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Bat Migration Research Report

Stable Isotope Analysis of Nathusius' Pipistrelle Fur Samples

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Contents

1 Summary

1.1 The extent of bat migration between continental Europe and the United Kingdom (UK) is poorly understood. Previous studies by BSG Ecology show that peak activity levels of Nathusius' pipistrelle *Pipistrellus nathusii* (a migratory species of bat) have been recorded from fixed point bat detectors deployed in coastal locations on the southern, eastern and western coastlines of the UK during the migration season for this species, and that this species has only been recorded in spring and autumn from bat detectors deployed on two offshore ferry routes in the southern North Sea in 2014 (BSG Ecology, 2013a-d; 2014b).

1.2 In 2013, the study was extended to include analysis of stable hydrogen isotope ratios (d^2H) of hair samples taken from Nathusius' pipistrelle bats found in the UK. Samples were taken from 25 Nathusius' pipistrelles that were captured / obtained from a range of sources including bat box checks, mist netting, harp traps, grounded bats and dead animals. The stable hydrogen isotope ratios of the hair samples were analysed using a method developed by Voigt *et al*. (2012).

1.3 The continental, and particularly latitudinal, patterns of hydrogen isotopes in rainfall (d^2Hp) are well known and predictable (Bowen, 2012) and these values are often reflected in newly-formed tissues of organisms (Hobson, 2012). In bats, d^2H is assimilated in fur keratin as it grows. Migratory bat species are known to moult (and grow new fur) in summer before migration therefore the summer habitat and/or breeding origin of bats can be inferred by identifying the d^2H of fur samples (Voigt *et al.*, 2012; Cryan *et al.,* 2004).

1.4 It is acknowledged that there can be local variation in d^2H of precipitation (d^2Hp) within a given area (C. Voigt *pers. comm.)*. However, the d^2Hp of the 25 samples ranged between -9.2 and +14.1 of the known d^2Hp of the location in which the bats were recovered. The results suggested that 4 %, i.e. one bat had been found north of where it spent the summer and moulted; 20 % (number = 5) stayed within the same d^2H precipitation region; 36 % (n = 9) had been found slightly further south or west of where the moult had occurred; and 40 % (n = 9) had been found substantially further south or west of where moult had occurred.

1.5 The variation within the sample is substantial and indicates that at least 40 % of the sample had moved a substantial distance since they had moulted the previous summer. In this case, the data strongly suggest that a proportion of the bats sampled were migratory and that, given the predominant migration direction of this species in Europe (south-west to north-east) and their distribution in the UK (largely in southern areas with no known breeding colonies in the north of the UK), this proportion of the sample may have originated from areas of northern or eastern Europe outside of the UK. The sample also indicates that some of the bats sampled are resident in the UK. The sample did not indicate a clear relationship between the sex of the bats and their summer range.

1.6 These findings concur with other evidence in support of the regular and predictable migration of Nathusius' pipistrelle in and out of the UK.

2 Introduction

2.1 Migratory movements of bats have been known and described for around the last 100 years (Popa-Lisseanu & Voigt, 2009). There has been an increase in interest in this area of research in recent years with a number of important publications on bat migration published in the last decade (e.g. Ahlen *et al*., 2009; Hutterer *et al*., 2005; McGuire *et al.*, 2011; McGuire *et al*., 2013; Popa-Lisseanu *et al*., 2012).

2.2 Birds and bats are the only flying animals to undertake seasonal return migration from breeding to non-breeding areas; however, migration in bats is far less common than in birds with less than 3 % of bats understood to be migratory[1] (in comparison to around 30 % of Palearctic[2] bird species), and only 12 species for which long-distance movements of more than 1000 km (one-way) have been recorded (Bisson *et al*., 2009). All of the latter belong to the family *Vespertilionidae*[3], are insectivorous and are found in temperate regions of Europe and North America.

2.3 In Europe, long distance migratory bat species include noctule *Nyctalus noctula*, Leisler's bat *Nyctalus leisleri*, Nathusius' pipistrelle and the parti-coloured bat *Vespertilio murinus*[4] (Hutterer *et al*., 2006), with typical migration distances in both spring and autumn thought to be 250 km to 1,000 km.

2.4 Knowledge of European bat migration is largely based on recoveries of ringed (or 'banded') individuals (Hutterer *et al*., 2005) as well as a handful of other studies. Over 1 million bats have been ringed (using metal rings with unique codes to mark individuals) in Europe and this has enabled the study of broad-scale bat migration patterns in Europe. This work has confirmed that long distance migrations for the four species mentioned above usually involve movements in a south-westerly direction in autumn (to wintering areas) and a north-easterly direction in spring (to breeding / summering areas).

2.5 Information on whether bats undertake seasonal migrations into and out of the UK is largely anecdotal. There is one record of a ringed Nathusius' pipistrelle moving between the UK and Continental Europe (Hargreaves, 2014), and numerous records of grounded bats of all these species (records of parti-coloured bat are rare and probably involve vagrants), bats being found on offshore platforms (Boshamer & Bekker, 2008) and being seen to fly in off the sea (e.g. BSG Ecology, 2013a). In 2001 Russ (Russ *et al.,* 2001) suggested Nathusius' pipistrelle make migratory movements into and out of the British Isles, however, no studies conclusively show the extent or scale of migration.

2.6 Previous studies by BSG Ecology have involved deploying bat detectors at fixed points at a number of coastal locations in the UK and on offshore ferries in the southern North Sea during 2012-2014. These are as follows:

- Dungeness, Kent in 2012 and 2013 (BSG Ecology, 2013a; 2013b)
- Sandwich Bay Bird Observatory, Kent, in 2013 (BSG Ecology, 2013b)
- The White Cliffs of Dover (National Trust), Kent, in 2013 (BSG Ecology, 2013b);
- Spurn Lighthouse, East Yorkshire, in 2013 (BSG Ecology, 2013c);
- Portland Bird Observatory, Dorset, in 2013 (BSG Ecology, 2013d);
- Ramsey Island, Pembrokeshire, in 2014 (see BSG Ecology, 2014a);
- Skomer Island, Pembrokeshire, in 2014 (see BSG Ecology, 2014a);
- Skokholm Island, Pembrokeshire, in 2014 (see BSG Ecology, 2014a);

[1] Migratory movements are identified as seasonal movements of more than 50 km.
[2] The Palearctic is one of eight ecozones that sub-divide the earth's surface. It includes Europe, Asia north of the Himalayas, north Africa and northern parts of the Arabian peninsula.
[3] The largest and best-known family of bats, containing over 300 species, and also known as 'evening' bats.
[4] The parti-coloured bat is not resident within the UK, however, vagrant records are occasionally recorded.

- Flandria Seaways vessel (DFDS freight) sailing between Felixstowe (Suffolk) and Vlaardinghen (Netherlands) in 2014 (see BSG Ecology, 2014b); and
- Pride of York vessel (P&O ferries) sailing between Hull (East Yorkshire) and Zeebrugge (Belgium) in 2014 (see BSG Ecology, 2014).

2.7 These studies show that peak activity levels of Nathusius' pipistrelle (a migratory species of bat) have largely been recorded from fixed point bat detectors in coastal locations during the likely migration season (principally April-May and September-October) for this species. This species has only been recorded from bat detectors deployed on two offshore ferry routes in the southern North Sea in spring and autumn 2014.

2.8 The study was extended to include analysis of stable hydrogen isotope ratios (d^2H) of hair samples taken from Nathusius' pipistrelle bats found in the UK. Stable isotope ratios of rain water are known to vary across continents according to distance from the coast, altitude and ambient temperature (Négrel & Giraud, 2011). This information can be used for tracking the migratory movements of bats because stable hydrogen isotopes are assimilated into inert keratin of fur once bats have moulted in summer before migrating in autumn (Voigt *et al*., 2014).

2.9 Recent studies in mainland Europe (Voigt *et al*., 2012; Voigt *et al*., 2014; Lehnert *et al*., 2014*)* have used stable hydrogen isotope analysis to investigate the provenance of migratory bat species, identifying both resident and migratory populations in each of the studies. To date (October 2014), analysis based on this method has not been conducted or published in the UK, and this is the first example of such a study based on Nathusius' pipistrelles found in the UK.

Aims of Study

2.10 Through the use of stable isotope analysis we aim to identify whether the stable hydrogen isotope ratios of Nathusius' pipistrelle fur samples differ from the recovery site of the bat, to the extent that these differences can be used to establish clear evidence of migration in individual bats recovered in the UK.

3 Methods

Obtaining Samples

3.1 In 2013 there were several projects undertaken to study Nathusius' pipistrelle throughout the UK. These included the Bedfont Nathusius' Project (led by Patty Briggs and Matt Dodds), the Cardiff Bat Group Nathusius' Pipistrelle Project (led by Richard Crompton) and an informal study of Nathusius' pipistrelle in Kent (led by John Puckett). Requests were made to these bat workers to amend their Natural England project licences to include collection of Nathusius' pipistrelle fur samples. Details of the collected samples are provided in Table 1.

Table 1: Details of collected samples.

No.	Date	Personnel	Location	Grid Ref.	Type
1	20/09/13	Richard Crompton	Cardiff Bay, South Wales	ST172736	Harp trap
2	21/09/13				
3	30/03/01	Patty Briggs	Walthamstow, north London	TQ357904	Dead
4-9	03/05/13		Bedfont Lakes, Heathrow	TQ078727	Box
10	10/08/13				Mist net
11	03/05/13				Box
12-23	23/09/13				Box
24	09/06/13	John Puckett	Herne Bay, Kent	TR156677	Grounded
25	18/07/13		Sandwich, Kent	TR301566	

3.2 Samples were obtained from 25 Nathusius' pipistrelle bats and a range of sources including bat box checks, mist nets, harp traps, grounded bats and dead animals. Samples were collected in March (earliest study record, 30/03/2001), May, June, July, August and September (latest study record, 23/09/2013). Fur samples were collected using scissors to cut two pieces of fur from the back of the bat. Care was taken not to cut at the very base of the fur to ensure that the insulating fur close to the skin was left intact. The hair samples were stored in sterile envelopes and/or plastic vials, in a dry location to prevent any fungal spores developing which could affect (damage/corrupt) the samples.

Analysing Samples

3.3 The stable hydrogen isotope ratios (d^2H) of the hair samples were analysed by Christian Voigt at the Leibniz Institute for Zoo and Wildlife Research, Berlin, Germany. By measuring fur samples in sequence with laboratory keratin standards of known stable isotope ratios of non-exchangeable hydrogen, it was possible to derive stable isotope ratios in the non-exchangeable portion of hydrogen in Nathusius' pipistrelle fur samples. The method followed to analyse the samples is described in detail by Voigt *et al* (2012). In summary, this included:

- Cleaning the fur of surface oil and contaminants using a 2:1 chloroform-methanol solution;
- Drying the samples in an oven at 50°C for 24 hours;
- Placing samples in an autosampler and flushing them with chemically pure helium; and
- Using a Delta V Advantage isotope ratio mass spectrometer connected via an interface with an elemental analyser to identify stable hydrogen ratios of each sample.

Interpretation of Data

3.4 The continental, and particularly latitudinal, patterns of hydrogen isotopes in rainfall (d^2Hp) are well known and predictable (Bowen, 2012; see **Figure 1**). The d^2Hp values are often reflected in newly-formed tissues of organisms (Hobson, 2012). In the case of bats, d^2H is assimilated in fur keratin as it grows. Studies have shown that migratory bats moult (and grow new fur) in summer before

migration therefore the summer habitat and/or breeding origin of bats can be inferred by identifying the d^2H of fur samples (Voigt *et al.*, 2012; Cryan *et al.,* 2004).

3.5 The d^2Hp of the recovery site (the location where the sample was taken) was identified by reviewing the map of rainwater isotopic zones shown in Figure 1 below. To enable differentiation between the isotopic zones for rainwater, the key d^2Hp values (where individuals are likely to have moulted in this study) have been labelled A-D. All bats were recovered within area B in the southern zone of the UK.

3.6 The zone where summering / moulting was expected to happen was identified and the difference in d^2Hp between the sample and expected origin were compared.

3.7 The known distribution of Nathusius' pipistrelle maternity colonies in the UK and documented migration routes in Europe are presented in **Figure 2**.

Figure 1: Rainwater isotopic zone map of Europe, showing d^2Hp and the recovery sites of the fur samples (adapted from Bowen (2012)).

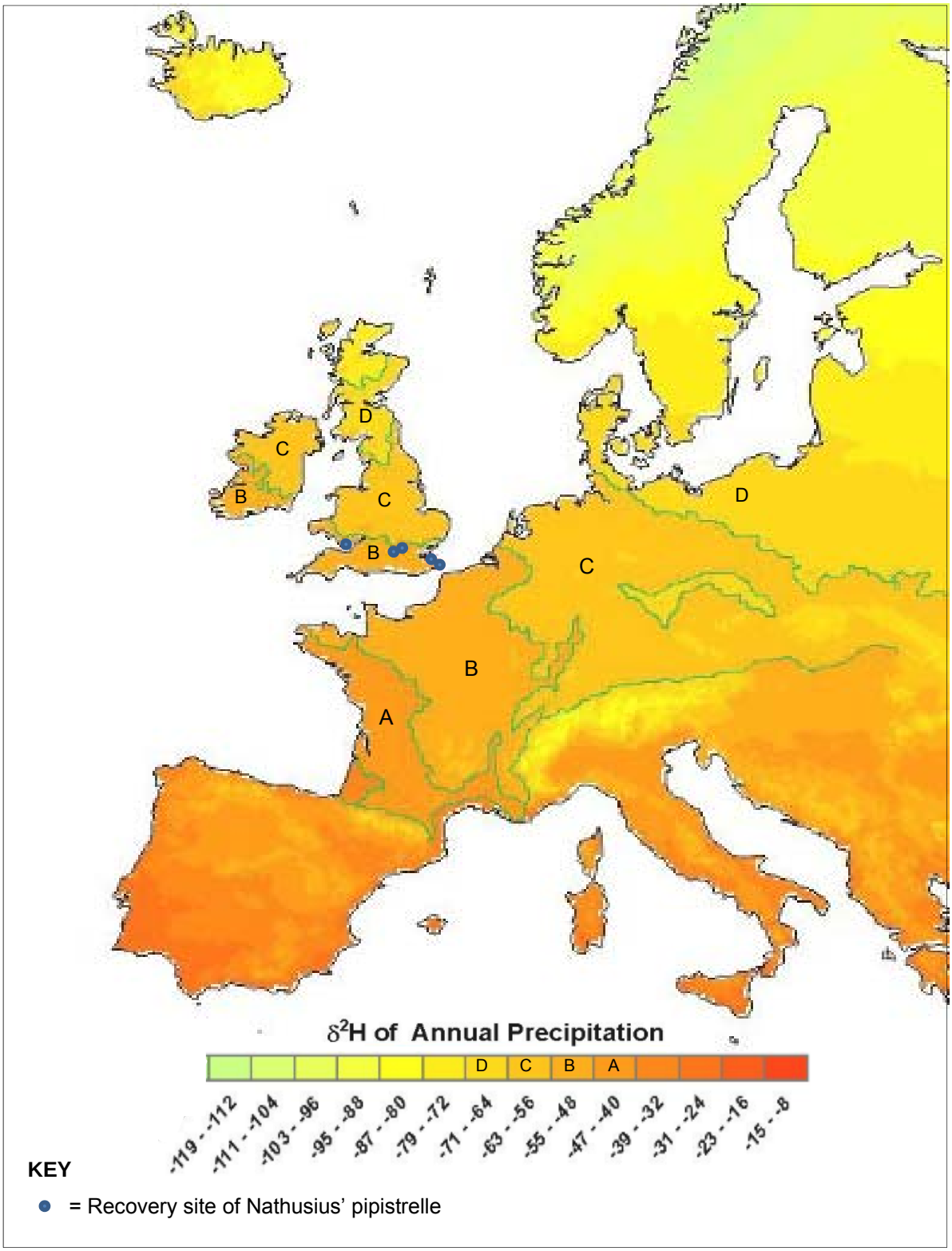
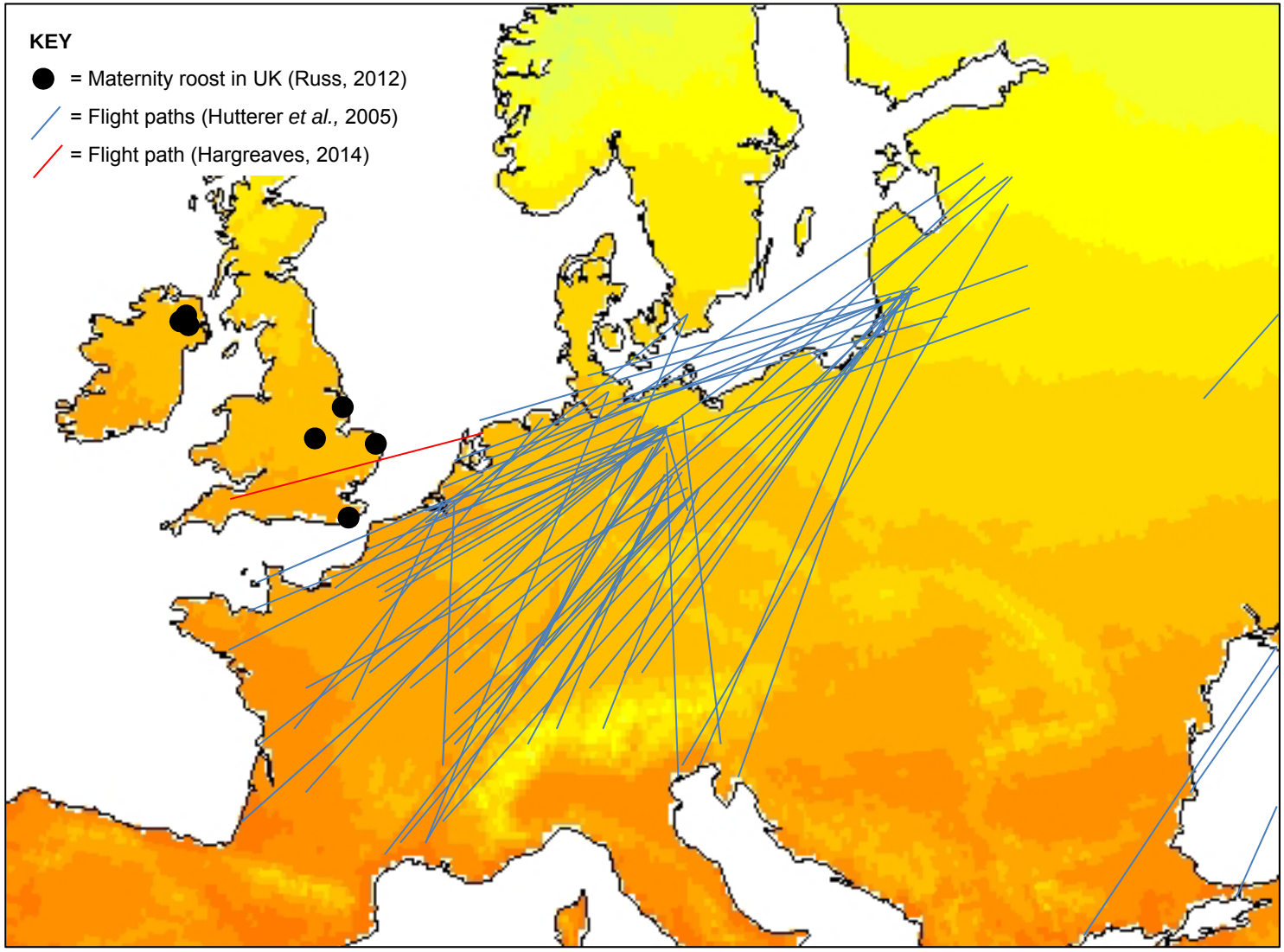


Figure 2: Documented long-distance movements of Nathusius' pipistrelle in Europe (Hutterer *et al*, 2005; Hargreaves, 2013) and known maternity roosts in the UK (Russ, 2012).



4 Results and Interpretation

4.1 The d^2H of the samples varied between -83.98 and -126.58. These d^2H values are not a direct reflection of the expected place of origin of the samples. A regression equation for Nathusius' pipistrelle has been established[5] (Voigt *pers. comm.,* November 2014) and was used to calculate the d^2Hp of the expected summer / moulting range of each bat, which varied between -43.8 and -68.10.

4.2 The d^2Hp of the recovery sites had little variation, being between -53 in Cardiff and -54 for the remainder of recovery locations.

4.3 On average, the d^2Hp of the fur samples was 6.1 units lower than those of local rainwater at the recovery locations.

4.4 The results are presented in the following three tables:

- Table 2 provides a summary of the d^2Hp data showing the recovery site and likely origin of the fur samples;
- Table 3 presents the data in more detail, including information relating to the origin, sex and timing of records; and
- Table 4 includes full details of the sample origin, biometric data and stable hydrogen isotope ratio analysis.

4.5 Table 2 provides information that allows the recovery site and the likely origin of the bat, based on the d^2Hp data, to be considered in combination (with reference to Figure 1). The data indicate:

- That a single bat was found north of where it spent the summer and moulted (A-B)
- Five individuals stayed within the same d^2H precipitation region (B-B)
- Nine had been found slightly further south or west of where they moult had occurred (C-B)
- Ten had been found substantially further south or west of where moult had occurred (D-B)

Table 2: Summary of the d^2Hp data showing recovery site and likely origin

Recovery Site	Likely Origin	Total	% of Total
B	A	1	4
	B	5	20
	C	9	36
	D	10	40
Grand Total		**25**	**100**

4.6 The additional information in Table 3 reveals:

- 60 % (n = 15 of 25) of the bats from which samples were taken were female. Females of several species of bat are more likely to migrate and/or to migrate longer distances than males (Kunz & Fenton, 2003; Giavi *et al*, 2014; Lehnert *et al,* 2014)). This has not been confirmed for Nathusius' pipistrelle to date.
- The single bat that was found north of where it was likely to have moulted (with a likely origin in d^2Hp region A) was found in Cardiff in September.
- The grounded bats in Kent in June and July were resident females, as indicated by the fact that they moulted within the same zone they were recovered (B-B).

[5] The regression equation is not finalised as the Leibniz Institute for Zoo and Wildlife Research are currently analysing more samples of individuals from Eastern Europe to refine the analysis.

- The dead animal found in Walthamstow in March 2001 had the second lowest d^2Hp value, indicating that it moulted in an area far north or east of Walthamstow (likely over 300 km).

Table 3: Summary of the d^2Hp data including the origin, sex and timing of bats from which samples were taken.

Recovery Location	Source	Likely Origin	Sex	Mar.	May	June	July	Aug.	Sept.	Grand Total
Cardiff	B	A	M						1	1
		C	M						1	1
Bedfont	B	B	F		1					1
			M						2	2
		C	F		1				3	4
			M		1				3	4
		D	F		4				4	8
			M					1		1
Walthamstow	B	D	M	1						1
Herne Bay	B	B	F			1				1
Sandwich	B	B	F				1			1
Grand Total				**1**	**7**	**1**	**1**	**1**	**14**	**25**

4.7 It is acknowledged that there can be local variation in d^2H of precipitation within a given area (C. Voigt *pers. comm.*, March 2014). It is likely that this variation explains the origins of the two bats found grounded in Kent in June and July 2013, where the d^2Hp was 0.5 and 2.3 lower than the d^2Hp of the recovery site. It is likely that these bats had not moved a significant distance between moult and subsequent recovery.

4.8 Overall, the d^2Hp of samples ranged between -9.2 and +14.1 of the known d^2Hp of the location in which the bats were recovered. This variation is substantial and is an indication that another parameter is responsible for the variance. In this case, the data strongly suggest that a proportion of the bats sampled moulted a considerable distance from where they were recovered, and had therefore undertaken a migratory movement. The strongest evidence for this are the 10 samples for which the d^2Hp of fur indicated that they moulted and assimilated d^2H particles with d^2Hp region D; at a location far north or east of the location at which they were recovered. Although, there are records of Nathusius' pipistrelle in flight, grounded and mating within region D in the UK, there are no known maternity colonies in Region D from which the individuals may have originated (see Figure 2). The most northerly known colony is within region C on the coast in east Lincolnshire, around 200 km south-west of region D and 160 km north of area B. Maternity colonies of Nathusius' pipistrelle have been recorded in Northern Ireland, approximately 300 km north-west of region B in Wales.

4.9 If the predominant migration direction of this species in Europe (south-west / north-east) is also taken into account, it is more likely that this proportion of the sample may have originated from areas of northern or eastern Europe outside of the UK, than more northerly latitudes of the UK.

Table 4: Full details of the samples' origin, biometric data and stable hydrogen isotope ratio data

Sample Number	Origin		Biometric Data					Stable Hydrogen Isotope Ratio Data				
	Date	Grid Ref.	Type	Sex	Weight	FA	5th digit	d^2H Results	d^2Hp of Recovery Site	d^2Hp of expected place of origin	Difference	Summary
1	20/09/13	ST172736	Harp trap	M	9.5	34.2	43.6	-83.98	-53	-43.8	-9.2	B > A
2	21/09/13	ST172736	Harp trap	M	8	33.2	48	-111.75	-53	-59.6	6.6	B > C
3	30/03/01	TQ357904	Dead animal	M	-	-	-	-125.03	-55	-67.2	12.2	B > D
4	03/05/13	TQ078727	Box	M	6.5	34.2	45.8	-105.36	-54	-56.0	2.0	B > C
5	03/05/13	TQ078727	Box	F	7.2	35	50	-97.46	-54	-51.5	-2.5	B > B
6	03/05/13	TQ078727	Box	F	8.11	35	51	-118.98	-54	-63.8	9.8	B > C
7	03/05/13	TQ078727	Box	F	7.55	33	48	-121.90	-54	-65.4	11.4	B > D
8	03/05/13	TQ078727	Box	F	7.6	33	46	-126.58	-54	-68.1	14.1	B > D
9	03/05/13	TQ078727	Box	F	7.7	33	45	-119.00	-54	-63.8	9.8	B > D
10	10/08/13	TQ078727	Mist net	M	8.8	33	42	-119.40	-54	-64.0	10.0	B > D
11	03/05/13	TQ078727	Box	F	8.41	33.5	45.6	-125.18	-54	-67.3	13.3	B > D
12	23/09/13	TQ078727	Box	F	8.5	36	47	-114.64	-54	-61.3	7.3	B > C
13	23/09/13	TQ078727	Box	F	9	37	44	-119.57	-54	-64.1	10.1	B > D
14	23/09/13	TQ078727	Box	M	7.6	33	43	-97.98	-54	-51.8	-2.2	B > B
15	23/09/13	TQ078727	Box	M	8	33.5	43	-112.45	-54	-60.0	6.0	B > C
16	23/09/13	TQ078727	Box	M	6.9	33.5	42	-116.65	-54	-62.4	8.4	B > C
17	23/09/13	TQ078727	Box	F	9.1	34.5	44	-113.23	-54	-60.5	6.5	B > C
18	23/09/13	TQ078727	Box	F	9.6	36.5	45	-119.24	-54	-63.9	9.9	B > D
19	23/09/13	TQ078727	Box	F	9.9	35	45	-120.40	-54	-64.6	10.6	B > D
20	23/09/13	TQ078727	Box	F	9.8	36	46	-114.02	-54	-60.9	6.9	B > C
21	23/09/13	TQ078727	Box	F	9.8	35	45	-120.88	-54	-64.8	10.8	B > D
22	23/09/13	TQ078727	Box	M	7.9	32.3	42.1	-96.75	-54	-51.1	-2.9	B > B
23	23/09/13	TQ078727	Box	M	7.8	33	44.7	-110.96	-54	-59.2	5.2	B > C
24	09/06/13	TR156677	Grounded	F	-	-	-	-100.89	-54	-53.5	-0.5	B > B
25	18/07/13	TR301566	Grounded	F	-	-	-	-97.73	-54	-51.7	-2.3	B > B

5 Conclusions

5.1 The d^2Hp of the recovery sites of individuals within this study had little variation (-53 and -54). However, the d^2Hp of the samples analysed in this study varied substantially (-43.8 and -68.10), suggesting that a parameter other than natural variation in d^2H in local rainwater at the recovery sites was responsible. In this case, the data strongly suggest that a proportion of the bats sampled moulted a considerable distance from where they were sampled, and had therefore undertaken a migratory movement.

5.2 The findings in this study concur with other evidence in support of the regular and predictable migration of Nathusius' pipistrelle in and out of the UK including:

- The low numbers of known maternity colonies in the UK yet high incidence of Nathusius' pipistrelle records (e.g. grounded bats) during the key migratory periods (May and September)
- Evidence that Nathusius' pipistrelle has adapted its range in response to recent climate change on a continental scale (Lundy *at al*. 2010)
- Peaks in Nathusius' pipistrelle activity recorded on static detectors at the coasts in Kent, east Yorkshire, Dorset, Pembrokeshire and ferries identified peaks of activity in the key migratory periods (BSG Ecology, 2013a-d; & BSG Ecology, 2014a-b);
- Offshore research identifying presence of Nathusius' pipistrelle between 15 km and 71 km offshore (Poerink *et al*, 2013; BSG Ecology, 2014b); and
- A bat ringed at Blagdon near Bristol in October 2012 and recovered in the Netherlands in December 2013 (Hargreaves, 2014).

5.3 With mounting evidence in support of Nathusius' pipistrelle migration between the UK and mainland Europe, it is apparent that coordinated international measures are required in order to maintain the favourable conservation status of migratory populations of this, and potentially other, bat species that regularly occur in the UK. This includes (i) conservation of maternity sites and (ii) identification and protection of migration routes and hibernation sites.

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