Section E: Supporting information for derogation licence

GREENBAT: MIGRATORY BATS AND OFFSHORE WIND ENERGY IN IRELAND

DR. SAMANTHA BALL

1. Table of Contents

Information

Title: GREENBAT: Migratory bats and offshore wind energy in Ireland

Project holders:
Dr. Samantha Ball and Dr. Allan McDevitt

Research Institute:
Marine and Freshwater Research Centre, Atlantic Technological University

Project Dates:
March 2025- November 2027

Project Funders:
Sustainable Energy Authority of Ireland (SEAI) and the National Parks and Wildlife Service (NPWS).

Species:
Nathusius' pipistrelles (*Pipistrellus nathusii*) and Leisler's bat (*Nyctalus leisleri*)

No of individuals: 256 (128 of each species)

2. Introduction

A. Objectives:

The overall aim of this project is to identify whether offshore windfarms (OWF) are a potential threat to migratory bat species in Ireland. In order to first address this question, it needs to be established whether or not Irish bat species are migrating in and out of Ireland in the first place. This will be achieved by collecting tissue and hair samples from current Irish populations of Nathusius' pipistrelles and Leisler's bats for the following objectives:

- *Method 1; Genomics*
  Using tissue samples collected from wing punches from Nathusius' pipistrelles and Leisler's bats, we will use double digest restriction-site associated DNA (ddRAD) sequencing for the detection and genotyping of thousands of single nucleotide polymorphisms (SNPs). This will allow us to determine the genomic structure of bat populations in each region (Ireland, UK, continental Europe) to identify potential gene flow/migration between the regions.

- *Method 2; Stable Isotope Analysis*
  Using hair samples collected by snipping a small number of hairs from the backs of Nathusius' pipistrelles and Leisler's bats, stable isotope analyses for five stable isotopes will be carried out, focusing on carbon, nitrogen, sulphur, hydrogen and oxygen, as complementary isotopic tracers. This will allow us to understand the origin of Irish individuals and to identify potential migration events.

B. Personnel:

Dr. Samantha Ball (Applicant):

BSc (Hons) Zoology, MSc Conservation and Biodiversity, PhD Ecology. Experience handling a range of wildlife including hares, badgers, sea birds, passerine birds, land crabs, sea turtles. The licence applicant will be assisted and trained by experienced bat handlers, including Dr. Emma Boston and Dr. Tina Aughney.

Dr. Emma Boston:

BSc (Hons), PhD; Emma has over 20 years' experience in capturing and handling bats using a variety of methods including a hand net, mist net and harp trap. Emma has held various licences to take wing biopsies from bats and to radio-track bats for the purposes of research.

Dr. Tina Aughney:

BSc (Hons) Environmental Science, PhD; Tina has worked as a Bat Specialist since 2000 and has undertaken extensive survey work for all Irish bat species. Tina has previously held licences for bat research here in Ireland.

C. Qualifications:

This work will be carried out to collect DNA and hair samples from two species of bats in Ireland. While this work does not constitute as surveys as such, trapped/ captured bats (harp traps, mist nets, hand nets or removed directly from bat boxes by hand) will be subject to individual disturbance.

Dr. Samantha Ball, lead applicant, has a BSc (Hons) Zoology, MSc Conservation and Biodiversity, PhD Ecology. Dr. Ball has also completed the LAST wildlife training course, the CIEEM '*Bat Ecology and Survey'* training course and the Bat Conservation Ireland '*Bat Identification and Survey Methods: Beginner to Intermediate Level'* training course.

Dr. Emma Boston has a BSc (Hons), PhD (Ecology). Dr. Boston is experienced in all sampling techniques being carried out in the GREENBAT project and has published several peer-reviewed scientific papers resulting from utilising these techniques. Dr. Boston has over 20 years' experience working with bats and is a leading Irish bat expert.

Dr. Tina Aughney has a BSc (Hons) Environmental Science and a PhD (Ecology). Dr Aughney is an experienced bat handler who teaches Bat Conservation Ireland's bat handling course and has worked as a bat specialist for over 25 years. Dr. Aughney is a leading bat expert in Ireland.

3. Background

In order to understand whether Irish bat species are migrating, biological samples (hair and tissue) need to be sourced from extant, wild Irish populations. As it is not possible to gain sufficient DNA from bat faecal samples for downstream genomics applications, the collection of tissue samples is necessary. Likewise, stable isotope ratios can vary across space due to variables such as rainfall patterns and

ambient temperature and the body tissue or hair/fur of animals are composed of the elements that animals consume (e.g. through food and water). Therefore, hair samples need to be collected for these analysis and will be comparable with other studies conducted elsewhere (Kruszynski et al. 2021, 2022).

At present, it is not possible to specify the exact locations where samples will be collected, as near-national coverage is required- particularly for Leisler's bats, which are widely distributed across Ireland. This species is known to switch roosts after occupying one for several days; therefore, sampling will be opportunistic and limited to sites where Section 23 and Section 34 licences have been granted to field experts Dr. Tina Aughney and Dr. Emma Boston. Although Leisler's bats are widespread, only *n = XX* roosts have been documented in Ireland, and capture efforts will focus on areas in proximity to these known roosts. Please refer to Figure 1 for the distribution of Leisler's bat roosts, as recorded by Bat Conservation Ireland as catching will take place in appropriate locations in proximity to these areas.

Nathusius' pipistrelles are becoming increasingly widespread in Ireland, though only a small number of roosts have been identified to date. Figure 1 illustrates the locations of these known roosts. Capture efforts will similarly target areas near these sites. Notably, one area known to be a hotspot for the species occurs at Killeshandra Lake, Co. Cavan, which will be a primary focus for Nathusius' pipistrelle sampling.

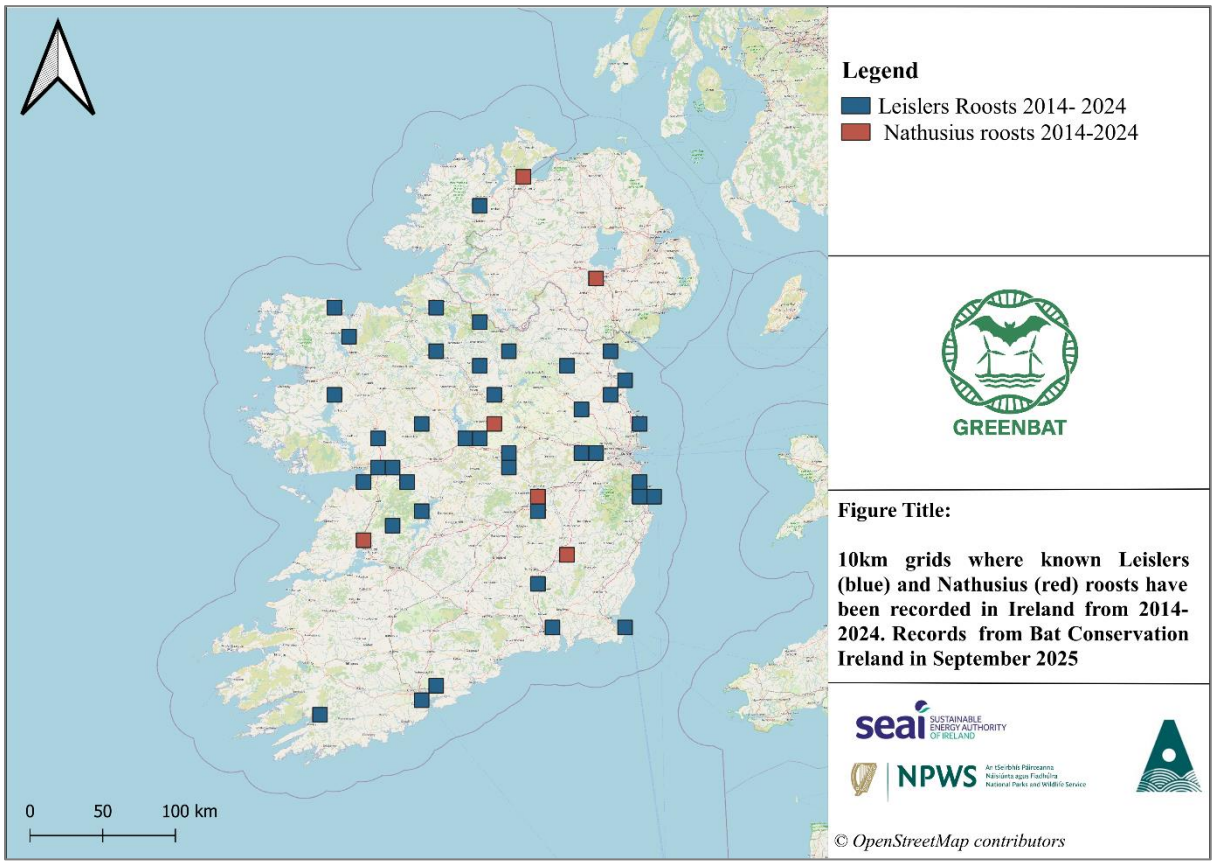

Figure 1. National grids (10km) where known Leislers (blue) and Nathusius (red) roosts have been recorded in Ireland from 2014- 2024. Records were obtained from Bat Conservation Ireland (BCI) in September 2025. Grids are being provided instead of absolute locations due data sharing agreements between ATU and BCI

4. Details of proposed activity

Bats (*Pipistrellus nathusii* and *Nyctalus leisleri*) will be captured using one of the following methods, requiring a derogation licence:

1. **Mist netting** is a widely used method for capturing bats for research purposes, whereby bats fly into nets set up along a flight path and become entangled. Mist nets will be setup to a height of 2.5-3m at dusk (Bat Conservation Ireland) when bats are most active and will be positioned perpendicular to flight paths to maximise capture rates.
2. **Harp trapping** is a widely used method for capturing bats for research purposes, whereby bats drop into a soft, canvas bag after coming into contact with '*vertically oriented banks of fine monofilament line'* (Institutional Animal Care and Use Committee 2023). Captured bats then crawl up the side of the bag where they are visible to the catchers, and rest in a seam. The use of harp traps is recommended in locations where high volumes of bats are expected (e.g., in proximity to roosts), as it is not required to untangle bats from nets. Harp traps are also recommended in areas where the flight corridor is small in size (e.g., forestry break). Therefore, harp traps will be utilised in areas where a high number of bats are expected.
3. **Hand net trapping** of bats is a standard, widely used practice to capture bats as they emerge from a known roost or bat box. Soft nets on extendable poles are placed over the exit site of a roost or bat box and bats enter the nets as they emerge at dusk. Bats are removed from the nets by hand, processed and released. This method is to be used at the time of emergence only and not for bats re-entering the roost/ bat box.
4. **Removing bats by hand** from artificial bat boxes during hours of daylight to allow for monitoring and sampling is a standard practice, utilised by several national organisations and researchers (e.g., Schaik et al. 2025). This method has the advantage that no traps (e.g., nets) are required for the capturing of bats, limiting opportunities for stress and injury. Artificial bat boxes of known occupancy will be accessed via ladder and opened to access the resting bats inside. Each bat will then be removed, one by one, using gloved hands for processing. Once samples and biometric data have been collected, the bat will be returned directly inside the bat box to prevent further disturbance and daytime flying.

All mist netting, harp trapping, hand netting and removal from bat boxes and subsequent handling will be overseen by, an external licensed, experienced professional until the point when the project lead is deemed suitably trained by said professional. This will include, but may not be limited to, Bat Eco Services (Bat Eco Services - Profile, Wildlife Surveys, Bat Surveys and Ecological Consultants Ireland), Scott Cawley Limited (Scott Cawley), NPWS rangers and Dr. Emma Boston.

Bats captured using one of the above methodologies will be bought forward for sampling if deemed appropriate (i.e., >6g in weight for *Pipistrellus nathusii* and >12g for *Nyctalus leisleri).* Both hair and tissue samples will be collected, outlined below:

1. To allow for stable isotope analysis, a small sample of hairs will be sampled from the back of the bat or between the shoulder blades to prevent unnecessary discomfort, to the size of 0.25 cm^2 (Kosonen 2013). This will be achieved using a small pair of scissors to gently remove hairs without stretching, pulling or snagging the skin. As we assume a minimum requirement of 0.3mg of hair for each element for SIA (Pylant et al. 2014) and as we intend on investigating five stable isotopes (carbon, nitrogen, sulphur, hydrogen and oxygen), a small tuft of hair consisting of c.10 hairs will be removed from each individual.

2. To allow for genomic analysis of whether our Irish bats are migrating, a tissue sample in the form of a wing biopsy will be taken. To facilitate this, an area of the wing membrane close to the body wall, avoiding veins, bones, and major arteries will be identified. A sterile biopsy tool (3mm) will then be used to collect the tissue sample by pressing the tool firmly on the identified part of the wing and twisting gently to detach the tissue from the wing.

Catching, handling and sampling will take place in proximity to any of the known *Pipistrellus nathusii* and/ or *Nyctalus leisleri* roosts, as recorded by Bat Conservation Ireland. These locations are highlighted at a 10km grid square resolution in Figure 1. For this reason, site plans can not be provided. Furthermore, strict operating procedures have been developed and will be followed in the interest of the bats comfort and safety.

5. Ecological survey and site assessment

NA- Not applicable to this application.

6. Evidence to support the derogation tests

Test 1:

In order to understand whether Irish bat species are migrating, biological samples (hair and tissue) need to be sourced from extant, wild Irish populations. As it is not possible to gain sufficient DNA from bat faecal samples for downstream genomics applications, the collection of tissue samples is necessary. Likewise, stable isotope ratios can vary across space due to variables such as rainfall patterns and ambient temperature, and the body tissue or hair/fur of animals are composed of the elements that animals consume (e.g. through food and water). Therefore, hair samples need to be collected for these analysis and will be comparable with other studies conducted elsewhere (Kruszynski et al. 2021, 2022). Therefore, as no alternative to sample collection from extant bats is available, a derogation license is required.

Test 2:

Below highlights the alternative options considered as part of the project, including a 'do-nothing' approach

- **'Do nothing':** If the research doesn't go ahead, then we will have a gap in our knowledge on bat migration in an Irish context. This information is essential to inform the design of offshore wind farms which is particularly relevant given the offshore windfarm developments currently proposed for the east and southeast coasts in Ireland.
- **Opportunistic sampling from cadavers:** This project will be using tissue samples for genomic based applications (ddRAD sequencing). To ensure accurate data, high quality DNA needs to be obtained from fresh samples in as many instances as possible. This is particularly important given the innovative use of ddRAD sequencing to identify migration potential in our target species. Furthermore, the short duration of time for the project whereby 2 years are remaining, means that relying on the opportunistic sampling from fresh cadavers is unlikely to yield the sample size required to detect migration (estimated at n= 128 per species).
- **Use of non-invasively collected faecal material:** Given the current level of knowledge on bat migration and the genomics of migratory species in Ireland, it is not yet possible to conduct genomic level research using non-invasive samples (i.e., faecal material). Dr. Emma Teeling, a

bat and molecular ecology specialist, was consulted (spring 2024) to assess the feasibility of obtaining sufficient DNA from bat faecal samples for downstream genomics applications, as a replacement to tissue samples. However, due to the lower quality and quantity of DNA typically extracted from faecal material, it was determined that this approach would not be suitable for our current study.

As the EC Guidance states in paragraph 3-61: *"derogations must be limited in time, place, numbers of specimens involved….."* a power analysis was conducted by the lead applicant (Dr. Ball) using the R package 'pwr' to determine the number of individual bats that would need to be sampled from to identify genomic clustering of the population. As the genomics of Irish populations of these species have not been studied, it is not known how many distinct genomic clusters (k) are present in the population. However, a similar study using ddRAD sequencing on barbastelle bats (Razgour et al. 2023) found two distinct genomic clusters within the UK. Therefore, data were simulated with 1-3 distinct genomic clusters to estimate the sample size needed for the present study. Assuming a medium effect size (f) of 0.25 and a significance level of 0.05, it was identified that one and two clusters (k=1, k=2) would require n=128 individual bats. For three distinct clusters, n=157 bats would be needed. A recent study (Schaik et al. 2025) on Nathusius pipistrelles in the Netherlands belongs to a single panmictic population, meaning there is no significant genetic sub-structuring. This suggests that the population is genetically diverse but not divided into distinct clusters. A study on Leisler bats in Ireland found two distinct genetic lineages (Boston et al. 2015). However, genomic clustering for both species remains unknown. Given the low genetic clustering of the aforementioned studies, we recommend sampling enough individuals to account for two clusters (k=2) for each species. This would amount to 128 bats of each species.

Test 3:

We will not be permanently removing, or causing any lasting damage, to any individuals in the population. All bats will be trapped, handled and have a tissue/ hair sample collected prior to being released back to the wild in the capture location. We do not expect there to be any mortalities recorded as part of this work with capture methods such as Harp traps generally considered to be safe for catching bats with a 0.3% mortality rate previously recorded (from >12,000 bats; Lemckert et al. 2006). There are no known published data to the project holders for injury rates relating to mist netting in microbats. The closest data that we have is for birds, where an average injury rate of 0.59%, and average mortality rate of 0.23% has been reported (Spotswood et al. 2011).

Bats can rapidly heal wounds in their wing membranes, which often naturally occur. Studies have demonstrated that wounds caused by wing punch biopsy are closed within 16 (Weaver et al. 2009) to 34 days (Pollock et al. 2016) of the sample being taken. Hair sample collection is expected to have no impact on the bats.

All capturing, handling and sampling will be overseen by a licensed, trained bat expert, until the point that the applicant is deemed to be competent.

7. Monitoring the impacts of the derogations

Section 3.4 of the Guidance document on the strict protection of animal species under the Habitats Directive states that national authorities must '*measure the impact of derogations (and the effectiveness*

of any compensation measures)'. In this case, the derogation supports research into the potential migration of Irish bat species in relation to offshore wind farm activity. As no compensation measures are in place, monitoring will rely entirely on research outputs.

Data will be generated from samples collected under this derogation using ddRAD sequencing (tissue) and stable isotope analysis (hair). These datasets will be analysed to detect evidence of migration by comparing Irish samples with those from collaborators in the UK and continental Europe. This analysis will serve as the monitoring mechanism to confirm whether the derogation was implemented correctly and achieved its objectives. Data will be generated and processed using the following workflows:
For the genomics component (ddRAD sequencing):

- Population structure will be assessed using Principal Component Analysis (PCA) and/or Discriminant Analysis of Principal Components (DAPC) to identify discriminant axes that maximise variation between regions while minimising within-region variation (Razgour et al. 2024)
- Admixture analysis using Bayesian clustering will identify potential migrants within each population (Dufresnes et al. 2023).

For the stable isotope analysis (SIA):

- Isotope ratios will act as environmental tracers to infer geographic origin based on isoscape models.
- The R package assignR (Ma et al. 2020) will predict sample origins by comparing observed isotope ratios with known isoscapes, indicating likely migration patterns in Irish bat populations.

The results will be disseminated through scientific publications in peer reviewed journals and a report for the NPWS and Sustainable Energy Authority of Ireland (SEAI).

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