AEWC Ltd

Animal Ecology & Wildlife Consultants

23-246
02/06/2025

Ref: Land to the East of Tilletts Lane 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 an HSI assessment and eDNA water sampling for two ponds present within 250m of the proposed development site at Land to the east of Tilletts Lane, Warnham, Horsham, West Sussex, in order to inform the proposed works at the site.

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

A Rapid Risk Assessment carried out as part of the PEA showed that, in the absence of mitigation, an offence was likely if GCN were present in ponds within 250m of the site, but not if they were present in ponds 250-500m from the site.

Given the results of the Rapid Risk Assessment, it was recommended that any ponds within 250m of site be subject to further GCN survey. Two ponds were identified within 250m of the site, these being Ponds 1 and 2 as per Figure 1.

Ponds 1 and 2 were therefore subject to Habitat Suitability Index (HSI) assessment and Environmental DNA (eDNA) survey. These were carried out on the 16th April 2025 by GCN licensed ecologist Natalie Arscott.

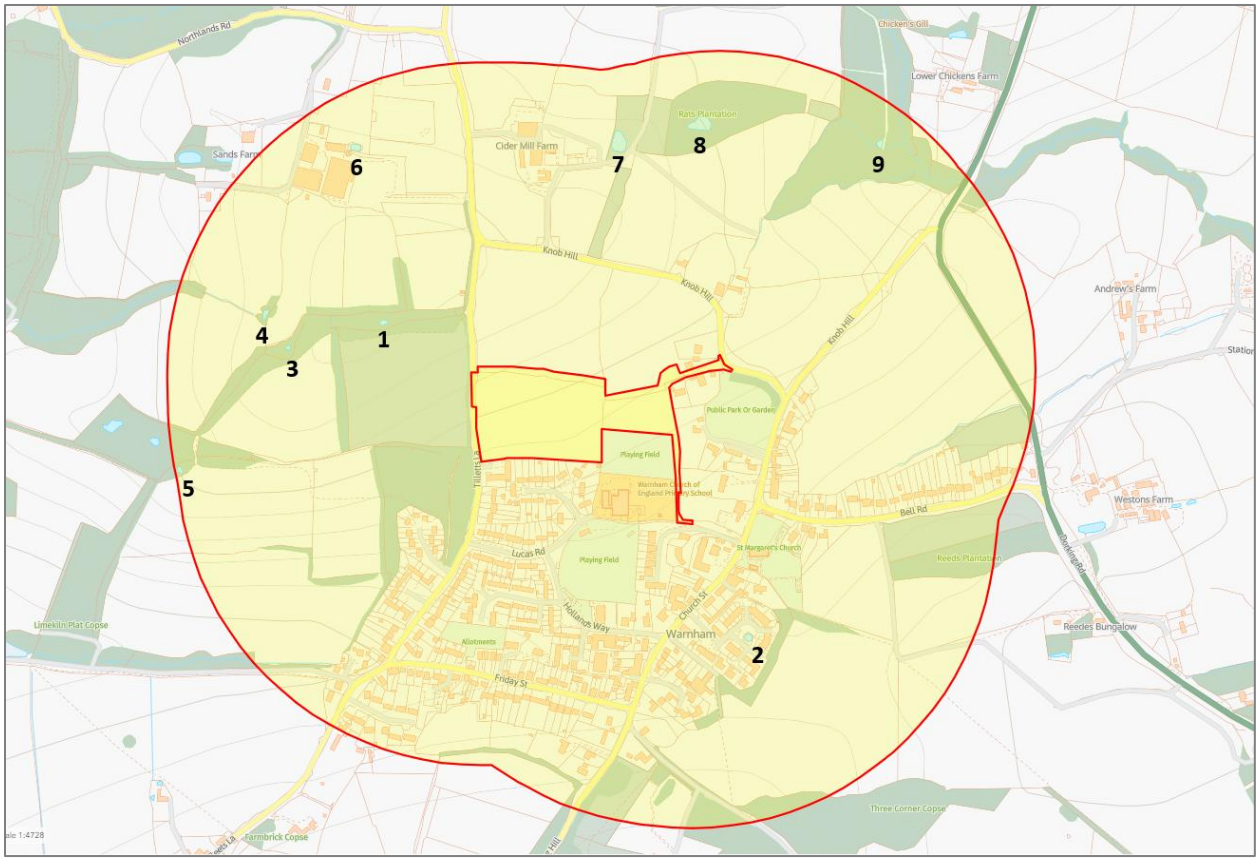


Figure 1: Ponds identified within 500m of the site.

HSI Assessment

Prior to eDNA sampling, an HSI was used to quantifiably assess whether the ponds were suitable for GCN.

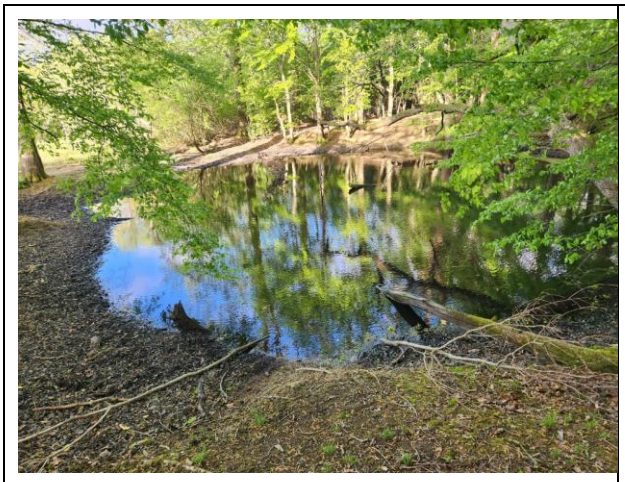
Pond 1 is situated within a belt of woodland, adjacent to Warnham butterfly fields, located 165m to the northwest of the site. It is approximately 100m^2 in size and mostly shaded, with poor water quality and low macrophyte cover. The surrounding terrestrial habitat is good quality. An HSI score of 0.64 was calculated which equates to 'average' suitability for GCN.

Pond 2 is situated within a residential area in Warnham village, located 205m to the southeast of the site. It is approximately 125m^2 in size and unshaded, with good water quality and high macrophyte cover. The surrounding terrestrial habitat is poor, with the pond being isolated by surrounding roads and development. An HSI score of 0.77 was calculated which equates to 'good' suitability for GCN.

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

Table 1 – HSI calculations

Pond ref	Pond 1	Pond 2
SI[1] - Location	1	1
SI[2] - Pond area	0.2	0.25
SI[3] - Pond drying	1	0.9
SI[4] - Water quality	0.33	1
SI[5] - Shade	0.5	1
SI[6] - Fowl	1	1
SI[7] - Fish	1	1
SI[8] - Ponds	1	1
SI[9] - Terrestrial habitat	1	0.33
SI[10] - Macrophytes	0.35	0.95
HSI Score	**0.64**	**0.77**
HSI Category	**Average**	**Good**



Photograph 1 – Pond 1.

Photograph 2 – Pond 2.

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 survey was undertaken on the 16th April 2025 to collect water samples from around the pond edges 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 standard samples are sent to a specialist approved laboratory for testing.

The results of the samples were received on the 6th May 2025 confirming the samples from both Ponds 1 and 2 to have been **negative for the presence of GCN DNA**.

A negative result confirms that GCN eDNA was not detected or is below the threshold detection level. 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.

The findings of the survey are shown in Figure 2 below.

Figure 2: eDNA survey results

Conclusions and Recommendations

Based on the result of the eDNA testing, it is considered that there are unlikely to be GCN breeding ponds within 250m of the site. Therefore, no further surveys or licensing for GCN are required.

This result significantly reduces the likelihood that GCN use terrestrial habitats within the site, however their presence cannot be definitively ruled out since GCN can potentially disperse up to 500m from breeding ponds, and there are local records for GCN within 2km of the site (but not within 500m). The status of ponds in terms of GCN presence can also change at any time if the species is present in the local vicinity.

The site also offers suitable habitat for common amphibians such as smooth newt, palmate newt, common toad, and common frog.

Based on the extent of suitable terrestrial habitat on the site and the potential for amphibians being present, **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 potential refugia. The method statement could be combined with the required reptile mitigation strategy and method statement, since reptile surveys undertaken by AEWC Ltd have confirmed the presence of slow worm and grass snake, as well as smooth newt, on-site (see Reptile Survey Report dated November 2024).

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

Best wishes,

Natalie Arscott
AEWC Ltd
Ecologist

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GCN Report

Technical Report

SureScreen Scientifics

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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 1685	Tilletts Lane - Pond 2	TQ 158 335	Pass	Pass	Negative	0/12
GCN25 1686	Tilletts Lane - Pond 1	TQ 152 340	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.