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Date: 12th December 2025
Reference: 82-168-L1-2

GREAT CRESTED NEWT SURVEY
HENTHORN ROAD, CLITHEROE

BACKGROUND

E3P were instructed by Gladman Developments Ltd to produce a Letter Statement detailing the results of a great crested newt *(Triturus cristatus)* eDNA survey at Henthorn Road, Clitheroe, hereafter referred to as "the site".

A Preliminary Ecological Appraisal (PEA) was undertaken in December 2025 (report reference: 81-168-R1). During the survey, seven waterbodies were identified within 250 m of the site, the locations of which can be found in Appendix I. Waterbody 5 was located approximately 140 m north. Waterbody 6 was located approximately 20 m east. Waterbody 7 was located approximately 50 m south.

GREAT CRESTED NEWT EDNA SURVEYS

The eDNA survey was undertaken on 15th April 2025 by Consultant Ecologists Max McCormick BSc (Hons) and Luke Shaw BSc (Hons). The survey comprised a water sample extraction and lab analysis for great crested newts (GCN) eDNA following the protocol set out in Appendix 5 of the DEFRA WC1067 Technical Advice Note. Water samples were retrieved from WB5, WB6 and WB7.

LIMITATIONS

Water samples were not taken for WB1, WB2, WB3 and WB4; due to the following constraints:

- WB1 is located on the southern bank of Pendelton Brook which acts as a significant barrier to dispersal due to deep flowing water. As such, should any great crested newts be present within WB1, it is considered extremely unlikely that they would commute to the site. WB1 was therefore discounted from further survey.

- WB2 is located adjacent to a public footpath, though access to the waterbody could not be gained due to it being fenced off as well as the dense woodland habitat and a significant terrain drop-off which made it unsafe to survey. WB2 has been identified within the catchment area of 'Flood zone 3', meaning a high probability of flooding. On this basis the pond, if present, would be unlikely to sustain a population of great crested newts due to the likelihood of flooding. Therefore, the lack of survey effort is not deemed to be a major constraint.

- WB3 and WB4 were dry at the time of the survey and as such were discounted from further surveys.

RESULTS

The results of the survey were received on 6th May 2025. All waterbodies (WB5, WB6 and WB7) tested **NEGATIVE** for the presence of great crested newts. Please see Appendix II for the eDNA Survey Results.

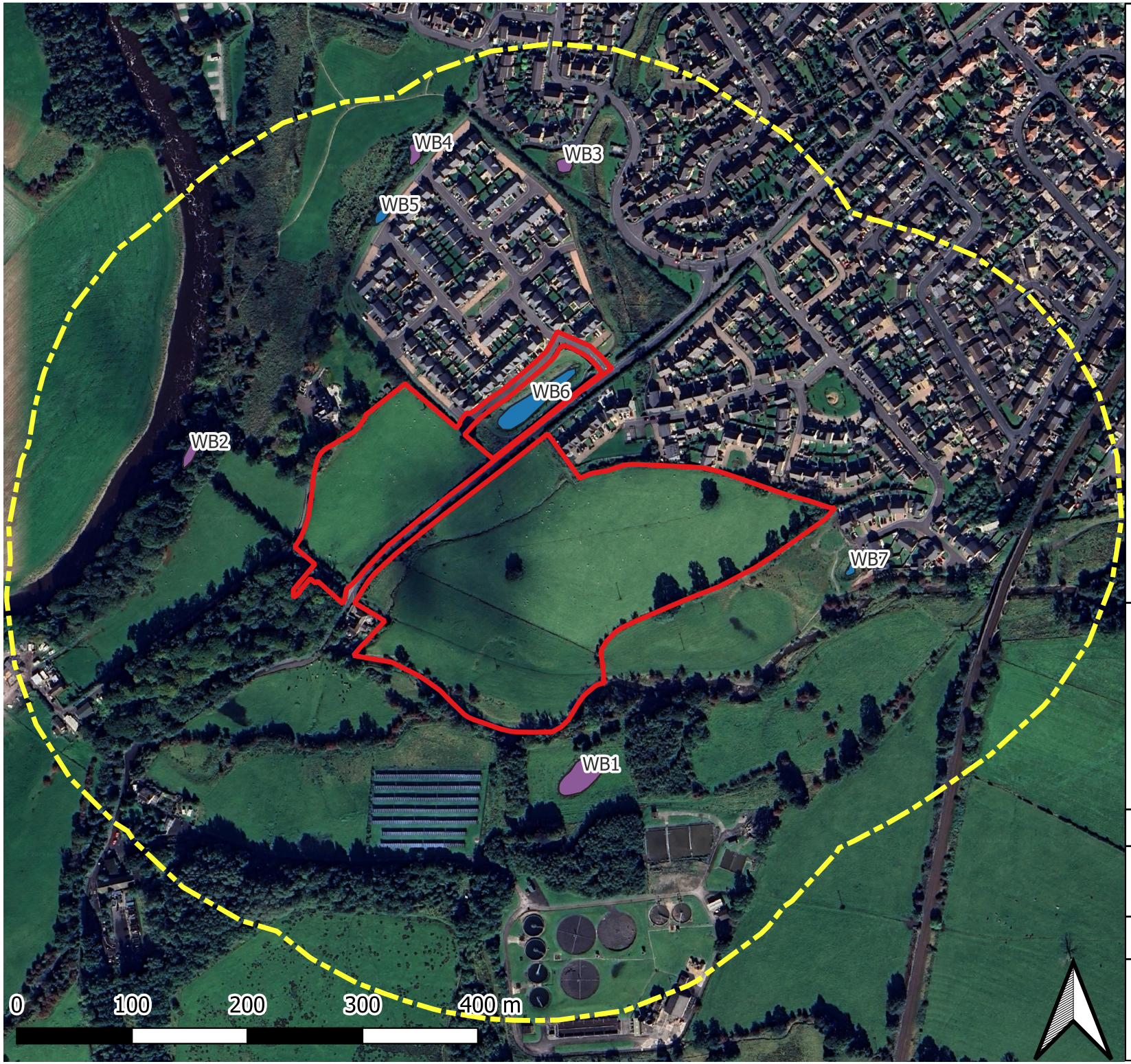
CONCLUSIONS AND RECOMMENDATIONS

Great crested newts have been confirmed absent from all waterbodies within 250 metres of the site boundary where no dispersal barriers exist. As there are no waterbodies within the site itself, and those within 250 metres have been ruled out from further consideration, no additional surveys or precautionary measures related to great crested newts are necessary for the proposed development to proceed.

Yours sincerely,
For and on behalf of E3P Ltd

Max McCormick
Consultant Ecologist

APPENDIX I
WATERBODY PLAN



Key:

- Red Line Boundary
- 250m from site boundary
- Potential waterbodies
- Discounted waterbodies

Notes

Issue: 1	Revision: 2	Date: 10/12/2025	Drawn: MM	Authorised: CK
Client: Gladman Developments Ltd			Job No. 82-168	Date: 10/12/2025
			Drawing No. 82-168-007	Scale: 1:4500 @ A4
Job title: Henthorn Road, Clitheroe			Drawing title: Waterbody Plan	

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APPENDIX II
GREAT CRESTED NEWT EDNA SURVEY RESULTS

Folio No:	372-2025
Purchase Order:	010955
Contact:	E3P
Issue Date:	06.05.2025
Received Date:	17.04.2025

GCN Report

Technical Report

SureScreen Scientifics

Folio No: 372-2025
Purchase Order: 010955
Contact: E3P
Issue Date: 06.05.2025
Received Date: 17.04.2025

SureScreen Scientifics

GCN eDNA Analysis

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 analyzing water samples, we can detect these small traces of environmental DNA (eDNA) to confirm GCN habitation or establish GCN absence.

Results

Lab ID	Site Name	OS Reference	Degradation Check	Inhibition Check	Result	Positive Replicates
GCN25 0833	Henthorn Road - WB6	SD 72962 40731	Pass	Pass	Negative	0/12
GCN25 0834	Henthorn Road - WB5	SD 72836 40895	Pass	Pass	Negative	0/12
GCN25 0837	Henthorn Road - WB7	SD 7324 40589	Pass	Pass	Negative	0/12

Matters affecting result: none

Reported by: Amy Bermudez

Approved by: Lauryn Jewkes

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SureScreen Scientifics

Methodology

The samples detailed above have been analyzed 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 tube which then undergoes DNA extraction. The extracted sample is then analyzed 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 attention to detail to prevent the 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 analytical security.

SureScreen Scientifics Ltd is ISO9001 accredited and participates in Natural England's proficiency testing scheme for GCN eDNA testing.

Interpretation of Results

Sample Integrity Check: 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. Any samples which fail this test are rejected and eliminated before analysis.

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.

Inhibition Check: **Pass/Fail.** The presence of inhibitors within a sample is assessed using a DNA marker. If inhibition is detected, samples are purified and re-analyzed. 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 the WC1067 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.
Inconclusive: Controls indicate inhibition or degradation of the sample, resulting in the inability to provide conclusive evidence for GCN presence or absence.