

Date: 01st July 2026

Reference: 82-893-L1-1

GREAT CRESTED NEWT EDNA SURVEY

TECHNICAL NOTE

Project	Land off Heywood Old Road, Middleton
Client	Equilibrium Architects Limited
E3P Project No.	82-893-L1-1
Subject	Great Crested Newt eDNA survey results
Author	Charlotte Fordham

1. BACKGROUND

E3P were instructed by Equilibrium Architects Limited to produce a Letter Statement detailing the results of a great crested newt (*Triturus cristatus*) eDNA survey at Land off Heywood Old Road, Middleton, hereafter referred to as 'the site'.

A Preliminary Ecological Appraisal (PEA) was undertaken by E3P in January 2026 (Report Reference: 82-893-R1). During the PEA, no waterbodies were identified within the site boundary. However, two waterbodies (WB1 and WB2) were identified within 250 m of the site boundary. A Habitat Suitability Index (HSI) assessed the waterbodies for their suitability as an aquatic habitat for Great Crested Newt (GCN). WB1 was assessed as **AVERAGE**, however, WB2 could not be accessed due to being located on private property. As such, the suitability of WB2 as an aquatic habitat for breeding GCN remains unknown. As such, GCN eDNA surveys were required to be undertaken on both waterbodies between mid-April and June (inclusive) to determine whether GCN were present or absent in the three waterbodies.

2. GREAT CRESTED NEWT EDNA SURVEYS

The eDNA surveys were undertaken on 18th May 2026 by Consultant Ecologist Daniel Syddall BSc (Hons) and Graduate Ecologist Charlotte Fordham BSc (Hons), and on 12th June 2026 by Consultant Ecologist Toby Leverret BSc (Hons) MSc and Graduate Ecologist Oliver Nicholson BSc (Hons). Daniel, Charlotte, Toby and Oliver all have experience undertaking numerous GCN eDNA surveys at a wide variety of sites across the UK.





The survey comprised a water sample extraction and lab analysis for GCN eDNA following the protocol set out in Appendix 5 of the DEFRA WC1067 Technical Advice Note.

- ✿ Water samples were retrieved from WB1.
- ✿ WB2 was found to be dry and therefore discounted from the assessment.

Please see Figure 2.1 for WB2 at the time of the site visit on 18th May 2026.

Figure 2.1 WB2 (as observed on 18th May 2026)



3. RESULTS

The results of the survey were received on 29th June 2026. WB1 tested **NEGATIVE** for the presence of GCN. Please see Figure 3.1 detailing the results of the survey.

Please see Appendix I for the full eDNA Survey Results and Appendix II for the Waterbody Plan, which shows the approximate locations of the waterbodies in relation to the site.



Figure 3.1 eDNA Results

Results

Lab ID	Site Name	OS Reference	Degradation Check	Inhibition Check	Result	Positive Replicates
GCN26 08602	Land off Heywood Old Road - WB1	SD 85418 07358	Pass	Pass	Negative	0/12

Matters affecting result: none

Reported by: Ruby Fielding

Approved by: Consuela Sopronyi

4. CONCLUSIONS AND RECOMMENDATIONS

Great crested newts have been confirmed as absent within WB1 via negative eDNA results. The upper dispersal limit of great crested newts is generally considered to be up to 250 m from a waterbody, with the exception of areas of high habitat connectivity. The habitats surrounding the site are predominantly agricultural and residential, and therefore are not deemed high connectivity habitats for these species. As such, great crested newts will not be present on-site in either their breeding or terrestrial phase, and no further surveys or precautionary working methods regarding great crested newts are required for development to proceed. The survey results will be valid for two years to inform a planning application.

Yours sincerely,

For and on behalf of E3P Ltd

Charlotte Fordham
Graduate Ecologist



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Appendix I: Great Crested Newt eDNA Survey Results



Folio No: 2541-2026
Purchase Order: 014403
Contact: E3P (Environmental Engineering Partnerships Ltd)
Issue Date: 29.06.2026
Received Date: 15.06.2026

GCN Report

Technical Report



SureScreen Scientifics

Folio No: 2541-2026
Purchase Order: 014403
Contact: E3P (Environmental Engineering Partnerships Ltd)
Issue Date: 29.06.2026
Received Date: 15.06.2026

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.

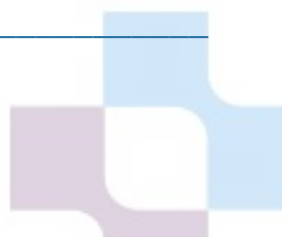
Results

Lab ID	Site Name	OS Reference	Degradation Check	Inhibition Check	Result	Positive Replicates
GCN26 08602	Land off Heywood Old Road - WB1	SD 85418 07358	Pass	Pass	Negative	0/12

Matters affecting result: none

Reported by: Ruby Fielding

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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:

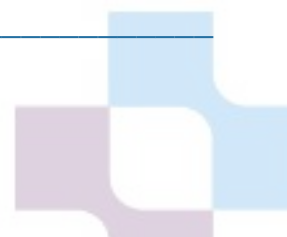
Presence of GCN eDNA (Positive/Negative/Inconclusive)

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.



Appendix II: Waterbody Plan



Key:

- Red line boundary
- 250m from site boundary
- Waterbodies

Notes

Issue: 1	Revision: 1	Date: 26/01/2026	Drawn: TL	Authorised: CK
Client: Equilibrium Architects Ltd			Job No. 82-893	Date: 26/01/2026
			Drawing No. 82-893-003	Scale: 1:4500 @ A4
Job title: Land off Heywood Old Road, Middleton (Redwood SEND)			Drawing title: Waterbody Plan	



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