Updating the distribution and status of the Nathusius pipistrelle (*Pipistrellus nathusii*) in Ireland

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Updating the distribution and status of the Nathusius pipistrelle (*Pipistrellus nathusii*) in Ireland: Final Report 2016

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INTRODUCTION

The Nathusius pipistrelle (*Pipistrellus nathusii*) is typically a migratory species, with movements of more than 1,900km recorded between summer and hibernation sites in mainland Europe (Hutterer *et al.,* 2005). In Ireland and the UK it has in the past been considered a vagrant due to the paucity of records (Stebbings, 1988), and more recently as a migrant winter visitor (Speakman *et al.,* 1991; Hutson 1993). During the 1990s evidence of mating was confirmed in south-west Britain, followed by the discovery of two maternity colonies in Lincolnshire, England, and County Antrim, Northern Ireland (Harris *et al.,* 1995; Hutson 1997; Russ *et al.,* 1998). Since then records have been steadily increasing, with evidence that these bats are resident year round in some parts of south eastern England and Northern Ireland. The increase in records, while likely in part due to initiatives such as the car-transect monitoring schemes in Ireland and Great Britain , is also possibly evidence that this species is expanding its range in response to changing climatic conditions in Europe (Lundy *et al.,* 2010; Roche *et al*., 2012).

In continental Europe, the Nathusius pipistrelle is found across western Europe to Asia Minor (Figure 1), with evidence of migration during autumn and winter from breeding colonies in Eastern Europe in a south-westerly direction to overwinter in Central and Southern Europe (Hutterer *et al.,* 2005). There are resident populations known in parts of Germany, Switzerland, Austria and possibly the Netherlands which are augmented during the winter months by incoming migrants (Arnold *et al.,* 1996) (Figure 2).

Figure 1. Distribution of *P. nathusii*

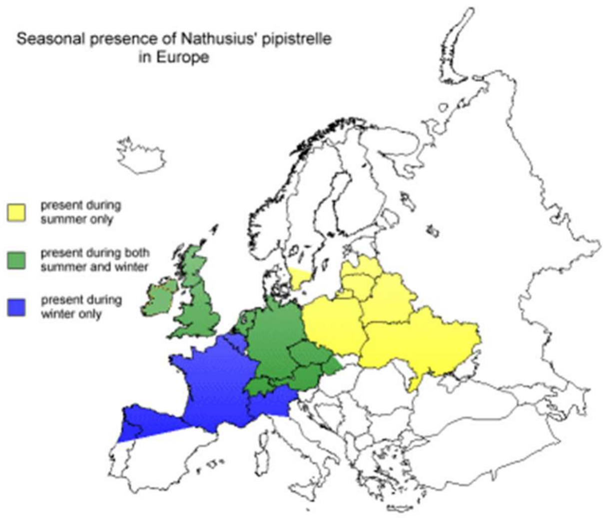


Figure 2. Seasonal presence of *P. nathusii* in Europe

(*Both taken from Jon Russ's website* www.nathusius.org.uk)

It seems likely that Ireland and Britain lie within this transitional zone (Russ *et al*., 2001). There is evidence in Great Britain of both resident and migratory populations, with the timing and peaks in detector records increasing during autumn and spring, as well as records of grounded bats on North Sea oil platforms, suggesting migration with Scandinavia (Russ *et al*., 2001). A recent discovery of a bat banded in south west England turning up in the Netherlands seems to confirm this (Daniel Hargreaves *pers. comm*).

As it stands the status of Nathusius pipistrelle and its current distribution in Ireland is comparatively unknown. In Northern Ireland it seems likely that resident populations exist, yet no roost records, or evidence of breeding in the Republic of Ireland have been documented. Furthermore, known roosts are not monitored so that their current status is unknown. Suggestions that Nathusius pipistrelles in Northern Ireland tend to form maternity colonies alongside Soprano pipistrelles, whose colonies consist of many hundreds of individuals, would make the identification of roosting sites of Nathusius pipistrelle problematic and could mean that many have been overlooked (Mark Smyth *pers. comm.*).

BatLife Europe declared 2015 the year of the Nathusius pipistrelle, and it was decided that here in Ireland we needed to form a consortium of bat researchers, professionals and volunteers in order to address the current gaps in knowledge. Under the umbrella of the 'All-Ireland Nathusius Pipistrelle Group', at Quercus, Queen's University Belfast we set out to undertake a body of research with the aim of determining the status and distribution of Nathusius pipistrelle in Ireland. Co-funding was made available by Quercus and the National Parks and Wildlife Service of the Department of Arts Heritage and the Gaeltacht.

Project objectives

In order to effectively conserve Nathusius pipistrelle populations in Ireland we first need to accurately map their distribution and understand their status as resident and/or migratory populations. To address these issues we proposed the following objectives:

i) Produce an up-to-date distribution map by collating all possible detector, specimen and roost records across the island of Ireland;
ii) Use this distribution data to model the predicted distribution by using both climatic factors and habitat variables;
iii) Utilise this model to identify key sites to target acoustic and catch surveys aimed at increasing records for this species; and identify and survey potential roost sites for Nathusius pipistrelle.
iv) Compare stable isotope signals among populations of Irish, British and European populations of Nathusius pipistrelle to look for evidence of recent migration.
v) Determine the genetic relationship among Irish, British and European populations of Nathusius pipistrelle to explore the patterns of genetic diversity and gene flow and perhaps reveal migration routes and colonisation history.

This is the final report detailing the results of the work completed towards these objectives.

METHODS

Collation of records

Records of *P. nathusii* were collated from data provided by CEDaR who hold the records from the Northern Ireland Bat Group (NIBG), the National Biodiversity Data Centre (NBDC) and Bat Conservation Ireland (BCI), who were able to provide grid referenced Car-Based Bat Monitoring scheme records. Records were also requested from ecological consultancies. The database was checked to remove any duplication or dubious records and included records of bats in the hand (grounded and caught under license), roost records and bat detector records.

Analyses of calls recorded during emergence and activity surveys, and calls recorded by the static detectors were undertaken using Kaleidoscope (version 2.2.2). Files were converted from .wac to .wav format and batch processed for automatic identification. Calls were then verified manually to ensure correct identification.

Site surveys and catching

Four known and three potential maternity colonies of Nathusius pipistrelle were surveyed.. Species were identified using a Petterson D240x detector and remotely deployed SM2 BAT+ detectors (Wildlife Acoustics Ltd.). Bats were captured under licence (Licence No.: DER/BAT 2015-32) using hand nets at roosts sites. Captured bats were identified and morphological measurements taken (including sex, age, reproductive status, forearm length and wing venation pattern); a hair sample for stable isotope analysis (see below) and two wing biopsy punches for molecular genetic analyses (see below) were taken from each captured Nathusius individual.

Species distribution modelling

We elected to use the same variables in the species distribution models as used in the BCI study by Lundy *et al*., (2011)(Table 1). These comprised land cover (http://www.eea.europa.eu), topography (http://www.diva-gis.org/Data), climate (http://www.worldclim.org/), riparian habitat (http://www.diva-gis.org/Data), and soil pH 9 (http://eusoils.jrc.ec.europa.eu/). A further variable encompassing human influence (http://sedac.ciesin.columbia.edu/wildareas) was used to offset for bias in survey effort due to the distribution of human population/human activity. Seven spatial scales were used: 500m, 1500m, 2500m, 4500m, 6500m, 10500m and 20500m.

A maximum entropy model (MEM) was fitted in MaxEnt (3.3.3k) (Elith *et al.,* 2011) using the updated distribution points collated in this study and the variables mentioned above. The model was built using the core distribution of this species in Northern Ireland and then projected to the entirety of Ireland. This was shown to produce more accurate models than building the model for the whole of Ireland. Ten replicates of models were run using bootstrap, each randomly selecting 25% of locations to test the model's predictive capability. Model performance was evaluated based on the AUC value (Fielding and Bell, 1997). An average of modelled output was used for the habitat suitability. When mapped, this shows a continuous classification of suitability, from low to high suitability. From this range, minimum probability of suitable habitat can be defined, and a threshold value of equal to the lower 10th percentile of the range is commonly used to define "suitable" and "unsuitable" habitat.

Table 1 – Variables used in the modelling (from Lundy *et al.,* 2011).

Explanatory variables		Units	Description
Land cover	Arable	m^2	Cover of non-irrigated arable land.
	Bare rock	m^2	Cover of bare rock.
	Bog, marsh, moor and heath	m^2	Cover for a composite of bog, marsh, moor and heath.
	Broad-leaved woodland	m^2	Cover of broad-leaf woodland.
	Conifer plantation	m^2	Cover of coniferous woodland.
	Forest	m^2	Cover of broad-leaved woodland, conifer plantations and

			mixed forest.
	Freshwater	m^2	Cover of open water.
	Mixed agriculture	m^2	Cover for a composite of complex cultivation patterns and land principally occupied by agriculture with significant natural vegetation.
	Mixed forest	m^2	Cover of vegetation formation composed principally of trees, including shrub and bush under storeys.
	Natural grass	m^2	Cover of low productivity grassland. Often situated in areas of rough, uneven ground. Frequently includes rocky areas, briars and heath.
	Pasture	m^2	Cover of dense grass cover, of floral composition, dominated by Graminaceae, not under a rotation system. Includes areas with hedges.
	Scrub	m^2	Bushy or herbaceous vegetation with scattered trees.
	Sparse vegetation	m^2	Cover of scattered vegetation is composed of gramineous and/or ligneous and semi-ligneous species.
	Urban	m^2	Cover of manmade structures and transport network.
Topography	Altitude	m	Elevation above sea level in metres.
Climate	Temp_{min}	°C	Minimum temperature of the coldest month.
	Temp_{max}	°C	Maximum temperature of the warmest month.
	Precipitation	mm	Total annual precipitation.
	Seasonality	Index	Standard deviation of mean monthly temperatures.
Other	Riparian corridor	m	Total length of river and water body edge including lakes, reservoirs, ponds, rivers, streams and canals.
	Soil pH	pH	Mean soil pH.
	Human influence	Index	Comprises population density, rail networks, major roads, navigable rivers, coastal shore lines, night-time stable light emissions, urban land cover and agriculture.

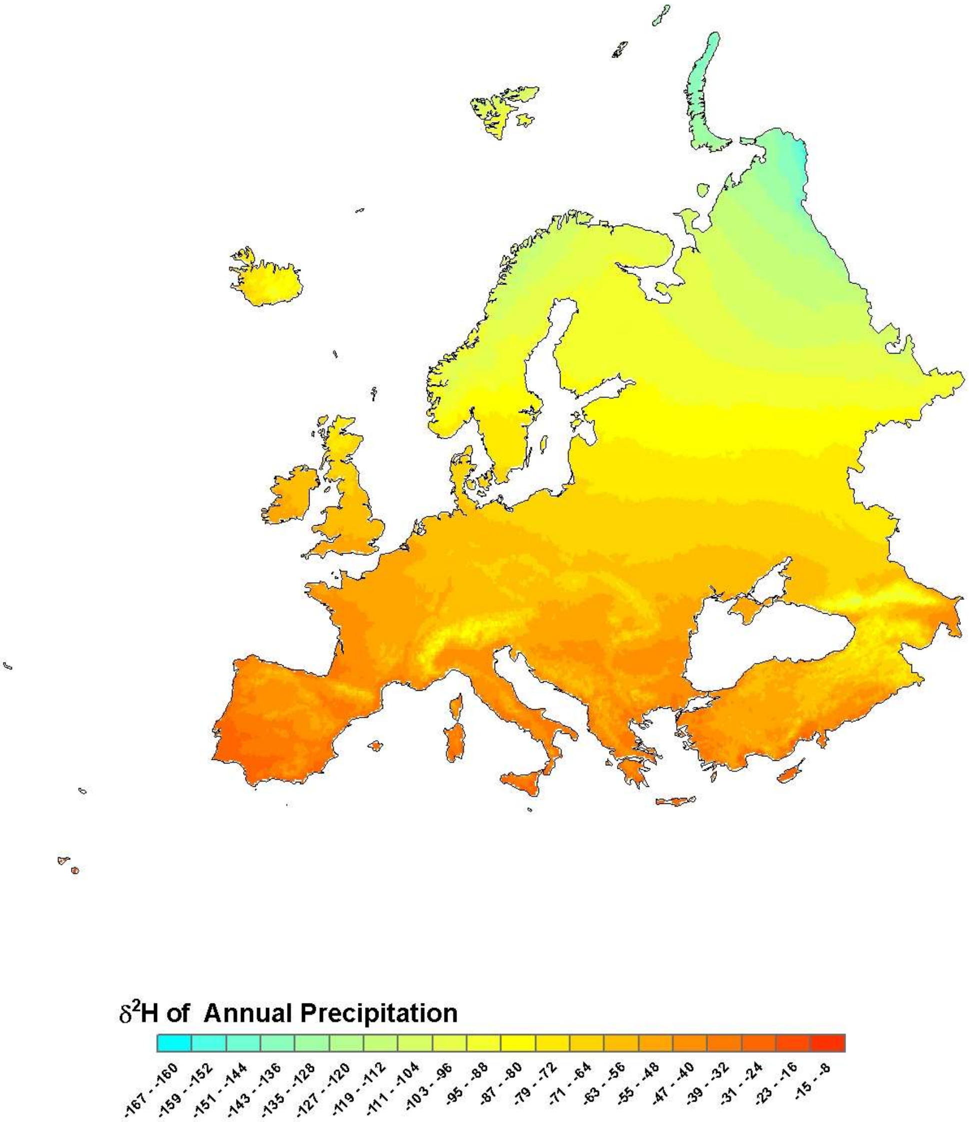
Modelled distribution led surveys

Based on the output of the distribution model described above we identified a number of sites within areas of high probability of presence for Nathusius pipistrelle in order to undertake acoustic and catch surveys. Acoustic surveys were undertaken using one or two remotely deployed SM2 BAT+ detectors (Wildlife Acoustics Ltd.) left overnight. At each site an attempt was made to catch Nathusius pipistrelles using a harp trap and an acoustic lure (manufactured by Frank Greenaway) following the success of this method for catching Nathusius pipistrelles in the UK. A harp trap was erected at a suitable site and the acoustic lure turn on at 30mins past sunset. Every 15 minutes the trap was checked and the call on the acoustic lure changed as recommended by Frank Greenaway. All bats captured were identified to species, and basic morphological measurements taken for the BCI's records.

Stable isotopes

Hair samples were collected from all captured Nathusius pipistrelles for stable isotope analysis. These samples were sent to Christian Voigt at the Leibniz Institute for Zoo and Wildlife Research, Berlin, Germany for analysis of ratios of the stable hydrogen isotope (δ^2H) (Voigt *et al.,* 2012). The latitudinal patterns of hydrogen isotopes in rainfall are well known and predictable (see Figure 2). By analysing the δ^2H assimilated in fur keratin of these bats we can infer their latitudinal location during the last moult, which generally occurs before winter migration, and thus gain information on their whereabouts last summer (Voigt *et al.,* 2012). By looking at the fur collected from adult bats in early summer we hope to determine if these bats had spent time at a different latitude the previous summer according to the δ^2H isotopic map. Samples taken from adult bats can also be compared to juveniles, whose fur would have grown in situ.

Figure 2. δ^2H isotopic map of Europe (taken from Bowen *et al.,*. 2012)



Molecular genetic analyses

Wing biopsy tissue samples were taken from all Nathusius pipistrelles captured in Ireland for DNA analysis. Two biopsies from each individual were taken under licence (Licence No.: DER/BAT 2015-32) and placed directly into ethanol until DNA could be extracted. At sites where capture was not possible faecal samples were collected, and samples sought from collaborators in England, Europe and Russia. DNA was extracted from tissues samples using a salt-chloroform method following Puechmaille *et al.,* (2009) and faecal samples extracted using a modified protocol for Qiagen QIAmp stool extraction kits (QIAGEN, Valencia, CA) in Professor Emma Teeling's laboratory.

Samples were amplified for a ~950 bp fragment of the mitochondrial DNA D-loop using the following PCR reactions: 25 µl volume containing 0.2 µM of both forward and reverse primers (mtDNA-R1-F and mtDNAF1- R — Puechmaille et al., 2011), 1X PCR buffer minus MgCl2 (Invitrogen, Carlsbad, CA), 1.5 µM MgCl2 (Invitrogen), 0.1 µM dNTPs, and 1U of Platinum Taq polymerase (Invitrogen). PCR amplification consisted of: denaturation at 95°C for 3 min, 10 cycles of denaturation at 95°C for 30secs, annealing at 65°C for 30secs minus 1°C per cycle, extension at 72°C for 60secs; followed by 35 cycles with 95°C for 30secs, annealing at 55°C for 30secs, extension at 72°C for 60sece. PCR products were purified using Exo-SAP-IT (Affymetrix Inc., Santa Clara, CA) and sequenced in a single direction with the forward PCR primer by Macrogen Inc. (Seoul, Korea). All sequences were aligned using CLUSTAL W in the program MEGA5 (Tamura et al., 2011) and collapsed into haplotypes using FaBox v1.35 (Villesen, 2007). A median joining network was produced to examine the relationships among haplotypes using all individual sequences in Network 5.0.0.0. (Fluxus Technology Ltd., United Kingdom).

In order to allow the exploration of both past and current connections with the UK and mainland Europe, twelve microsatellite primers developed for *P. pipistrellus and P. pygmaeus* (Petri *et al.,* 1997; Burland *et al.,* 1998; Mayer *et al.,* 2000; Vonhof *et al.,* 2002; Racey *et al.,* 2007) were tested for use in *P. nathusii*. The twelve loci were amplified in two multiplex reactions for all tissue and faecal samples. The first multiplex included: Ppip02, Ppip04 (Racey *et al.,* 2007), EF1 (Vonhof *et al.,* 2002), Paur05 (Burland et al., 1998), NN8 (Petri *et al.,* 1997), P217 (Mayer *et al.,* 2000); and the second multiplex included the following: Ppip04, Ppip06 (Racey *et al.,* 2007), EF4, EF6 (Vonhof *et al.,* 2002), NN18 (Petri *et al.,* 1997), P219 (Mayer *et al.,* 2000). In each case, PCR was carried out in 10 µl multiplex reactions containing 5 µl TypeitTM Microsatellite PCR Kit Master Mix (QIAGEN, Valencia, CA). Primer concentration, fluorescent label and multiplex information are shown in Table 1. PCR amplification conditions were as follows: 95ºC for 5min; 30 cycles of 95ºC for 30 s, 57ºC for 90 s, 72ºC for 30 s and a final extension at 72ºC for 30 min using a Biometra TProfessional. Resulting PCR products were run on an ABI PRISM 3130xl Genetic Analyser 16 Capillary system (Applied Biosystems) and sized with internal DNA size standard (600 LIZ — Applied Biosystems) using the program Genemapper v. 4.0 (Applied Biosystems).

RESULTS

Collation of records

A database of all combined records for the species was constructed (n = 345). After removal of replicate records, and records where no location was determined, there were a total of 278 reliable presence records. The species is most commonly encountered in the summer months, with a particular increase in records in July and August due to the timing of the Car-Based Bat Monitoring scheme records (Figure 3). The occurance primarily in the summer months suggests these are breeding populations across Ireland. In addition, records have been significantly increasing yearly (R2=0.289, F1, 18=8.643, p=0.009) since the first record in 1996, although some fluctuation is visible (Figure 4). The distribution of records is wide and the Nathusius' pipistrelle occurs across Ireland, with records in all four quadrants. There is, however, an apparent bias to the north and east, indicating a stronghold for the species in Northern Ireland (see Figure 5).

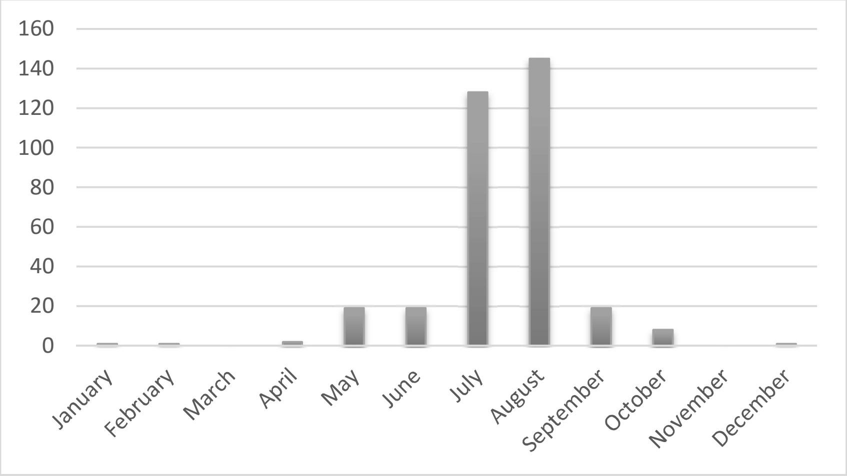


Figure 3 Error! No text of specified style in document.**.** Record numbers by month.

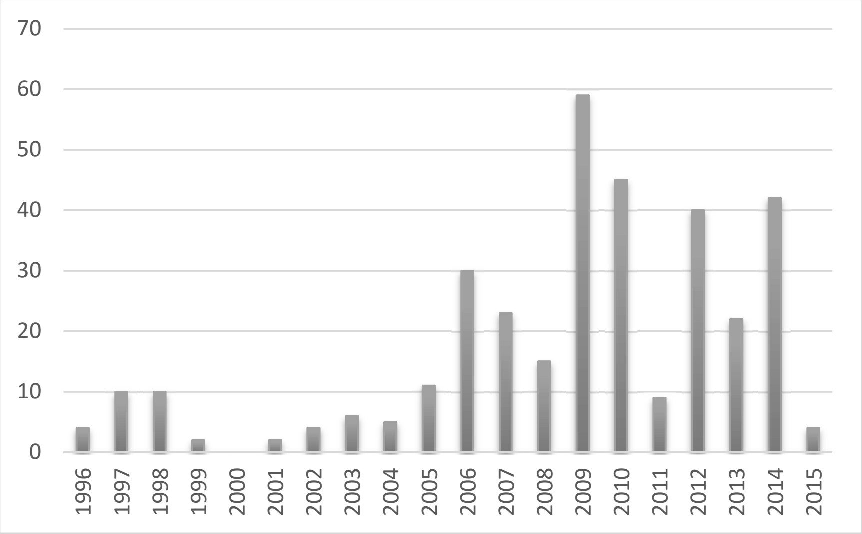


Figure 4. Record numbers by year.

Site surveys and catching

Emergence surveys were undertaken at four previously known roost sites of Nathusius pipistrelle and three potential roost sites in Northern Ireland between June and September 2015 (Shane's Castle, Co. Antrim; Clothworthy House Co. Antrim; Old Mill, Co. Antrim; Crom Estate, Co. Fermanagh; Ardress House, Co Armagh; Benburb Castle, Co. Armagh; Greysteele, Co. Londonderry (Figure 5)). Nathusius pipistrelles were confirmed to be present at six sites, and roosting was confirmed at four of these, including two sites where no previous roost had been identified. At one site the potential roost was confirmed to be that of soprano pipistrelle (Figure 5).

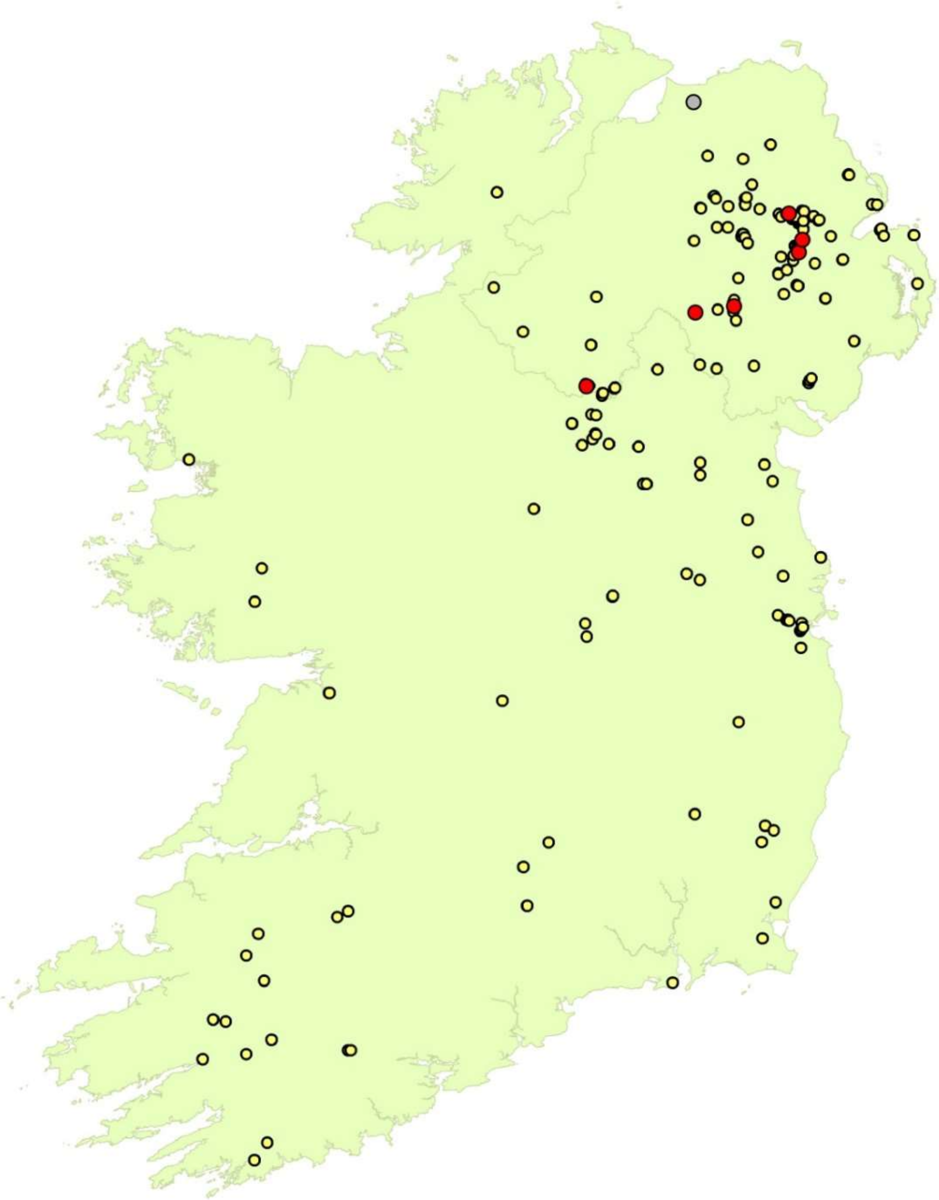


Figure 5. Distribution of Nathusius' Pipistrelle in Ireland as of 2015. Red circles represent sites surveyed where Nathusius roosts were confirmed or strongly suspected, and the grey circle represents a potential site now confirmed not to be Nathusius pipistrelle.

The transect surveys undertaken at Crom Estate, Ardress House and Benburb Castle are shown in Figure 6 a,b,c; including the position of two static SM2BAT+ detectors deployed for one week at each site. At Crom Estate, Nathusius pipistrelles were not found roosting in the sites previously described, nor were any other roosts confirmed. However they were recorded along the transect close to the river and a soprano pipistrelle roost in an old school house close to the bridge. They were also recorded on the static SM2 close to the turf house each night of the survey, suggesting they remain in the area and are likely roosting in one of these locations. At Ardress House, the SM2 deployed in the courtyard near the entrance of a known large soprano pipistrelle roost recorded a record number of Nathusius' pipistrelle passes, and mating calls confirming a roost, for the first time at this site. Benburb Castle had not previously been surveyed for bats. A roost of soprano pipistrelles and brown long eared bats was discovered within the Castle during this survey and Daubenton's bats, Natterer's bats, common pipistrelles, Leisler's bats and Nathusius pipistrelles were also recorded on the SM2 detectors. There were a larger number of Nathusius calls including numerous male social calls along the cliff face below the castle, suggesting Nathusius are mating and potentially roosting at this site.

Figure 6a, b, c. Static detector locations at (a) Crom estate, (b) Ardress House and (c) Benburb castle denoted by red dots. The yellow line denote the transects and spot checks along transects are denoted by purple dots.

Nathusius bats where caught at Clotworthy House on 30th June 2015. The roost was estimated to contain over 500 Nathusius pipistrelles, and we sampled 25 for stable isotope and genetic analyses. Twelve of these were female, and all showed signs of recent lactation, there were four adult males sharing the maternity roost, and nine juveniles of both sexes. It was also noted that all of the juveniles were large, fully furred, and free flying at this date in early summer, suggesting they were born quite early in the season. Additionally, numerous soprano pipistrelles were captured, suggesting that in addition to sharing roost space, the two species were using the same exit/entry points.

Species distribution modelling

Models were constructed using 278 presence records. Variables with greatest percentage contribution to each model, and the AUC value for each are summarised in Table 2. AUC values for all models achieved >90% suggesting that models constructed are useful for predicting species occurrence. Lundy *et al.,* (2011) previously found favoured habitats for the species to be broad-leaved woodland, pasture and freshwater. The scales that show these to be most relevant are 500m and 10500m, hence we ran models at 500m, 4500m and 10500m (Figure 7a, b, c).

In each model, seasonality consistently showed a positive association with bat occurrence, however this decreased with scale, being most important within 500m. Freshwater on the other hand increased in importance with scale, becoming more important in determining presence within a 10.5km radius. Maximum temperature appears to be a relatively important factor at smaller spatial scales, as does precipitation with a negative relationship. Broad-leaved woodland and association with urban areas showed positive trends at each scale, while bog, marsh, moor and heath consistently showed a negative trend which become more important when considering a larger scale.

There is a clear north-easterly bias in the predicted distribution of Nathusius pipistrelle in Ireland, with a concentration of highly suitable areas in and around Lough Neagh and the midlands. By testing our model on the limited core range and then projecting it across the island we have tried to avoid as much as possible any bias created by the current dominance of records in the north. And thus are happy that these models are a good representation of where we may expect to find Nathusius pipistrelle.

Figure 7a,b,c. Landscape associations modelled using maximum entropy for Nathusius pipistrelle in Ireland at (a) 500m, (b) 4500m and (c) 10500m.

Table 2. The selected predictive variables used in Maximum Entropy Modelling of Nathusius occurrence at three spatial scales (m) are shown together with their % contribution to the model (% cont). Three relationships with variables are shown: positive associations (+ve), negative associations (-ve) and quadratic (+ve/-ve). The Area Under the Curve (AUC) statistic provides the percentage correct classification of the model.

	Spatial scale (m)					
	500		**4500**		**10500**	
AUC	0.9		0.91		0.904	
Variables	% cont	Relationship	% cont	Relationship	% cont	Relationship
Altitude	7	+ve/-ve	3.7	+ve/-ve	2.9	+ve
Arable	2	-ve	3.3	-ve	7.7	+ve/-ve
Bog, marsh	2.4	-ve	5.2	-ve	6.8	-ve
Broad-leaved	6.7	+ve/-ve	6.9	+ve	3.8	+ve
Conifer	3.8	-ve	3.3	-ve	-	-
Freshwater	2.3	+ve/-ve	20.6	+ve/-ve	31.3	+ve/-ve
Human influence	5.4	+ve/-ve	-	-	-	-
Mixed agri	2.1	-	4.6	+ve/-ve	-	-
Mixed forest	-	-	-	-	2.1	+ve/-ve
Moor, heath	3.5	+ve/-ve	-	-	12	-ve
Natural grassland	3.3	+ve/-ve	-	-	2	+ve/-ve
Pasture	-	-	2.6	+ve/-ve	-	-
Precipitation	8.3	-ve	6.1	-ve	4.4	-ve
Riperian corridor	-	-	2.6	+ve	3.9	+ve
Scrub	-	-	-	-	-	-
Seasonality	15.6	+ve	12.3	+ve	6.8	+ve
Temp max	14.4	+ve/-ve	6	+ve	-	-
Temp min	-	-	2.5	-ve	2.6	-ve
Urban	14.1	+ve/-ve	13.5	+ve/-ve	5.1	-ve

Landscape model led acoustic and catch surveys

Ten woodland sites close to waterbodies within areas predicted by our model to have a high probability of presence for Nathusius pipistrelle at the 500m scale were chosen to undertake catch and acoustic surveys as a means to test the model and increase records for this species (Figure 8). One additional site was acoustically surveyed only with the help of volunteers.

While no Nathusius pipistrelles were captured using the acoustic lure and harp trap method, it was shown to work well for other species and allowed us to capture 77 individuals over 13 nights survey, including: *Pipistrellus pygmaeus* (62), *Myotis daubentonii* (7), *M. mystacinus* (2), *M. nattereri* (4) and *Plecotus auritus* (2). All individuals were measured and records were given to BCI. At a number of sites we did detect Nathusius pipistrelles on the SM2 detectors, two of which held no previous records.

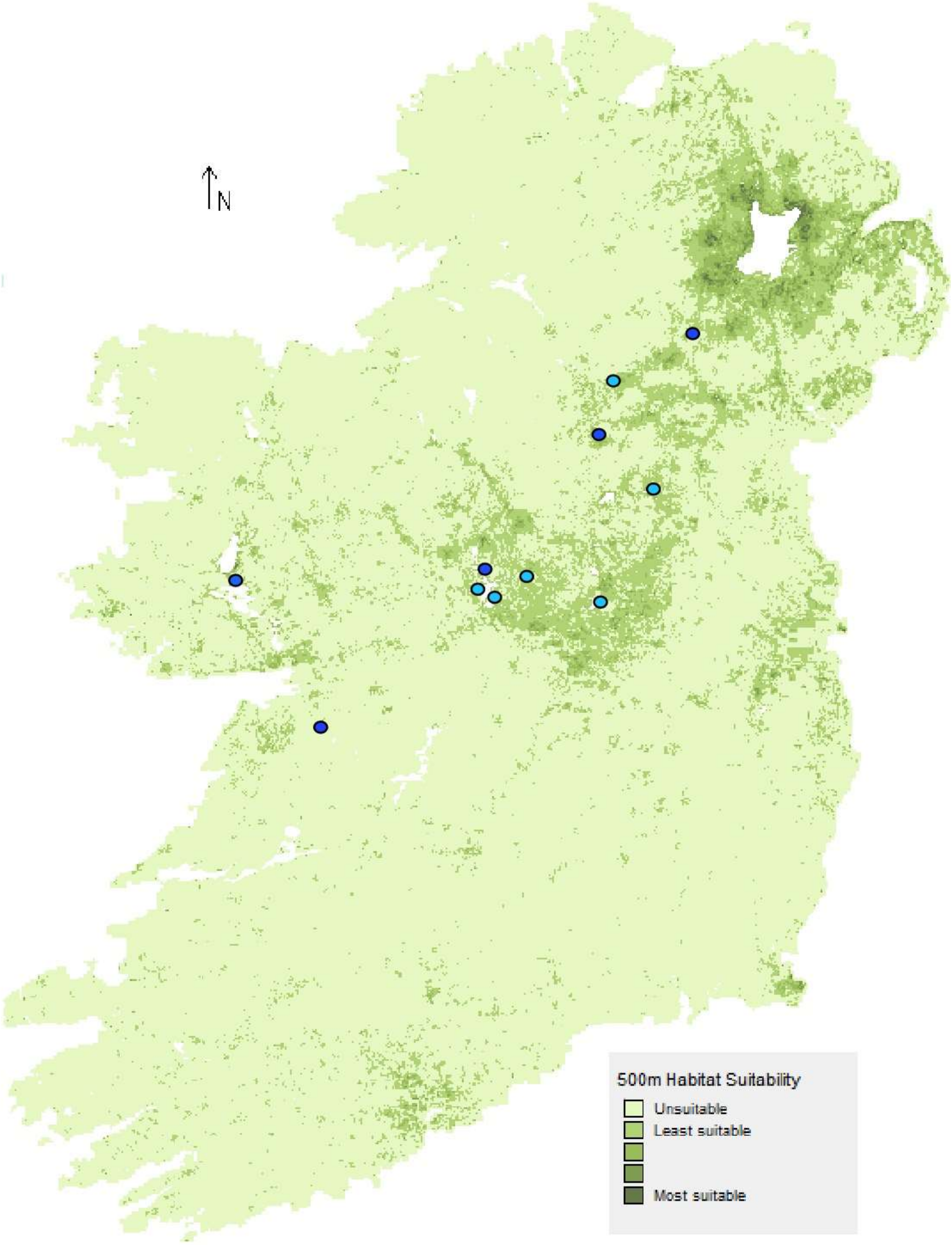


Figure 8. Sites surveyed with the acoustic lure, harp trap and SM2 bat detectors. Light blue circles indicate sites where no Nathusius bats were detected, and the dark blue circles indicates sites where Nathusius bats were detected.

Landscape Model led roost surveys

The next step is to undertake surveys of potential roost sites for Nathusius pipistrelle, particularly in the Republic of Ireland where no records are known. Since it has been shown that this species does cohabit with soprano pipistrelles it is possible that maternity roosts of Nathusius pipistrelle may have been overlooked in the past. In this study we have collated records of known soprano pipistrelle roost sites and those of unknown pipistrelle species from BCI and NIBG. These sites have been filtered to only those records where over 50 individual bats were recorded and to those which were recorded after the year 2000. These records were then overlaid on the landscape model and only those selected which fell within the area predicted to be suitable for Nathusius pipistrelle at the broad scale of 10500m to make it more inclusive (Figure 9). A survey of a number of these sites with the help of volunteers in both the Republic of Ireland and Northern Ireland is planned for spring 2016 to attempt to identify new maternity roosts of Nathusius pipistrelle in Ireland.

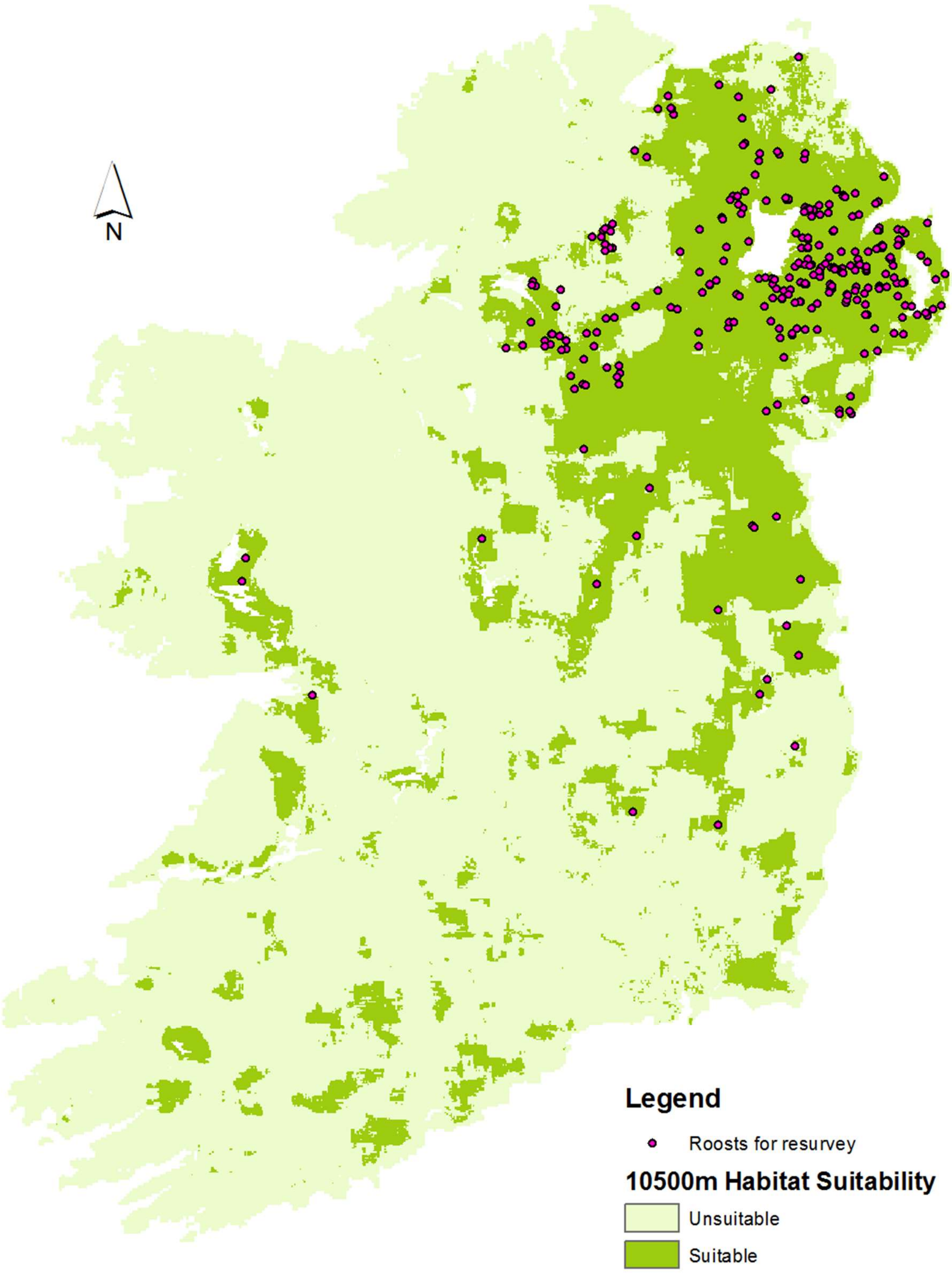


Figure 9. Potential roost sites to be surveyed which fall within the predicted suitable habitat for Nathusius pipistrelle from the 10500m model.

Stable isotopes

A total of 25 hair samples from Irish Nathusius pipistrelles were collected from 9 adult females, 3 adult males, 10 juvenile males and 3 juvenile females at Clothworthy House in Co. Antrim. Eighteen of these samples from both adult and juvenile Nathusius pipistrelles were analysed for stable hydrogen isotopes (δ^2H). The raw δ^2H values for each individual are shown in Table 3. All show very similar values, including females and males, and between adults and juveniles.

The expected range of values for hydrogen isotopes in precipitation (δ^2Hp),for Northern Ireland are between -63 to -56 according to the index by Bowen *et al.,* (2012). Once the raw values of the stable hydrogen isotope from hair samples is converted using a regression equation established for Nathusius pipistrelle (C. Voigt *pers. comm.*) all values for these bats captured in Co. Antrim fall within a range of -50 to -59, with many around -56. This is within the expected range for this region and suggests that the adult bats were present in an area with the same isotope signal as this site in Co. Antrim when they last moulted given the similarity to the signal in juvenile bats and the expected local δ^2Hp conditions.

Table 3. Raw δ^2H results for each individual.

Bat ID	Date	Country	County	Age	Sex	Rep.	dH_Results
NPCH03	30/06/2015	N. Ireland	Antrim	F	A	Lac	**-107.498**
NPCH04	30/06/2015	N. Ireland	Antrim	F	A	Lac	**-110.156**
NPCH05	30/06/2015	N. Ireland	Antrim	F	A	Lac	**-111.852**
NPCH06	30/06/2015	N. Ireland	Antrim	F	A	Lac	**-102.252**
NPCH07	30/06/2015	N. Ireland	Antrim	F	A	Lac	**-110.439**
NPCH08	30/06/2015	N. Ireland	Antrim	M	A	N/A	**-101.728**
NPCH09	30/06/2015	N. Ireland	Antrim	F	A	Lac	**-106.852**
NPCH13	30/06/2015	N. Ireland	Antrim	F	A	Lac	**-110.247**
NPCH14	30/06/2015	N. Ireland	Antrim	F	A	Lac	**-107.023**
NPCH16	30/06/2015	N. Ireland	Antrim	M	A	N/A	**-103.415**
NPCH20	30/06/2015	N. Ireland	Antrim	M	A	N/A	**-101.467**
NPCH22	30/06/2015	N. Ireland	Antrim	F	A	Lac	**-111.825**
NPCH01	30/06/2015	N. Ireland	Antrim	M	J	N/A	**-104.431**
NPCH02	30/06/2015	N. Ireland	Antrim	F	J	N/A	**-101.805**
NPCH10	30/06/2015	N. Ireland	Antrim	M	J	N/A	**-102.721**
NPCH19	30/06/2015	N. Ireland	Antrim	F	J	N/A	**-94.396**
NPCH24	30/06/2015	N. Ireland	Antrim	M	J	N/A	**-99.864**
NPCH25	30/06/2015	N. Ireland	Antrim	F	J	N/A	**-102.245**

Molecular genetic analysis

The only bats captured in Ireland during this study were those from the maternity colony at Clotworthy House, Co. Antrim. Wing biopsy tissue samples were obtained and DNA extracted and amplified for mitochondrial DNA sequencing from the 12 adults only to avoid psuedoreplication. Faecal samples were contributed from England (25), Germany (16), Lativa (11) Russia (8) and Ukraine (2) (see Appendix 1). Many of the samples collected in England, Germany and Latvia were from bats captured as part of banding or telemetry studies, thus while these were faecal samples information was known about the individuals and they are associated with a ring number.

From the 96 samples amplified and sequenced for the mitochondrial D-loop, 71 (9 from Ireland) provided sufficiently good quality sequence to allow analysis. These 71 sequences from a 490bp region of mitochondrial D-loop represented 40 haplotypes which are represented as a haplotype network (Figure 10). All of which differed by no more than 10bp. This plus the star shaped pattern in the core of the haplotype network suggests these all share a fairly recent common ancestor. The lack of geographic restriction of haplotypes and haplotype sharing across countries and continents suggests a recent colonisation and/or constant population mixing, which may be expected from a migratory species. For example, the most common haplotype encountered was found in bats from England, Latvia, Germany and the Ukraine.

In Ireland the 9 samples from Nathusius pipistrelles captured in Co. Antrim represented a total of 6 haplotypes. Two of these are shared with bats from England, Germany, Latvia and Russia. This high diversity, plus the distribution of these haplotypes throughout the haplotype network which suggests these populations either colonised Ireland recently in good numbers, or that they are still part of a migrant population connected to the rest of Europe. Similarly, there was high diversity in the bats captured in England, where out of 25 bats captured, there were a total of 21 haplotypes, which again supports either recent colonisation or seasonal migration to mainland Europe. One of the English samples is from a banded bat originally tagged in Latvia. This individual shares its haplotype with others bats from England and Germany.

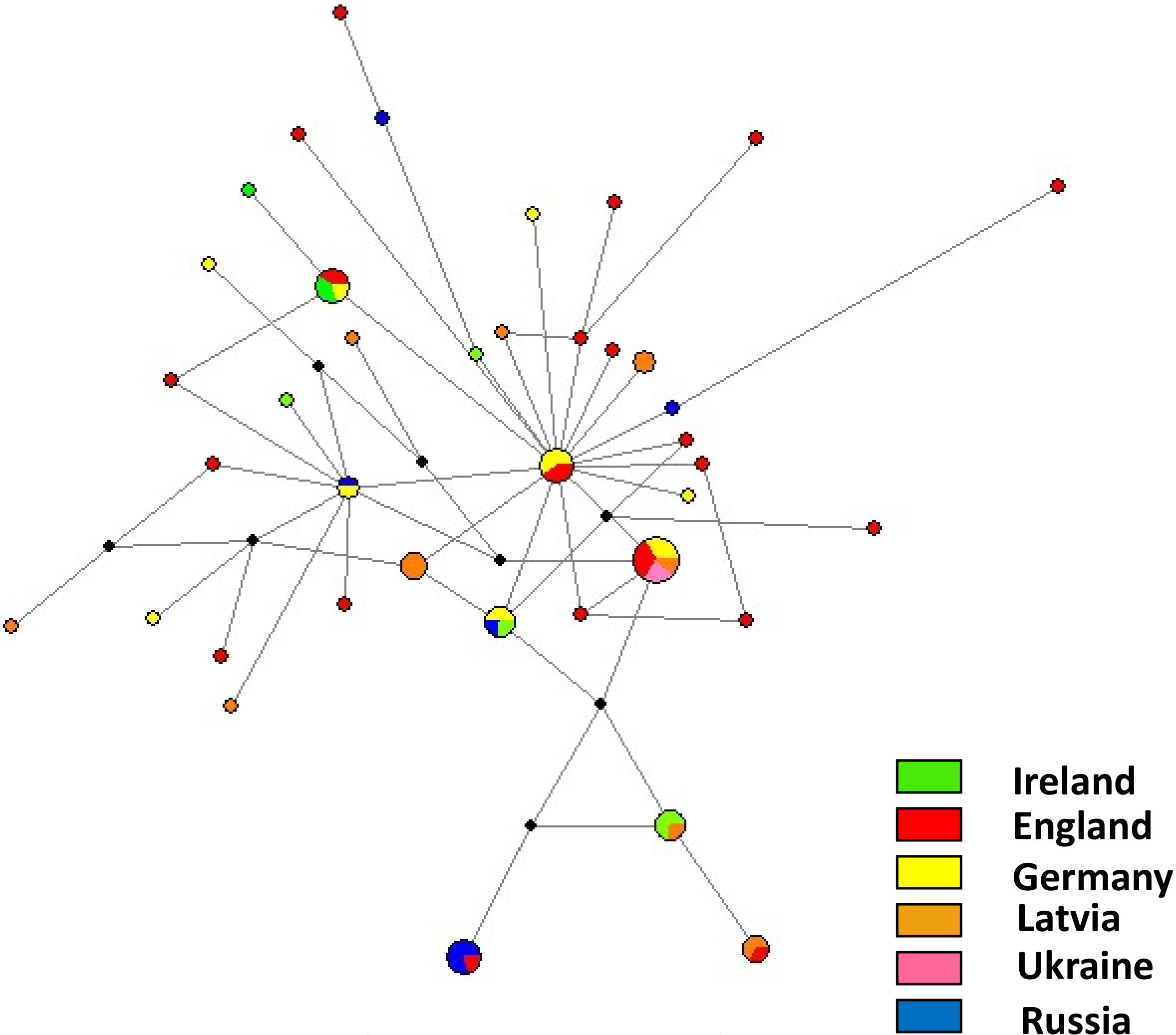


Figure 10. Haplotype network of 71 individual sequences of Nathusius pipistrelles representing 40 haplotypes from a 490bp region of the mitochondrial D-loop.

Of the twelve microsatellites tested in *P. nathusii*, only 5 amplified bands, and only 2 consistently with sufficient variability for analysis. As such, it was not possible to undertake any analysis with these microsatellite markers. Further optimisation of a number of these markers could be attempted for the use in *P. nathusii*, but it is likely that in order to undertake a good population study that markers specific to the species would need to be developed.

DISCUSSION

In this study we have produced an updated database of distribution records for Nathusius pipistrelle in Ireland through the collation of records from various sources and from the re-survey of known roosts and surveys of potential roost sites. In doing so we have identified two new roost sites for Nathusius pipistrelle in Northern Ireland, but so far none in the Republic of Ireland. All records will be provided to the NBDC and CEDaR. This updated distribution maps consists of 278 records for this species in Ireland, and includes an increasing number in the south, supporting the theory that this species is expanding its range in Ireland (Lundy *et al.,* 2010)

Furthermore, we have improved upon the landscape model produced by Lundy *et al.,* (2011) including a total of 278 records and modifying the model testing procedure to produce a number of landscape suitability models for Nathusius pipistrelle at varying scales with over 90% detection accuracy. In order to make the model comparable to those produced by Lundy *et al.,* (2011) for the Bat Landscape Project, this model will need to be projected across the entire area with the average probability calculated on a 5 km grid and the threshold value equal to the lower 10th percentile of the range used to calculated the land area with a habitat suitability score above this to provide the species' core area. This can be done fairly easily and would make this model available with those for the other species at the NBDC. In this study, however, we tested the finer scale 500m landscape model in an attempt to try the method of catching Nathusius with the harp trap and lure here in Ireland, and to improve our records for this species further. We identified 11 woodland sites to survey using the harp trap and lure and acoustic equipment. Of the twelve nights survey we did not capture any Nathusius pipistrelles. Yet with the SM2 detectors deployed at these same sites overnight we recorded Nathusius at five sites out of 11. Two of these were in sites which previously had no records for this species. While we did not capture Nathusius using the acoustic lure and harp trap methodology, we believe it is a useful method and did capture 71 bats of six species across twelve night's survey. While this method does work it would require a very intensive survey effort to be useful in improving our distributional data for this species given its scarcity. A more appropriate method to identify more records may be instead to use the model to direct roost surveys within highly favourable areas from a list of known soprano or unknown pipistrelle roosts. Since in Northern Ireland the majority of the known roosts are shared with soprano pipistrelles, this could be an effective method for identifying new roost records. This approach could be especially useful in the Republic of Ireland where currently no roost records are known, although, according to the model, areas of highly suitable habitat exists. We have produced a list of potential roost sites which fall within the predicted area of the 10500m scale landscape suitability model. A preliminary survey of some of these sites is to be planned for this coming spring of 2016.

The forth objective of this study was to capture specimens of Nathusius pipistrelle in Ireland and take samples for stable isotope analysis. Recent studies in Europe have shown that stable hydrogen isotope (δ^2H) analysis can help inform on the origin of migratory species (Voigt *et al.,* 2012), and has been used in a recent study in the UK to look for evidence of migration in Nathusius pipstrelles (BSG Ecology, 2014). By comparing the δ^2H pattern in the eighteen specimens captured in Co. Antrim to the expected latitudinal patterns of hydrogen isotopes in rainfall for Northern Ireland we find that all specimens had a signal which fell within the expected signal for that area. Similar signals were found in males and females, and in adults and juveniles. This information, in addition to the observation of large, furred, flying juveniles in late June, and the observation that these bats remain on site into late October (*Pers. obs*.), suggests that Nathusius pipistrelles are resident in Northern Ireland year round. While it is possible that the same individuals return to the site year on year during the summer, and thus give this δ^2H signal, it would mean that the females would have had to migrate fairly early to this maternity site in order to have given birth in May.

The final objective of this study was to utilise molecular genetics to inform the relationship between Irish and other European populations of Nathusius pipistrelle, and to provide insights into the question of migration in Irish populations. The samples collected were to be examined using both mitochondrial and microsatellite markers, each of which can provide different information on the demographic history of populations. Unfortunately, of the 12 microsatellite markers tested for this species, 10 proved not to amplify well or be sufficiently informative to allow population genetic analysis. It is this data that would have provided information on the extent of ongoing gene flow among the sampled populations of Nathusius pipistrelle. Further work and primers more specific to this species will be needed to get good population level data.

We were able to amplify and sequence the hypervariable D-loop of the mitochondrial however, and undertake the first phylogeographic study of this species in Europe. From the 71 individuals sequenced we identified 40 haplotypes, and a network which suggests a recent common ancestor of all individuals, that have either colonised Europe fairly recently, and/or have had continually mixed populations, which may be expected from a migratory species. The maternity colony in Co. Antrim appears to be highly diverse with respect to the mitochondrial D-loop which supports the theory that this species is a recent colonist to Northern Ireland, as proposed by Lundy *et al.,* (2010). However, it is not possible to determine from this data whether or not migration among other European populations is ongoing. Interestingly, mtDNA analysis from the samples collected in England show a similar pattern, while there we do have evidence of two migration events between England and the Netherlands and England and Latvia (Daniel Hargreaves, *pers. comm.*).

CONCLUSIONS

To conclude, the Nathusius pipistrelle is becoming more commonly recorded across Ireland in recent years as the records indicate and along with the work by Lundy *et al.,* (2010), the molecular signal in the mitochondrial DNA supports the theory of recent colonisation to Ireland. According to the habitat suitability modelling there is suitable habitat for this species beyond its current distribution in Ireland, and this model could help us identify more records for this species and potentially maternity colonies. The fact that this species is recorded primarily in summer months in Ireland, and that maternity colonies are known in Northern Ireland tells us that this species is indeed breeding in Ireland. So the question only remains as to whether they are migrating to hibernate elsewhere over the winter months, i.e. is our population resident year round or are some or all of these bats summer migrants. The stable isotope data suggests that the adults were likely present at this same site last year when the previous moulting took place, and gives a hint that these are in fact stable resident populations, or at least faithful migrants to the same colonies.

The conservation priority for this species in Ireland should be to identify maternity colonies in order to protect these summer populations. In order to conclusively answer the migration question we should also be looking for evidence of hibernation by these bats in Ireland and/or actual evidence of bats migrating across the Irish Sea during late autumn and early spring. Perhaps a banding study such as the one ongoing in the UK might be the best way to answer this conclusively or a larger population genetic study which would include the development of Nathusius specific markers.

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APPENDIX 1

List of samples collected and donated from various collaborators across Europe, including ID in this project, collector name(s), date of collection, country, location, sample type, sex if known, survey type and ring no. if the bat is part of a banding study. The bat denoted with the * beside the ring number is an individual re-captured in England having been tagged in Latvia.

ID	Collector	Date	Country	Location	Sample	Sex	Survey	Ring No.
NPCH03	Emma Boston, Mark Smyth	30/06/2015	N. Ireland	Clotworthy House, Antrim	Tissue	Female	Individual	
NPCH05	Emma Boston, Mark Smyth	30/06/2015	N. Ireland	Clotworthy House, Antrim	Tissue	Female	Individual	
NPCH07	Emma Boston, Mark Smyth	30/06/2015	N. Ireland	Clotworthy House, Antrim	Tissue	Female	Individual	
NPCH08	Emma Boston, Mark Smyth	30/06/2015	N. Ireland	Clotworthy House, Antrim	Tissue	Male	Individual	
NPCH09	Emma Boston, Mark Smyth	30/06/2015	N. Ireland	Clotworthy House, Antrim	Tissue	Female	Individual	
NPCH13	Emma Boston, Mark Smyth	30/06/2015	N. Ireland	Clotworthy House, Antrim	Tissue	Female	Individual	
NPCH16	Emma Boston, Mark Smyth	30/06/2015	N. Ireland	Clotworthy House, Antrim	Tissue	Male	Individual	
NPCH20	Emma Boston, Mark Smyth	30/06/2015	N. Ireland	Clotworthy House, Antrim	Tissue	Male	Individual	
NPCH22	Emma Boston, Mark Smyth	30/06/2015	N. Ireland	Clotworthy House, Antrim	Tissue	Female	Individual	
EBNP3	Patty Briggs	20/09/2015	England	Bedfont Lakes Country Park, Middlesex	Faecal		Bat box	
EBNP9	Patty Briggs	11/10/2015	England	Bedfont Lakes Country Park, Middlesex	Faecal		Bat box	
EBNP11	Ksenia Kravchenko	13/08/2015	Ukraine	Kharkiv	Faecal		Individual	
EBNP12	Ksenia Kravchenko	17/08/2015	Ukraine	Kharkiv	Faecal		Individual	
EBNP18	Lyndon Roberts	01/10/2015	Russia	Biological Station Rybachy, Kaliningrad	Faecal		Maternity	
EBNP19	Lyndon Roberts	01/10/2015	Russia	Biological Station Rybachy, Kaliningrad	Faecal		Maternity	
EBNP20	Lyndon Roberts	01/10/2015	Russia	Biological Station Rybachy, Kaliningrad	Faecal		Maternity	
EBNP23	Lyndon Roberts	01/10/2015	Russia	Biological Station Rybachy, Kaliningrad	Faecal		Maternity	
EBNP26	Lyndon Roberts	01/10/2015	Russia	Biological Station Rybachy, Kaliningrad	Faecal		Maternity	
EBNP27	Lyndon Roberts	01/10/2015	Russia	Biological Station Rybachy, Kaliningrad	Faecal		Maternity	
EBNP29	Lyndon Roberts	01/10/2015	Russia	Biological Station Rybachy, Kaliningrad	Faecal		Maternity	
EBNP36	Lyndon Roberts	01/10/2015	Russia	Biological Station Rybachy, Kaliningrad	Faecal		Maternity	
EBNP38	Jane Harris	19/11/2015	England	Salhouse, Norfolk	Faecal		Individual	
EBNP46	Dr Caroline Hillier	01/10/2015	England	Hartlepool, Durham	Faecal	Female	Individual	
EBNP47	Oliver Lindecke	17/09/2015	Germany	Havelberg, Stadtforst, Kastenkontrolle	Faecal	Male	Bat box	O98210
EBNP48	Oliver Lindecke	17/09/2015	Germany	Havelberg, Stadtforst, Kastenkontrolle	Faecal	Male	Bat box	V09112

EBNP49	Oliver Lindecke	17/09/2015	Germany	Havelberg, Stadtforst, Kastenkontrolle	Faecal	Male	Bat box	V09113
EBNP50	Oliver Lindecke	26/08/2015	Latvia	Pape, Rucava	Faecal	Female	Individual	SA2186
EBNP51	Oliver Lindecke	26/08/2015	Latvia	Pape, Rucava	Faecal	Female	Individual	SA2214
EBNP53	Oliver Lindecke	26/08/2015	Latvia	Pape, Rucava	Faecal	Male	Individual	SA2242
EBNP55	Oliver Lindecke	29/08/2015	Latvia	Pape, Rucava	Faecal	Female	Individual	SA2443
EBNP57	Oliver Lindecke	29/08/2015	Latvia	Pape, Rucava	Faecal	Female	Individual	SA2446
EBNP58	Oliver Lindecke	29/08/2015	Latvia	Pape, Rucava	Faecal	Female	Individual	SA2461
EBNP61	Oliver Lindecke	29/08/2015	Latvia	Pape, Rucava	Faecal	Female	Individual	SA2451
EBNP69	Oliver Lindecke	29/08/2015	Latvia	Pape, Rucava	Faecal	Female	Individual	SA2436
EBNP70	Oliver Lindecke	03/09/2015	Latvia	Pape, Rucava	Faecal	Female	Individual	SA2757
EBNP71	Oliver Lindecke	03/09/2015	Latvia	Pape, Rucava	Faecal	Male	Individual	SA2762
EBNP76	Oliver Lindecke	03/09/2015	Latvia	Pape, Rucava	Faecal	Male	Individual	SA2766
EBNP81	Daniel Hargreaves, Sally Ann Hurry, Heidi Cooper-Berry	10/10/2015	England	Rye Harbour Nature reserve, Sussex	Faecal	Female	Individual	H8698
EBNP82	Daniel Hargreaves, Sally Ann Hurry, Heidi Cooper-Berry	10/10/2015	England	Rye Harbour Nature reserve, Sussex	Faecal	Female	Individual	H8700
EBNP84	Daniel Hargreaves, Sally Ann Hurry, Heidi Cooper-Berry	27/09/2015	England	Rye Harbour Nature reserve, Sussex	Faecal	Female	Individual	A7444
EBNP85	Daniel Hargreaves, Sally Ann Hurry, Heidi Cooper-Berry	02/08/2015	England	Rye Harbour Nature reserve, Sussex	Faecal	Male	Individual	
EBNP86	Daniel Hargreaves, Sally Ann Hurry, Heidi Cooper-Berry	27/09/2015	England	Rye Harbour Nature reserve, Sussex	Faecal	Male	Individual	H8625
EBNP87	Daniel Hargreaves, Sally Ann Hurry, Heidi Cooper-Berry	27/09/2015	England	Rye Harbour Nature reserve, Sussex	Faecal	Male	Individual	A7449
EBNP88	Daniel Hargreaves, Sally Ann Hurry, Heidi Cooper-Berry	10/10/2015	England	Rye Harbour Nature reserve, Sussex	Faecal	Male	Individual	H8687
EBNP89	Daniel Hargreaves, Sally Ann Hurry, Heidi Cooper-Berry	27/09/2015	England	Rye Harbour Nature reserve, Sussex	Faecal	Male	Individual	H8627
EBNP90	Daniel Hargreaves, Sally Ann Hurry, Heidi Cooper-Berry	27/09/2015	England	Rye Harbour Nature reserve, Sussex	Faecal	Female	Individual	
EBNP91	Daniel Hargreaves, Sally Ann Hurry, Heidi Cooper-Berry	17/10/2015	England	Rye Harbour Nature reserve, Sussex	Faecal	Female	Individual	H8876
EBNP92	Daniel Hargreaves, Sally Ann Hurry, Heidi Cooper-Berry	27/09/2015	England	Rye Harbour Nature reserve, Sussex	Faecal	Male	Individual	
EBNP93	Daniel Hargreaves, Sally Ann Hurry, Heidi Cooper-Berry	02/08/2015	England	Rye Harbour Nature reserve, Sussex	Faecal	Male	Individual	
EBNP94	Daniel Hargreaves, Sally Ann Hurry, Heidi Cooper-Berry	02/08/2015	England	Rye Harbour Nature reserve, Sussex	Faecal	Male	Individual	
EBNP95	Daniel Hargreaves, Sally Ann Hurry, Heidi Cooper-Berry	27/09/2015	England	Rye Harbour Nature reserve, Sussex	Faecal	Male	Individual	A7446
EBNP98	Daniel Hargreaves, Sally Ann Hurry, Heidi Cooper-Berry	05/09/2015	England	Rye Harbour Nature reserve, Sussex	Faecal	Male	Individual	H8684
EBNP99	Daniel Hargreaves, Sally Ann Hurry, Heidi Cooper-Berry	27/09/2015	England	Rye Harbour Nature reserve, Sussex	Faecal	Female	Individual	A7443
EBNP100	Daniel Hargreaves, Sally Ann Hurry, Heidi Cooper-Berry	10/10/2015	England	Rye Harbour Nature reserve, Sussex	Faecal	Female	Individual	H8692
EBNP101	Daniel Hargreaves, Sally Ann Hurry, Heidi Cooper-Berry	05/09/2015	England	Rye Harbour Nature reserve, Sussex	Faecal	Male	Individual	

EBNP102	Daniel Hargreaves, Sally Ann Hurry, Heidi Cooper-Berry	10/10/2015	England	Rye Harbour Nature reserve, Sussex	Faecal	Male	Individual	SA4722*
EBNP104	Daniel Hargreaves, Sally Ann Hurry, Heidi Cooper-Berry	27/09/2015	England	Rye Harbour Nature reserve, Sussex	Faecal	Male	Individual	H8628
EBNP105	Daniel Hargreaves, Sally Ann Hurry, Heidi Cooper-Berry	10/10/2015	England	Rye Harbour Nature reserve, Sussex	Faecal	Male	Individual	H8694
MF 73	Marcus Fritze, Bernd Ohlendorf and colleagues	09/07/2015	Germany	Cheine, Cheiner Moor, Kastenkontrolle	Tissue	Female	Individual	O 94949
MF 74	Marcus Fritze, Bernd Ohlendorf and colleagues	09/07/2015	Germany	Cheine, Cheiner Moor, Kastenkontrolle	Tissue	Female	Individual	O 64799
MF 80	Marcus Fritze, Bernd Ohlendorf and colleagues	09/07/2015	Germany	Cheine, Cheiner Moor, Kastenkontrolle	Tissue	Female	Individual	O 94274
MF 95	Marcus Fritze, Bernd Ohlendorf and colleagues	10/07/2015	Germany	Jederitz, NSG Jederitzer Forst, Kastenkontrolle	Tissue	Female	Individual	V 07665
MF56	Marcus Fritze, Bernd Ohlendorf and colleagues	18/07/2015	Germany	Havelberg, Stadtforst, Kastenkontrolle	Tissue	Male	Individual	O 71675
MF 69	Marcus Fritze, Bernd Ohlendorf and colleagues	27/06/2015	Germany	Helme-Stausee Kelbra, Krautwehr	Tissue	Female	Individual	V 07556
MF 65	Marcus Fritze, Bernd Ohlendorf and colleagues	27/06/2015	Germany	Helme-Stausee Kelbra, Krautwehr	Tissue	Female	Individual	V 07558
MF 67	Marcus Fritze, Bernd Ohlendorf and colleagues	27/06/2015	Germany	Helme-Stausee Kelbra, Krautwehr	Tissue	Female	Individual	V 07559
MF1	Marcus Fritze	01/05/2015	Germany	Kiesgrube Berga	Tissue	Female	Individual	
MF2	Marcus Fritze	01/05/2015	Germany	Kiesgrube Berga	Tissue	Female	Individual	
MF47	Marcus Fritze, Janine Dombrowski	01/08/2015	Germany	Samtens Kastenrevier, Rügen	Faecal	Male	Bat box	
MF46	Marcus Fritze, Janine Dombrowski	01/08/2015	Germany	Samtens Kastenrevier, Rügen	Faecal	Male	Bat box	
MF44	Marcus Fritze, Janine Dombrowski	01/08/2015	Germany	Samtens Kastenrevier, Rügen	Faecal		Bat box	