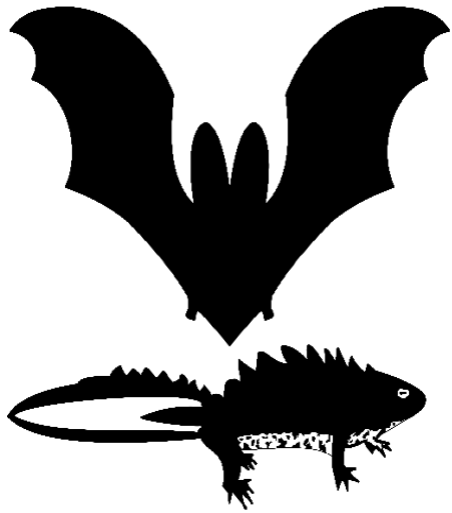




AEWC^{Ltd}

Animal Ecology & Wildlife Consultants

25-105
29/07/2026

Ref: Wiltshire's Farm Great Crested Newt HSI and eDNA

To whom it may concern;

AEWC Ltd were commissioned by Batcheller Monkhouse on behalf of their client to undertake eDNA water sampling for the wet ditch present within 250m of the proposed development at Wiltshire's Farm, Pickhurst Lane Codmore Hill, Pulborough, RH20 1DA to help inform the proposed works at the site. See Figure 1.

A total of nine waterbodies were identified as part of the Preliminary Ecological Appraisal (PEA) within the accepted maximum great crested newt (GCN) dispersal distance of 500m. These were numbered 1 – 9. See Figure 2.

A Rapid Risk Assessment undertaken as part of the PEA indicated that, in the absence of mitigation:

- An offence would be **highly likely** if GCN breeding ponds are present within 100m of the site boundary;
- An offence would be **likely** if breeding ponds are present between 100 and 250m of the site;
- An offence would be **highly unlikely** if all breeding ponds are located more than 250m from the site, given the scale of impact.

Based on the findings of the Rapid Risk Assessment, it was recommended that only the ditch to the south of the site (No.1) and drain to the north (arrow) be subject to further GCN survey. All other potentially suitable waterbodies are located greater than 250m from the site.

A visit was undertaken on the 17th April 2026 by qualified ecologists Monika Cuskova to undertake HSI assessment and, where appropriate, eDNA water sampling of these.

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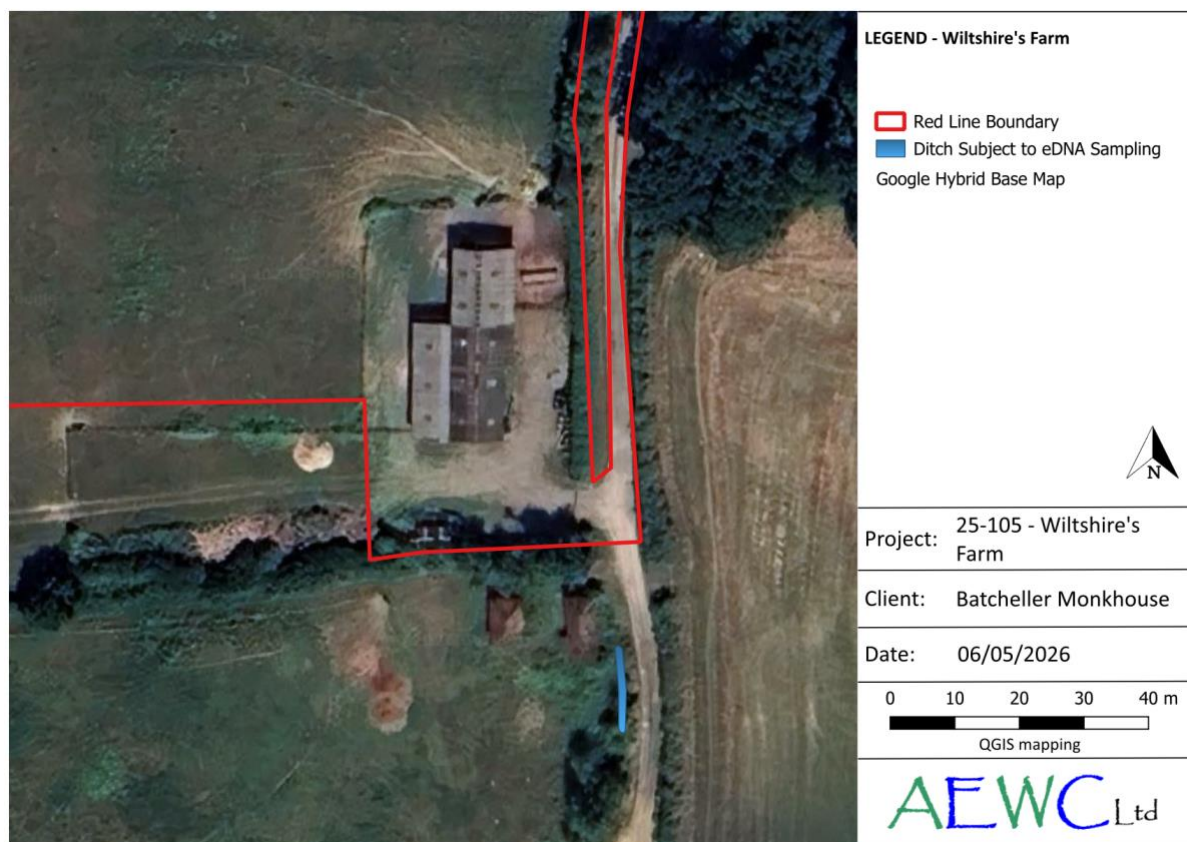


Figure 1: Ditch subject to eDNA survey

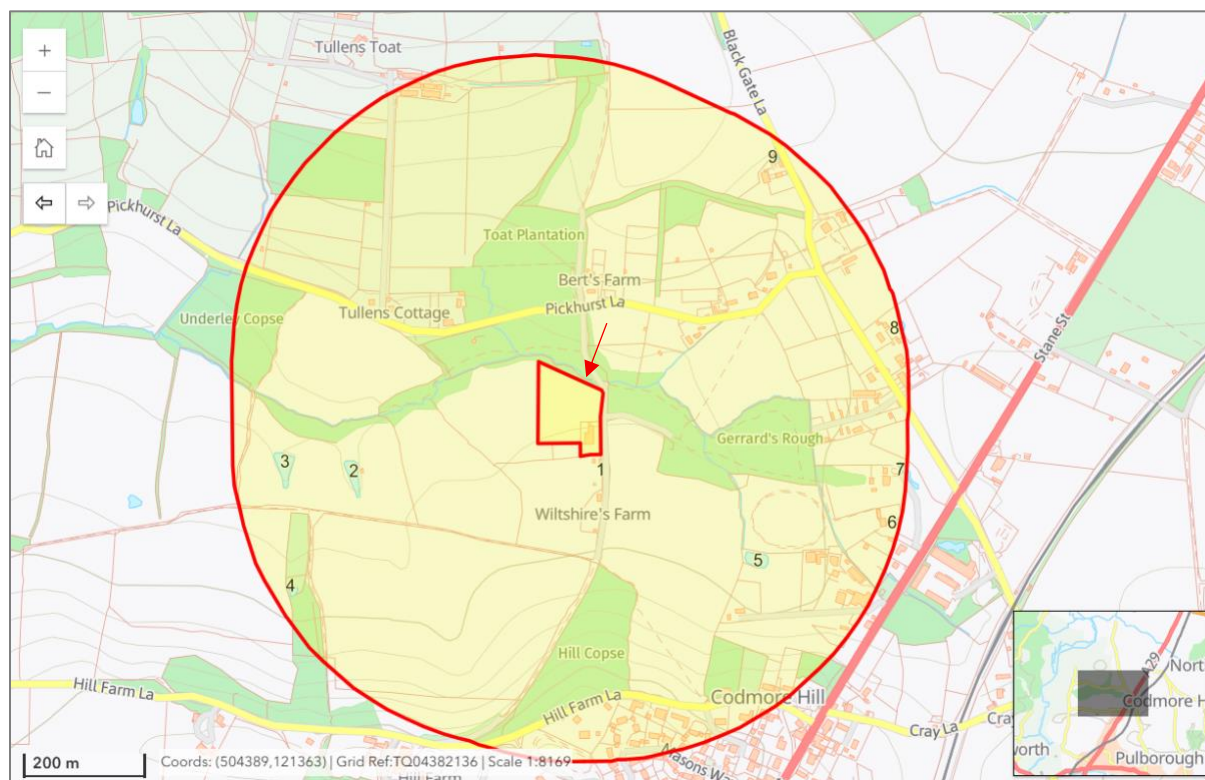


Figure 2: Waterbodies identified within 500m of the site.

HSI Assessment

Prior to eDNA sampling, an HSI is used to quantifiably assess whether the waterbodies are suitable for GCN.

The ditch to the south (ref 1) is surrounded by scrub and grassland. It is approximately 55m² in size, with no more than 60% shading. The ditch supports moderate water quality and approximately 85% macrophyte cover. No evidence of waterfowl or fish was recorded. The surrounding terrestrial habitat provides suitable habitat within the dense tall grassland and scrub. An HSI score of 0.71 was calculated which equates to 'good' suitability for GCN.

The northern drain is located within woodland. It is flowing, very shallow, and fully shaded. Water quality was assessed as poor, and no evidence of waterfowl or fish was recorded. An HSI score of 0.44 was calculated which equates to 'poor' suitability for GCN. Given that the water was flowing and was very shallow (<20cm deep), it is considered that this watercourse would be completely unsuitable for breeding GCN.

The full HSI calculations are provided in Table 1 and photographs are provided below.



Photograph 1 – Ditch (No.1) to the south of the site.



Photograph 2 – Drain (arrow) to the north of the site.

Table 2 – HSI calculations

Pond ref	1	Arrow
SI ¹ - Location	1	1
SI ² - Pond area	0.05	0.05
SI ³ - Pond drying	1	0.5
SI ⁴ - Water quality	0.67	0.33
SI ⁵ - Shade	1	0.2
SI ⁶ - Fowl	1	1
SI ⁷ - Fish	1	1
SI ⁸ - Ponds	0.89	0.89
SI ⁹ - Terrestrial habitat	1	0.67
SI ¹⁰ - Macrophytes	0.95	0.3
HSI Score	0.70	0.46
HSI Category	Good	Poor

Environmental DNA

Environmental DNA (eDNA) surveys can be used to find out if GCN are present and whether to conduct population size class surveys on ponds and other waterbodies.

The drain to the north was considered unsuitable for GCN and was therefore not subject to eDNA survey.

The ditch to the south was considered suitable for GCN and was therefore subject to eDNA survey.

The survey was undertaken in the daytime by Monika Cuskova on 17th April 2026 to collect water samples from around the ditch edge in line with the methodology outlined by Defra, during the period when newts are likely to be present (this depends on location and weather conditions). Natural England will only accept eDNA survey results from samples collected between 15 April and 30 June.

The water samples are then mixed, and a standard sample is sent to a specialist approved laboratory for testing.

The results of the sample were received on the 5th May 2026 confirming the sample to have been **negative for the presence of GCN DNA. The laboratory report is appended to this letter.**

A negative result confirms that 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 it does not exclude the potential for GCN presence below the limit of detection.

Conclusions and Recommendations

Based on the results of the eDNA test it is considered that the site is unlikely to be used by terrestrial GCN and no further surveys for GCN are required.

There remains a low risk of GCN being present, since GCN can potentially disperse up to 500m from breeding ponds. The site could also be used by common amphibians such as common toads and small newts. Therefore, **it is recommended that a precautionary working method statement regarding amphibians is produced to cover the proposed works.** This should include precautionary working methods to safeguard amphibians, such as ecological supervision, controlled vegetation and topsoil clearance, and the careful dismantling of any potential refugia.

If you have any queries, please do not hesitate to contact us.

Best wishes,

Monika Cuskova

AEWC_{Ltd}
Ecologist

Folio No: 460-2026
Purchase Order: 25-105-MC
Contact: AEWCLtd
Issue Date: 05.05.2026
Received Date: 20.04.2026

GCN Report

Technical Report



SureScreen Scientifics

Folio No: 460-2026
Purchase Order: 25-105-MC
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Issue Date: 05.05.2026
Received Date: 20.04.2026

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 00592	Wilthire's Farm, Pickhurst Lane, RH20 ! DE - DITCH	TQ 05355 20671	Pass	Pass	Negative	0/12

Matters affecting result: none

Reported by: Chelsea Warner

Approved by: Alice Cunnington

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.

