For and on behalf of
Boningale Homes Limited

Great Crested Newt eDNA Survey – Technical Note

Land at Tilstock Rd, Tilstock

NGR: SJ 542 380

Prepared by
Cass Design Consultants Ltd
Liverpool

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Prepared by:	Alasdair Grubb BSc (Hons) ACIEEM
Reviewed By	Daniel Ross BSc (Hons)
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Cass Design Consultants Ltd

Tel: 01234 261266

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1.0 INTRODUCTION

1.1 Cass Design Ltd (Cass) was commissioned by Boningale Homes Limited to carry out environmental DNA (eDNA) analysis in relation to land at Tilstock Road, Tilstock (hereafter referred to as the 'Site').

1.2 The Survey was commissioned following recommendations made in the Preliminary Ecological Appraisal Report (Cass, April 2024). The Site location is shown in Figure 1.

1.3 All ponds on Site and within 250m were recommended for survey to establish the status of great crested newt (GCN). Pond locations are shown in **Figure 1**.

2.0 METHODS

2.1 Great crested newt eDNA sampling surveys were conducted on all accessible waterbodies that were identified as having potential suitability to support great crested newt during the Site walkover and desk-based assessment. The survey was carried out on the 15th of April 2024, within the acceptable survey period for this technique. The eDNA survey methodology is accepted by Natural England (NE) as a reliable technique for determining the presence / likely absence of great crested newt within a waterbody through detection of traces of great crested newt DNA within the water. All surveys were undertaken following best practice guidelines[1].

2.2 The presence or likely absence of great crest newt from each of the surveyed water bodies was determined based on the results of the eDNA analysis. If eDNA is detected this provides confirmation of presence and the relevant water bodies are likely to represent a development constraint that requires further consideration. If eDNA is not detected, then this provides high confidence that there is no reasonable likelihood of great crested newt being present in the relevant water bodies.

2.3 The surveyed ponds were subjected to assessment for suitability for use by breeding great crested newts using the Habitat Suitability Index[2] (HSI).

3.0 LIMITATIONS & ASSUMPTIONS

3.1 An ecological survey represents a 'snapshot' in time of the ecological condition of a site. The ecological character of a site can change substantially throughout both the course of a year, and from

[1] Briggs, J., Ewald, N., Valentini, A., Gaboriaund, C., Griffiths, R.A., Foster, J., Wilkinson, J., Arnett, A., Williams, P. & Dunn, F. (2014) Analytical and methodological development for improved surveillance of the Great Crested Newt. Appendix 5. Technical advice note for field and laboratory sampling of great crested newt (Triturus cristatus) environmental DNA. Freshwater Habitats Trust, Oxford

[2] 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.

year to year impacting on the extent and quality of habitats potential to support protected species.

3.2 Only on-site ponds and those under the ownership of the client were subject to eDNA survey, as some of the off-site ponds are not in the ownership of the client and access was not possible.

3.3 Ponds 1 and 4 were connected by high water levels at the time of the survey – as such, only one eDNA sample was taken, to cover both ponds: Given the small size of the ponds, this is appropriate.

4.0 RESULTS

4.1 The results of eDNA analysis on the surveyed ponds are presented in the table below. With the lab report provided in **Appendix 1**.

Pond reference	eDNA result	HSI Score
Pond 1	Negative	Poor
Pond 2	Negative	Good
Pond 3	Negative	Below average
Pond 4	Negative	Poor
Pond 5	Negative	Good
Pond 6	Not surveyed	
Pond 7	Not surveyed	
Pond 8	Positive	Good
Pond 9	Negative	Good

5.0 DISCUSSION

5.1 Of the ponds surveyed, all but one returned a negative result.

5.2 Pond 8 returned a positive result (3/12 replicates). This pond was assessed as offering 'Good' suitability for GCNs using the HSI. This pond lies 150m from the Site to the north-east.

5.3 The on-site pond (Pond 1) returned a negative eDNA result and was assessed as of 'Poor' suitability

for GCNs.

5.4 The two ponds which could not be surveyed (Ponds 6 and 7) are separated from the site by Tillstock road which will likely offer a barrier to amphibian movement.

5.5 The positive result returned from one of the surveyed ponds, confirms GCN presence within the immediate vicinity of the Site. However, the onsite pond and other ponds (closer to the Site) returned negative results.

FIGURES

Figure 1: *Pond Locations and References*

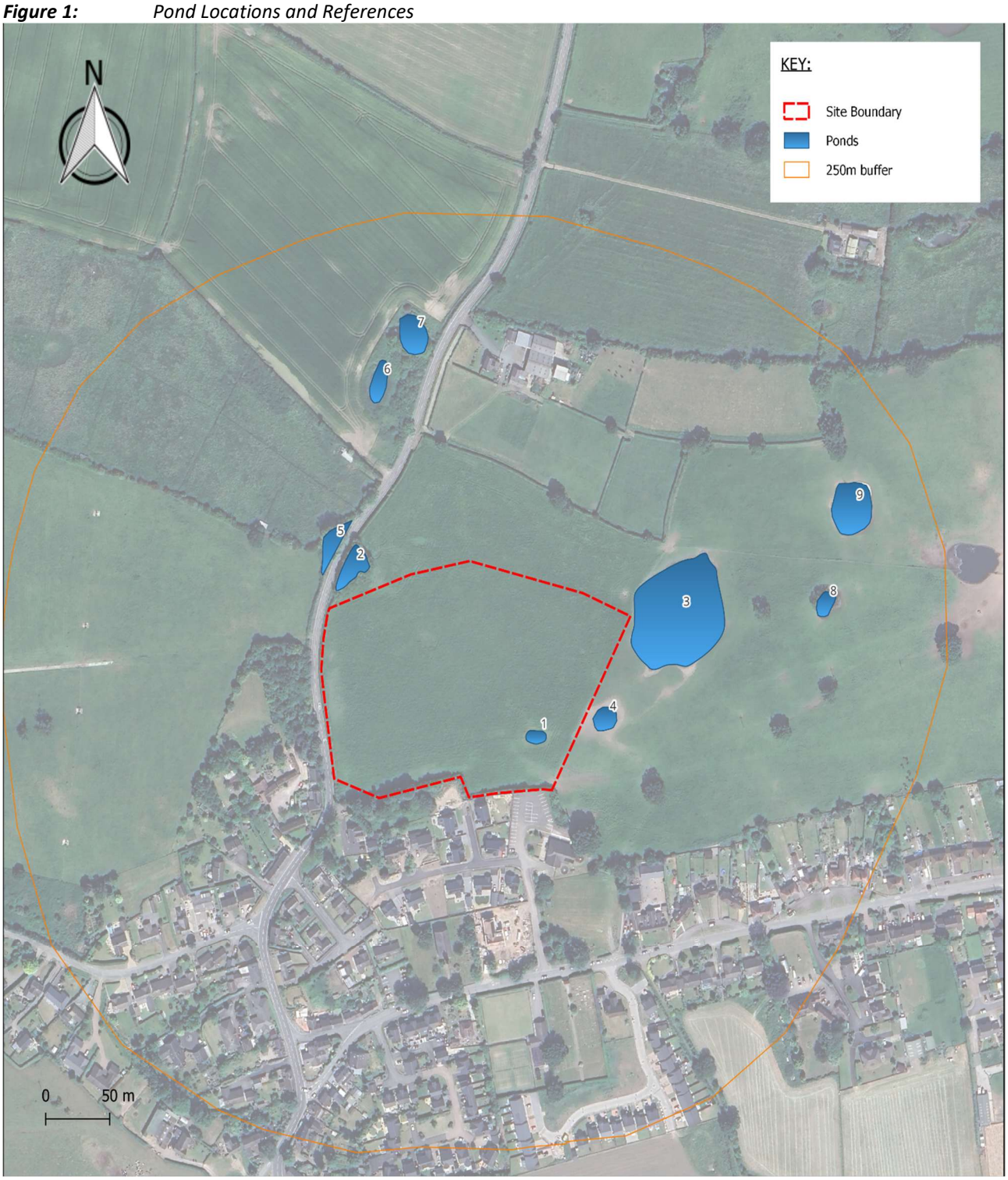

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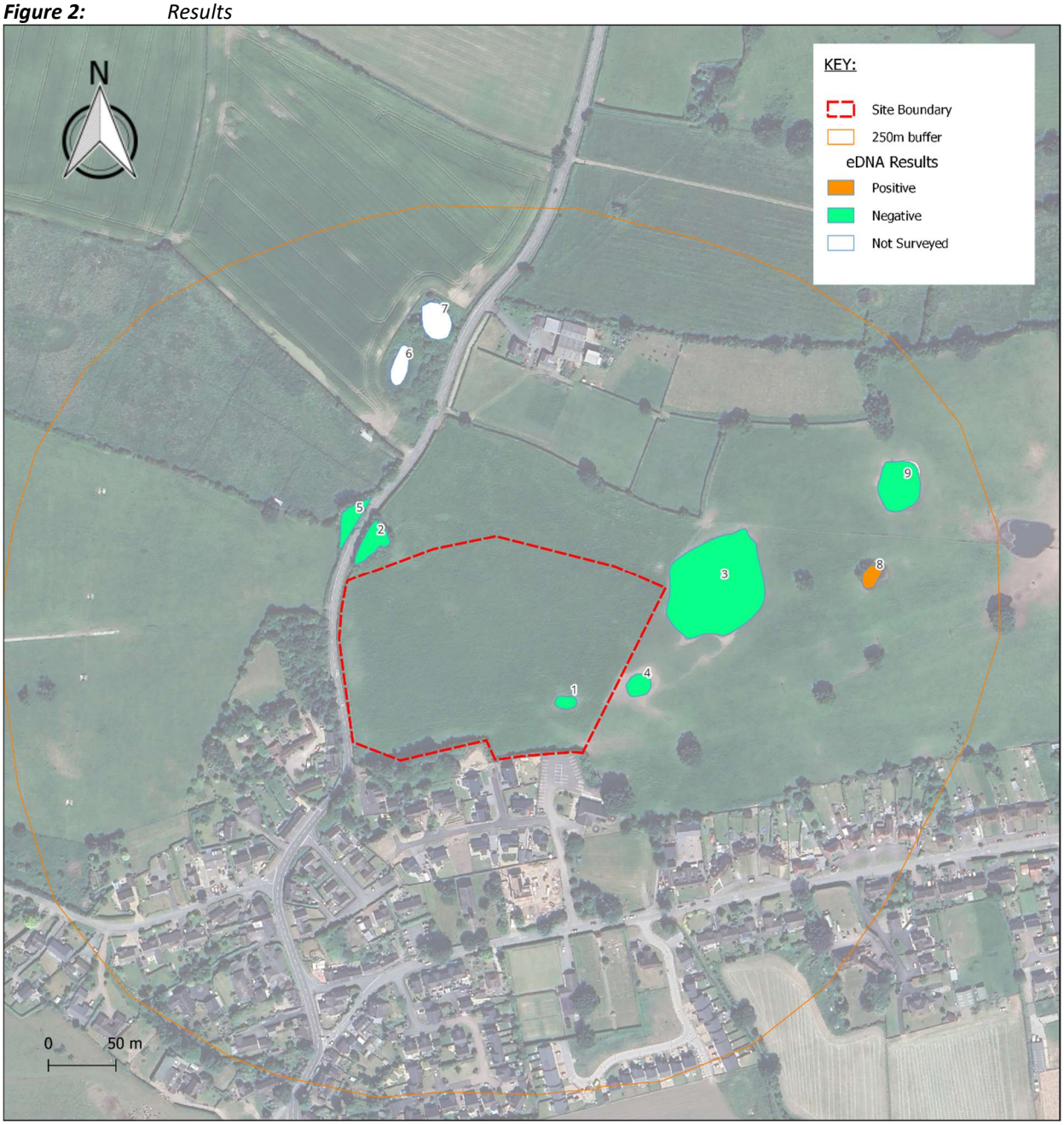
Figure 2: *Results*



APPENDICES

APPENDIX 1 eDNA LAB RESULTS

Folio No: 187-2024
Purchase Order: Til 01
Contact: WildEye Environmental Ltd
Issue Date: 29.04.2024

GCN Report

Technical Report

SureScreen Scientifics

Folio No: 187-2024
Purchase Order: Til 01
Contact: WildEye Environmental Ltd
Issue Date: 29.04.2024

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
2118	Tilstock Pond 5	SJ5417538159	Pass	Pass	Negative	0/12
2119	Tilstock Pond 2	SJ5420038137	Pass	Pass	Negative	0/12
2120	Tilstock Pond 1 + 4	SJ54345738021	Pass	Pass	Negative	0/12
2121	Tilstock Pond 3	SJ5444838105	Pass	Pass	Negative	0/12
2122	Tilstock Pond 9	SJ5458638177	Pass	Pass	Negative	0/12
2123	Tilstock Pond 8	SJ5456538113	Pass	Pass	Positive	3/12

Matters affecting result: none

Reported by: Daisy Chambers

Approved by: Christopher Troth

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.

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Bedford

5 Abbey Court, Fraser Road,
Priory Business Park Bedford, MK44 3WH
t: 01234 261 266 **e:** mail@cass-design.co.uk

Leeds

Princes Exchange, Princes Square,
Leeds, LS1 4HY
t: 0113 280 5808 **e:** mail@cass-design.co.uk

Liverpool

Studio 204B The Tea Factory
82 Wood Street, Liverpool, L1 4DQ
t: 0151 707 0110 **e:** mail@cass-design.co.uk

London

Unit 107 Clerkenwell Workshops, 31 Clerkenwell
Close, London, EC1R 0AT
t: 0203 761 5396 **e:** mail@cass-design.co.uk