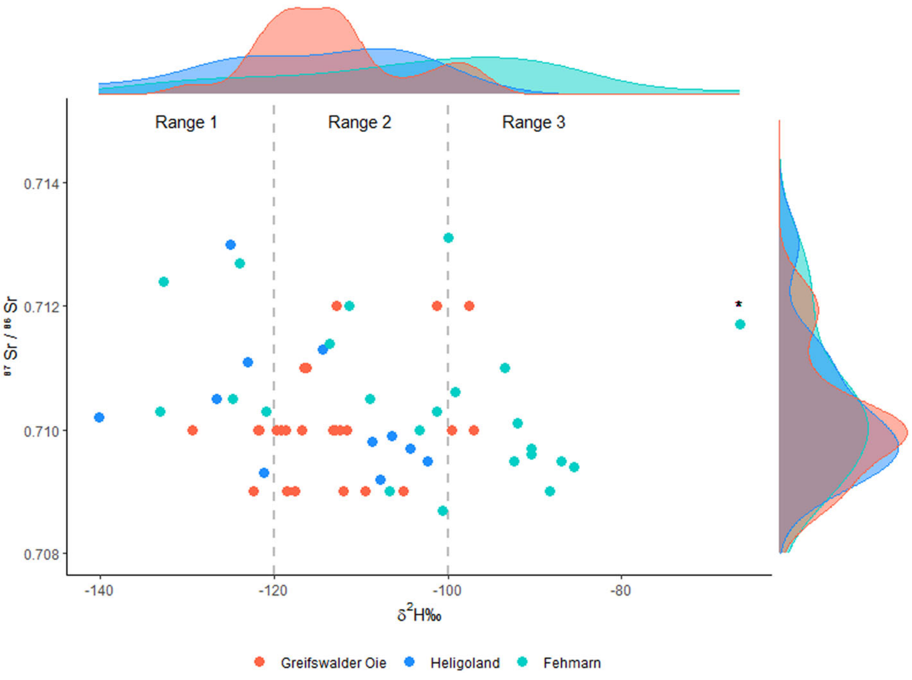

FIGURE 3 δ^2H (‰) and ^{87}Sr/^{86}Sr raw values of 59 Nathusius' pipistrelles captured during the migratory period on offshore islands in Germany. Ranges 1 to 3 represent the ranges in assignment maps. Note the outlier shown (*) was not used for further analysis

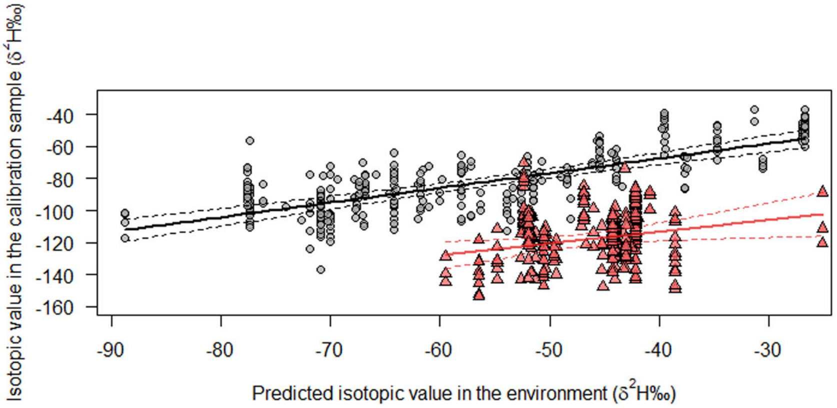

FIGURE 4 Relationship between the stable hydrogen isotopic ratios (δ^2H values) of fur keratin in 458 Nathusius' pipistrelles captured during the non-migration period in Europe and the isotopic ratios of monthly precipitation (δ^2H values) in their environment (red triangles). In addition, we plotted 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

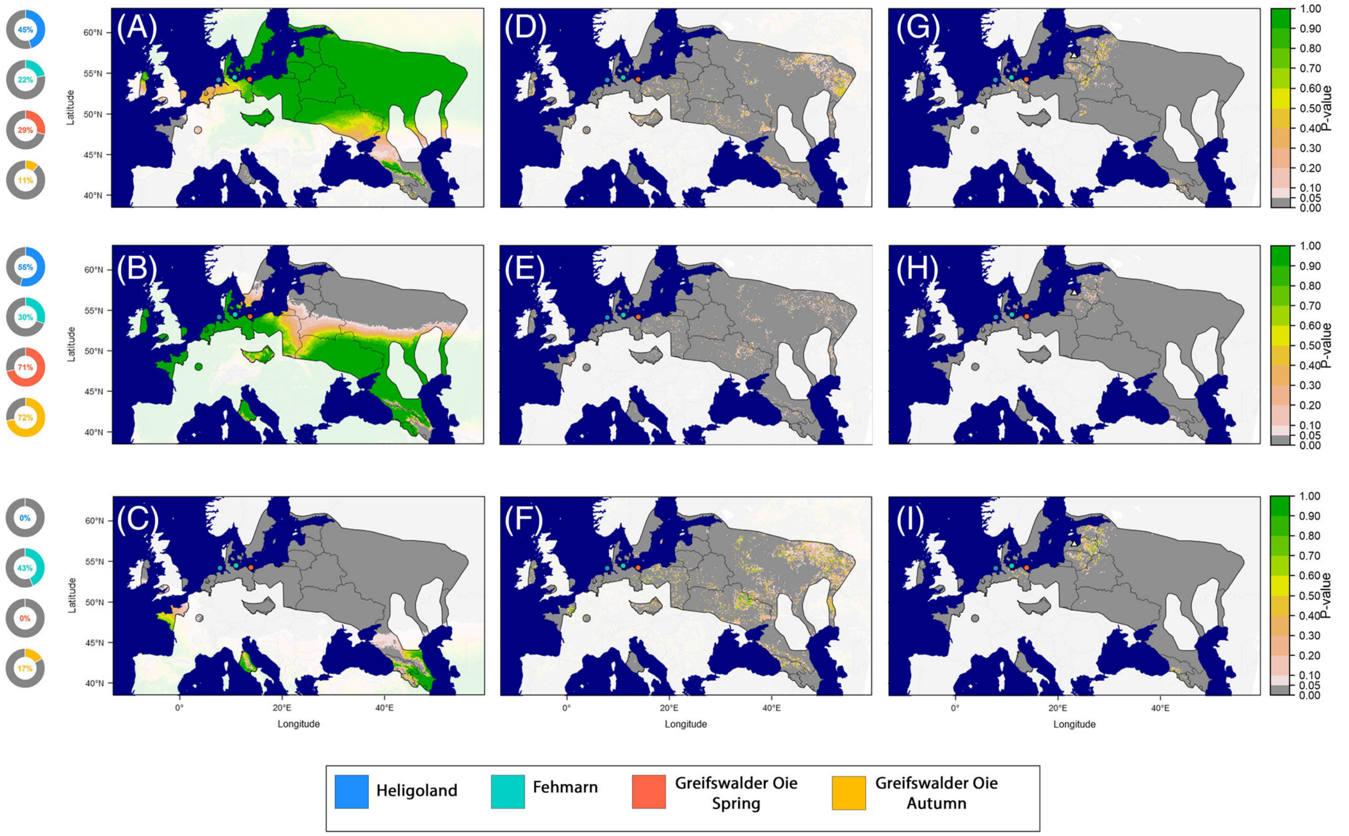

FIGURE 5 Maps depicting the likely summer origin of 58 Nathusius' pipistrelles captured on three offshore islands in Germany (Heligoland, Fehmarn, Greifswalder Oie). Geographic assignments were based on keratin δ^2H values (A, B, C), raw ^{87}Sr/^{86}Sr values (D, E, F) and ^{87}Sr/^{86}Sr values corrected 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$_{\mathrm{corr}}$; 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.

ACKNOWLEDGEMENTS

The authors thank Anja Luckner and Yvonne Luckner for stable hydrogen isotope analysis at the Leibniz-IZW. The authors thank many volunteers across Europe for providing fur samples for referencing stable isotope ratios of Nathusius' pipistrelles. The authors also thank the following institutions for offering the infrastructure and the permits to conduct work: Jordsand für den Schutz der Seevögel and Stella Klasan from Natur eV (Station Greifswalder Oie), NABU Wasservogelreservat Wallnau and Stiftung Naturschutz Schleswig-Holstein. Finally, the authors thank the reviewers who helped improve the manuscript. This study was funded by the BfN to NABU Mecklenburg-Vorpommern. C.K. was supported by a fellowship from the Brazilian National Council for Scientific and Technological Development (CNPq, process no. 290079/2015-2).

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1002/rcm.9031

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.

How to cite this article: Kruszynski C, Bailey LD, Courtiol A, et al. Identifying migratory pathways of Nathusius' pipistrelles (*Pipistrellus nathusii*) using stable hydrogen and strontium isotopes. *Rapid Commun Mass Spectrom*. 2021;35:e9031. https://doi.org/10.1002/rcm.9031