

Supporting Information for natterjack toad (*Epidalea calamita*) derogation 2026

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1. Introduction

1.1. Objective of the Proposed Works

The objective of this activity is to conduct a comprehensive assessment of the population biology and conservation status of the regionally endangered natterjack toad (*Epidalea calamita*) in Ireland. This project, funded by the NPWS, serves to directly support statutory reporting under the EU Article 17 framework and to provide data for my PhD.

The specific field objectives include:

- **Range-wide Monitoring:** Quantifying annual breeding effort across the species' entire natural Irish range (approximately 200 ponds) using standardised egg-string counts.
- **Health Surveillance:** Screening Irish populations of natterjack toads, common frogs (*Rana temporaria*), and smooth newts (*Lissotriton vulgaris*) for emerging infectious diseases, specifically chytridiomycosis (*Bd* and *Bsal*) and ranaviruses, via dermal swabbing.
- **Microbiome Characterization:** Collecting dermal swabs from both wild and captive-reared toadlets (from Fota Wildlife Park and Dingle Aquarium) to evaluate the impact of head-starting on skin microbial diversity and post-release resilience.
- **Method Validation:** Calibrating novel, non-invasive monitoring tools, including Passive Acoustic Monitoring (PAM) and camera-trapping, against traditional spawn survey data to provide scalable proxies for population size and breeding phenology.
- **Intervention Evaluation:** Utilizing pond-water eDNA sampling at translocation and head-start release sites to determine the long-term occupancy and success of these conservation measures.

1.2. The following personnel are responsible for the execution of the proposed field activities:

- **Rachel Haynes (Derogation applicant):** 2nd Year PhD Candidate (QUB). MRes in Amphibian Extinction Risks. Experienced in amphibian handling, swabbing, and surveying (Ex-Zookeeper, worked on various amphibian reintroductions; previous 2025 derogation holder).
- **Dr. Neil Reid (Lead supervisor):** Senior Lecturer in Conservation Biology (QUB). Expert in evidence-based conservation and statistical sampling design.
- **Dr. Marina Reyne (Supervisor):** Research Fellow (QUB). Leading authority on Irish natterjack toads; author of *Irish Wildlife Manual No. 107*.
- **Dr. Ferdia Marnell (Supervisor):** Senior Scientist (NPWS). Lead amphibian specialist for the Irish Government.
- **Field Assistants:** Four 2nd-year Wildlife Biology BSc students (Kai Cochrane, Clara Cummins, Ríoghnach Timmins and Liam Ryan) from Munster Technological University (MTU) will assist under the direct supervision of Rachel Haynes and Dr. Neil Reid. All students will undergo rigorous, minimally invasive handling and biosecurity training prior to field commencement.

2. Background to proposed activity

2.1. Location and Ownership

The proposed activities will take place across the entire natural Irish range of the natterjack toad (*Epidaeia calamita*). Fieldwork will take place in County Kerry, where populations are restricted to coastal dune and grassland habitats.

- Survey Sites: A total of 172 ponds across 23 sites were monitored in previous seasons (2024–2025) and will continue to be the focus of the 2026 activities.
- Ownership: The study area encompasses a mosaic of publicly managed lands (Special Areas of Conservation) and private agricultural holdings. Access to private sites is facilitated by the NPWS Pond Creation Scheme, designed to maintain breeding ponds through landowner cooperation.

2.2. Type of and Need for the Proposed Activity

This activity is a specialised conservation research and statutory monitoring program. Despite decades of intervention, Irish natterjack populations continue to decline in both range and reproductive output.

The need for this activity:

1. Statutory Obligation: The project directly supports Article 17 framework reporting and provides the evidence base for the next NPWS Irish Wildlife Manual.
2. Pathogen Surveillance: There is a critical need for systematic screening of emerging infectious diseases (EIDs), including Chytridiomycosis (Bd and Bsal) and Ranaviruses, which have not been comprehensively assessed in the species' core Irish range.
3. Conservation Optimization: This research addresses a significant knowledge gap regarding the skin microbiome of wild vs. captive-reared toads. Understanding these microbial communities is essential for improving the success of the long-standing head-starting and translocation programs.

2.3. Planning History and policy context

The proposed activities consist of biological recording, non-invasive acoustic monitoring, and minimally invasive dermal swabbing. These actions do not involve any land alteration, construction, or infrastructural development. As such, the project does not require planning permission, and there is no relevant planning history of development at the survey locations.

This work is firmly situated within the following policy frameworks:

- EU Habitats Directive (92/43/EEC): Specifically, Article 12 (Strict Protection) and Article 16 (Derogations) for Annex IV species.
- National Biodiversity Action Plan: Directly addressing the need for evidence-based management of Ireland's rarest amphibian.
- Irish Wildlife Manuals: The methodology builds upon and updates the standardised protocols established in previous cycles.

2.4. Zoning in Relevant Development Plans

The study areas are located within zones designated for 'Natural Heritage,' 'Conservation,' or 'Agriculture' in the Kerry County Development Plans. The proposed scientific activities, consisting of seasonal pond visits and non-destructive sampling, are fully compatible with these zonings and the conservation objectives of the overlapping SAC designations.

3. Full details of proposed activity to be covered by the derogation

3.1. Method Statement and Monitoring Framework

The proposed activities consist of a multi-methodological approach designed to quantify the contemporary conservation status of the natterjack toad across its natural Irish range. The fieldwork is structured into several core components:

3.1.1. Range-wide Breeding Monitoring

- **Scope:** Approximately 200 ponds across the species' entire natural Irish range will be surveyed.
- **Frequency:** Each pond is visited every 7-10 days between late March and the end of July.
- **Protocol:** Surveyors conduct visual counts of egg strings to quantify annual breeding effort. These standardised counts serve as the primary proxy for population size and reproductive output.

3.1.2. Disease Surveillance and Microbiome Characterization

- **Target Species:** Natterjack toads (*Epidalea calamita*), common frogs (*Rana temporaria*), and smooth newts (*Lissotriton vulgaris*).
- **Procedure:** Individuals are temporarily captured for dermal swabbing. Swabs are analyzed via qPCR to screen for chytridiomycosis (*Bd* and *Bsal*) and ranaviruses.
- **Microbiome Research:** Dermal swabs are also used for 16S rRNA metabarcoding to characterize the skin bacterial communities of both wild and captive-reared individuals.

3.1.3. Evaluation of Conservation Interventions

- **eDNA Sampling:** Water samples are collected from ponds that have previously received translocations or head-started releases.
- **Protocol:** Quantitative eDNA (qPCR) assays are applied to detect the presence of natterjack toads, particularly sub-adults or non-breeding adults that are often missed by traditional spawn surveys.

3.1.4. Remote Sensing and Novel Tool Validation

- **Passive Acoustic Monitoring (PAM):** Autonomous recording devices are deployed to capture nocturnal calling activity patterns.
- **Camera Trapping:** Cameras are placed on poles in the centre of breeding ponds to provide continuous visual confirmation of calling toads.
- **Calibration:** These remote-sensing metrics are calibrated against manual egg-string counts to validate their use as scalable, non-invasive monitoring tools.

3.1.5. Environmental Assessment

- **Water Chemistry:** To account for environmental drivers of population trends, measures of pond nutrification (nitrite, nitrate, phosphorus, and ammonia) are recorded twice per season (mid-season and end-season).

3.2. Site Plan and Extent

- **Geographic Extent:** The project covers 172 previously surveyed ponds, mapped on Survey123 (Fig. 1), plus the newly created natterjack ponds, as advised by Ferdia Marnell.

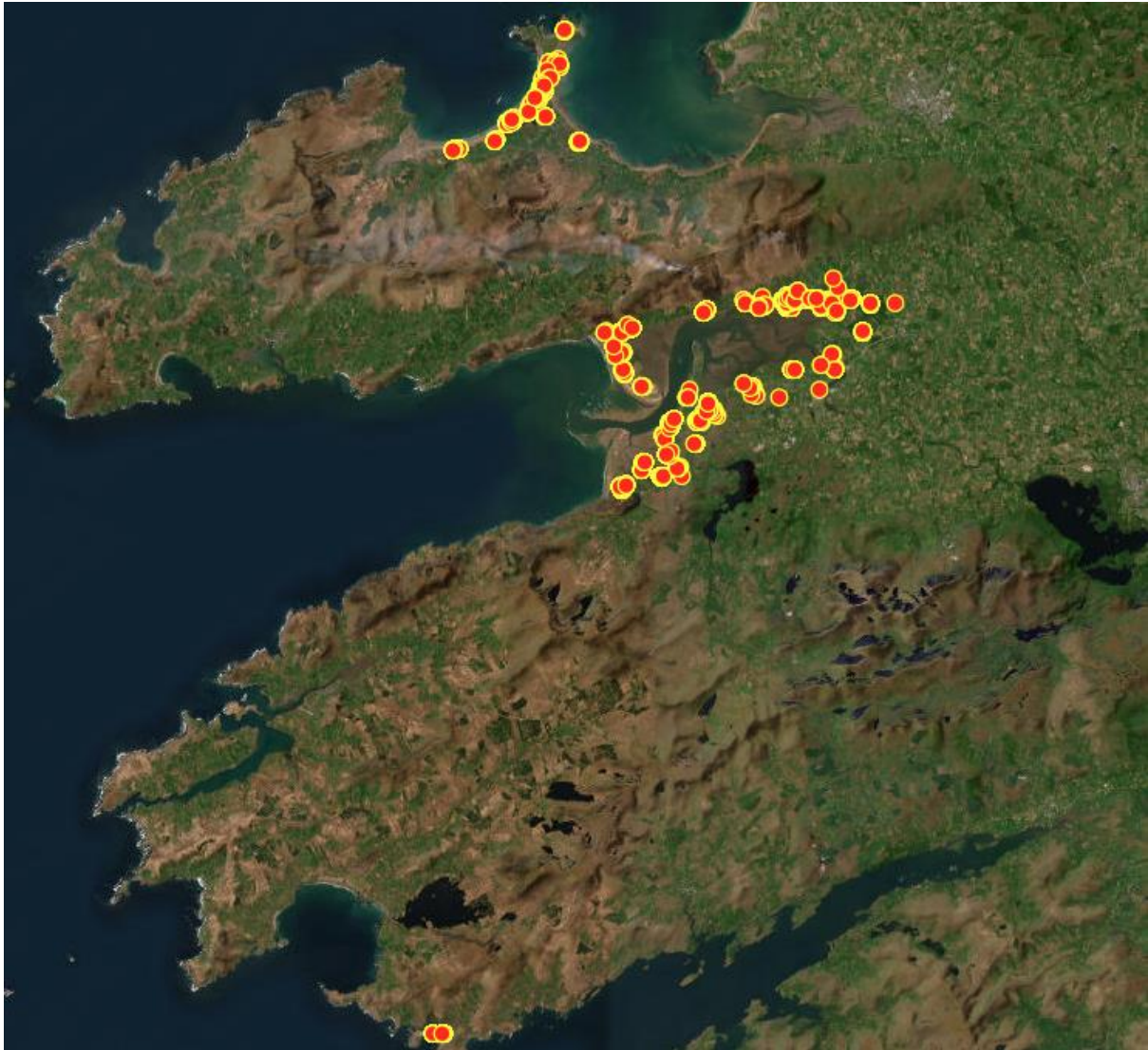


Fig. 1 Survey123 map export of the distribution of ponds monitored for Natterjack toads in 2024 and 2025.

4. Ecological Survey and site assessment (given due to need for handling and swabbing)

4.1. Pre-existing information on species and local status

The natterjack toad (*Epidalea calamita*) is Ireland's rarest amphibian, restricted almost entirely to the coastal areas of County Kerry, with a small translocated population in Co. Wexford. Historical data from the last two decades (Sweeney et al. 2013; Reyne et al. 2019) indicates a population under significant pressure. While pond creation schemes have increased the number of potential breeding sites, occupancy remains inconsistent, and the species is classified as IUCN Regionally Endangered in Ireland.

Current data suggests that while the species is maintained at a favourable status (Article 17 Report, 2019) due to range stability, the actual breeding effort is in decline. In 2024, surveys identified a concerning trend of low egg-string counts relative to the number of available ponds. The population is considered highly vulnerable to stochastic events, habitat succession, and potential disease outbreaks (Chytrid/Ranavirus).

4.2. Objectives of survey

1. **Demographic Assessment:** To quantify the 2026 breeding effort and estimate adult population size through sex-ratio determination.
2. **Health Baseline:** To establish the first comprehensive pathogen baseline (Bd, Bsal, Ranavirus) for the Irish natterjack range.
3. **Conservation Validation:** To evaluate the success of the NPWS head-starting programme by comparing the skin microbiomes of captive-reared vs. wild toadlets.
4. **Technological Calibration:** To validate Passive Acoustic Monitoring (PAM) and eDNA as scalable, lower-disturbance monitoring alternatives.

4.3. Methodology of Survey

The survey area encompasses 23 distinct sites across the natural range, with the largest metapopulation found in Maharees peninsula. The habitat consists of fixed dunes, slack systems, and coastal grazing marshes. A total of 172 specific ponds (both natural and artificial) have been identified for the 2026 cycle.

Protocol: Methodology follows the standardized national protocols in Irish Wildlife Manual No. 107 (Reyne et al. 2019).

- **Timing:** Surveys are conducted between March and July, primarily during daylight for spawn counts. Deployment and collection of acoustic devices and camera traps will also take place during the day, despite data being recorded overnight.
- **Equipment:** Sterile Rayon-tipped swabs, powder-free nitrile gloves, Survey123 for digital data capture, and Song Meter Micro (PAM) units.
- **Uncertainties:** High inter-annual variability in rainfall affects pond hydroperiod and toad detectability. This is mitigated by the high frequency of visits (every 7–10 days).

4.4. Survey Results (2024-2025 Summary)

- **2024:** 172 ponds surveyed; 23 sites covered.
- **2025:** Maintained consistent monitoring across the same range under Permit DER-NATTERJACK TOAD-2025-02.

- **Findings:** Preliminary results suggest that while the range is stable, breeding effort is concentrated in a small subset of high-quality ponds, reinforcing the need for the targeted microbiome and water chemistry research proposed for 2026.

4.5. Population Size Class Assessment

Based on previous cycles, the Irish population is considered 'Small to Moderate' but fragmented. Most sites support fewer than 50 breeding females annually. By determining the sex ratio of handled individuals in 2026, this project will move beyond egg-string counts to provide a more robust estimate of total adult biomass and effective population size.

5. Evidence to support the Derogation Tests

5.1. Test 1: Reason for Derogation

The application relies on Regulation 54(2)(a) (Interests of protecting wild flora and fauna) and 54(2)(d) (Purpose of research, education, and re-introduction).

- **Evidence for 54(2)(a):** The Natterjack toad is Regionally Endangered. Current evidence (Reyne et al. 2021) suggests long-standing conservation actions have failed to halt declines. This activity provides the diagnostic data (specifically pathogen screening and microbiome analysis) required to detect and mitigate the species from catastrophic disease outbreaks (e.g., *Bd* or *Bsal*), which have devastated populations globally but remain uncharacterized in the Irish range.
- **Evidence for 54(2)(d):** The activities are part of a structured PhD research program (QUB/NPWS) aimed at optimizing the head-starting and translocation programmes. Swabbing and sexing are fundamental to determining the health and demographic viability of these re-introduction efforts.

5.2. Test 2: Absence of Alternative Solutions

Following the 2025 Guidance (Section 3.2), the following alternatives were considered and rejected as unsatisfactory:

Alternative	Impact	Reason for Rejection
Do nothing	Neutral (short-term) / Negative (long-term)	Fails to meet statutory goals. Without 2026 data, Ireland cannot fulfil Article 17 reporting. Furthermore, an undetected disease outbreak could cause a population collapse that would remain unnoticed without active surveillance.
Visual-only Survey (No handling)	Low Disturbance	Scientifically insufficient. Visual surveys cannot confirm sex in natterjacks (vocal sacs are not visible from above) and cannot detect sub-clinical pathogens. This would result in inaccurate population models and a lack of biosecurity data.
eDNA-only Surveillance	Very Low Disturbance	Limited resolution. While eDNA can detect presence/absence, it cannot characterize the individual-level microbiome or provide the high-resolution pathogen load data necessary to evaluate the health of head-started individuals post-release or distinguish between life stages and sex ratios.

To conclude, capture and swabbing is the only technically feasible way to achieve the research objectives while maintaining the standards set by the *Irish Wildlife Manual*.

5.3. Test 3: Impact of a Derogation on Conservation Status

The impact on the population is deemed to be net positive.

- **Geographic scale:** The impact is localised to individuals at 23 sites. Given the 2019 Favourable status, the temporary handling of ~300 individuals represents a negligible risk to the national population.
- **Pre-mitigation impact:** Potential temporary physiological stress and a theoretical risk of cross-contamination of pathogens.

- **Mitigation measures:**
 1. **Aseptic technique:** Use of fresh, powder-free nitrile gloves for every individual and sterile Rayon-tipped swabs.
 2. **Handling efficiency:** Total handling time per individual will be restricted to **<2 minutes** to minimise stress.
 3. **Training:** All field assistants (MTU students) will be supervised by the applicant (experienced in amphibian handling/zookeeping) to ensure Best Practice as per EAZA Guidelines (2025).
- **Post-mitigation impact:** The net effect is Positive. The data gathered will directly inform the NPWS on how to improve pond management and head-starting protocols, ensuring the long-term maintenance of Favourable Conservation Status.

The success of these specific mitigation measures is well-documented; identical biosecurity and handling protocols have been used in Irish natterjack monitoring cycles since 2011 (Sweeney et al. 2013; Reyne et al. 2019) with no evidence of handling-induced mortality or pathogen spread recorded.

6. Monitoring the impacts of the derogations

To ensure the derogation is implemented correctly and the conservation objectives are achieved without detrimental impact, the following monitoring and verification measures will be employed:

6.1. Procedural Verification and Quality Control

- **Field Logs:** A standardized digital log (via Survey123) will be maintained for every individual handled. This includes recording the exact time of capture and release to verify that the <2-minute handling limit is strictly adhered to.
- **Supervisory Audits:** Dr. Neil Reid and/or Dr. Marina Reyne will conduct periodic field site visits to audit the handling and swabbing techniques of the applicant and the MTU placement students. This ensures consistent application of the EAZA Best Practice Guidelines (Pedersen et al 2025) and biosecurity protocols.
- **Equipment Checks:** Daily inspections of PAM units and camera traps will ensure data integrity and verify that equipment placement does not interfere with the natural movement or breeding behaviour of the toads.

6.2. Scientific Evidence of Objective Achievement

- **Data Validation:** The success of the Research and Education objective will be measured by the successful generation of a pathogen baseline (qPCR results) and the characterization of the skin microbiome (16S metabarcoding).
- **Comparative Analysis:** By comparing 2026 egg-string counts with the 2024 and 2025 data (collected under Permit DER-NATTERJACK TOAD-2025-02), the project will provide scientifically based evidence of population trends to the NPWS.

6.3. Corrective Measures

- **Health Triggers:** If any signs of clinical disease (e.g., lethargy, skin sloughing, or unusual mortality) are observed during surveys, handling will cease immediately at that site.
- **Emergency Reporting:** Any such findings will be reported to the NPWS (Dr. Ferdia Marnell) within 24 hours to initiate an emergency biosecurity response or veterinary assessment.
- **Biosecurity Refinement:** If a breach in biosecurity is suspected, all field gear will be subjected to an intensive sterilization cycle (Virkon S soak) before any further site visits occur.

6.4. Proposed Reporting

The data collected using this permit will inform the contents of the most up to date NPWS Irish Wildlife Manual, which I will submit to NPWS in October 2026.