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23rd April 2020

Dear Gary,

Subject: Berry Moor House Farm - GCN eDNA Survey Letter Report

Introduction

Middleton Bell Ecology were contracted to undertake a Great Crested Newt (GCN) *Triturus cristatus* pond assessment and eDNA survey to inform a planning proposal to renovate the farmhouse at Berry Moor House Farm, Silkstone Common (Ordnance Survey Grid Reference: SE 29435 03393). This work was commissioned by the client, Gary Hensby on 3rd April 2020.

Methodology

On the 16th April 2020 Robert Bell (MCIEEM; GCN Survey Licence: 2015-19174-CLS-CLS) carried out a daytime inspection of site water features which were subject to a Habitat Suitability Indices (HSI) assessment (Oldham *et al.*, 2000) and a GCN eDNA test.

Great crested newts are habitat specialists and their status in a given water-body is influenced by the existence of particular features (e.g. fish, heavy shading) and/or the absence of others (e.g. suitable terrestrial habitat within 500 m). The HSI provides a numerical value (ranging from 0 to 1) that indicates the suitability of a waterbody for great crested newts. The higher the HSI score, the more suitable (or closer to optimum habitat conditions) the waterbody may be considered for great-crested newts. However, it should be noted that the HSI score is no substitute for presence or absence surveys.

Water samples for the eDNA test were taken in accordance with methodology approved by Natural England (Biggs *et al.*, 2015). A total of 20 water samples were taken from across the three water features identified, being careful to stir the water but not disturb the sediment. These samples were mixed together, and six samples were taken from the

combined liquid and added to the laboratory sample pots/vials (per pond). All samples were taken using in-date sterile kits provided by the laboratory at SureScreen Scientifics.

Results

Three water features are present within 500m of the site, these are shown on Figure 1. All water features were very small and lacked aquatic vegetation. The pond was low and appeared likely to dry up in the near future. The other two features comprise water troughs, with frog *Rana temporaria* tadpoles noted from the second water trough. All three features were considered to offer Poor suitability for breeding GCN, with HSI results presented in Appendix 1. Images of the water features are shown in Plates 1-3 with their locations shown in Figure 1.

Figure 1. Recorded water features

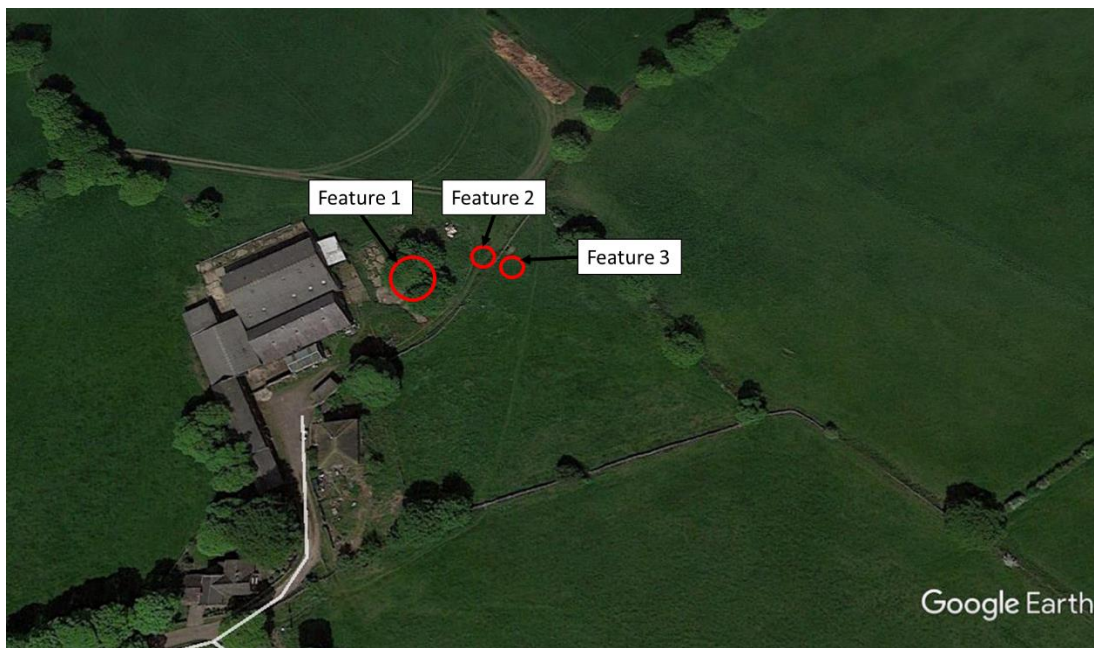


Plate 1. Feature 1



Plate 2. Feature 2



Plate 3. Feature 3



The eDNA test recorded GCN as being absent from the three water features tested. The eDNA test result is presented in Appendix 2.

Legislation

Great crested newts are a European Protected Species (EPS) and as such receive protection under The Conservation of Habitats and Species Regulations (2017) and the Wildlife and Countryside Act (1981) (as amended). It is illegal to kill, injure, capture, handle or disturb them, and the places they use for breeding, resting, shelter and protection are protected from being damaged or destroyed. GCN are included as a species of principal importance under Section 41 of the NERC Act (2006).

Assessment

The HSI assessment showed the three water features display Poor suitability for breeding GCN, with the eDNA test showing this species was absent from the water samples taken across the sampled water features.

Great crested newt are not considered to be a receptor to the proposed scheme.

Yours sincerely,



Robert Bell MCIEEM
Principal Ecologist

References

Biggs, J *et al.* (2015) Using eDNA to develop a national citizen science-based monitoring programme for the great crested newt *Triturus cristatus*. Biological Conservation. 183. 19-28.

Oldham R.S., Keeble J., Swan M.J.S. & Jeffcote M. (2000). Evaluating the suitability of habitat for the Great Crested Newt (*Triturus cristatus*). Herpetological Journal 10(4), 143-155.

Appendix 1. Pond HSI Results

Pond No.	Grid Reference/ Project	Location	Area (m2)	Pond Permanence	Water Quality	Shade (%)	Waterfowl	Fish	Pond Density	Terrestrial Habitat Quality	Macrophyte Cover (%)	HSI Sum	Final HSI Score	Prediction (Likelihood of GCN)
1	Berry Moor Farm	1	0.05	0.1	0.33	1	1	1	0.65	0.67	0	0.00	0.00	Poor
2	Berry Moor Farm	1	0.05	0.9	0.33	1	1	1	0.65	0.67	0	0.00	0.00	Poor
3	Berry Moor Farm	1	0.05	0.9	0.33	1	1	1	0.65	0.67	0	0.00	0.00	Poor

Appendix 2. eDNA Test Result

Folio No: E6775
Report No: 1
Purchase Order: BERRY1
Client: MIDDLETON BELL ECOLOGY
Contact: Robert Bell

TECHNICAL REPORT

ANALYSIS OF ENVIRONMENTAL DNA IN POND WATER FOR THE DETECTION OF GREAT CRESTED NEWTS (TRITURUS CRISTATUS)

SUMMARY

When great crested newts (GCN), *Triturus cristatus*, inhabit a pond, they continuously release small amounts of their DNA into the environment. By collecting and analysing water samples, we can detect these small traces of environmental DNA (eDNA) to confirm GCN habitation or establish GCN absence.

RESULTS

Date sample received at Laboratory: 20/04/2020
Date Reported: 21/04/2020
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
1108	Berry Moor Pond	SE 29423 03356	Pass	Pass	Pass	Negative	0

If you have any questions regarding results, please contact us: ForensicEcology@surescreen.com

Reported by: Chris Troth

Approved by: Sarah Evans



METHODOLOGY

The samples detailed above have been analysed for the presence of GCN eDNA following the protocol stated in DEFRA WC1067 'Analytical and methodological development for improved surveillance of the Great Crested Newt, Appendix 5.' (Biggs et al. 2014). Each of the 6 sub-sample tubes are first centrifuged and pooled together into a single sample which then undergoes DNA extraction. The extracted sample is then analysed using real time PCR (qPCR), which uses species-specific molecular markers to amplify GCN DNA within a sample. These markers are unique to GCN DNA, meaning that there should be no detection of closely related species.

If GCN DNA is present, the DNA is amplified up to a detectable level, resulting in positive species detection. If GCN DNA is not present then amplification does not occur, and a negative result is recorded.

Analysis of eDNA requires scrupulous attention to detail to prevent risk of contamination. True positive controls, negative controls and spiked synthetic DNA are included in every analysis and these have to be correct before any result is declared and reported. Stages of the DNA analysis are also conducted in different buildings at our premises for added security.

SureScreen Scientifics Ltd is ISO9001 accredited and participate in Natural England's proficiency testing scheme for GCN eDNA testing. We also carry out regular inter-laboratory checks on accuracy of results as part of our quality control procedures.

INTERPRETATION OF RESULTS

SIC: **Sample Integrity Check** [Pass/Fail]

When samples are received in the laboratory, they are inspected for any tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to inconclusive results.

DC: **Degradation Check** [Pass/Fail]

Analysis of the spiked DNA marker to see if there has been degradation of the kit or sample between the date it was made to the date of analysis. Degradation of the spiked DNA marker may lead indicate a risk of false negative results.

IC: **Inhibition Check** [Pass/Fail]

The presence of inhibitors within a sample are assessed using a DNA marker. If inhibition is detected, samples are purified and re-analysed. Inhibitors cannot always be removed, if the inhibition check fails, the sample should be re-collected.

Result: **Presence of GCN eDNA** [Positive/Negative/Inconclusive]

Positive: GCN DNA was identified within the sample, indicative of GCN presence within the sampling location at the time the sample was taken or within the recent past at the sampling location.

Positive Replicates: Number of positive qPCR replicates out of a series of 12. If one or more of these are found to be positive the pond is declared positive for GCN presence. It may be assumed that small fractions of positive analyses suggest low level presence, but this cannot currently be used for population studies. In accordance with Natural England protocol, even a score of 1/12 is declared positive. 0/12 indicates negative GCN presence.

Negative: GCN eDNA was not detected or is below the threshold detection level and the test result should be considered as evidence of GCN absence, however, does not exclude the potential for GCN presence below the limit of detection.

