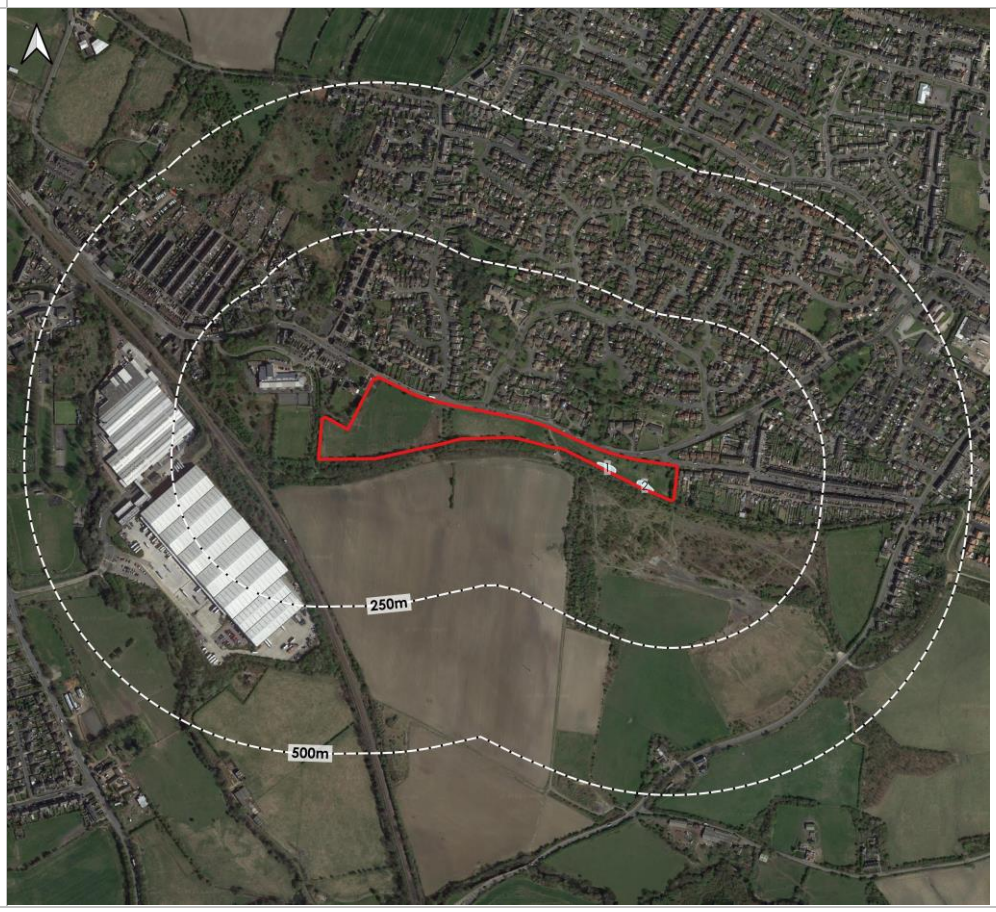
Site Inspection Report

Land off Darton Lane, Mapplewell

Project:	Land off Darton Lane, Mapplewell	**Site Inspection Reference:**	SI-6517-01
Date of inspection:	25.04.2023	**No. of Pages:**	9
Inspection carried out by:	Victoria Baker BSc (Hons) MSc MCIEEM Senior Ecologist		
Background	As part of Brooks Ecological's Preliminary Ecological Appraisal Report (ER-6517-01A, February 2023), two standing waterbodies (Waterbodies 1-2) were found within the Site boundary. No other standing waterbodies were found on mapping within a 500m radius of the Site boundary. If Great Crested Newt (GCN) were found to be present within either of the waterbodies, the development risks impacting upon either breeding or terrestrial habitat available to them.		
Figure 1 Map showing the standing waterbodies which fall within a 500m radius			

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Task description:	eDNA survey An eDNA surveys were carried out to establish the status of GCN within the water bodies. This involved taking water samples from 20 different locations around the pond's margin, focusing on areas where newts are most likely to gather. The survey utilised sterile kits supplied by Surescreen Scientifics laboratory and followed methodology as advised in the Natural England Technical Advice Note (WC1067).
Results	Water samples were collected on the 25th April 2023. At the time of the survey visit, waterbody 2 was found to be completely dry and was subsequently not sampled. Waterbody 1 was found to hold minimal amounts of water but was subject to sampling. The results were returned on the 8th May 2023 - see Appendix 1 for full report. A **negative** result was returned for Waterbody 1 meaning that GCN DNA was not detected within the water samples collected. The results can be used to demonstrate the likely absence of GCN from Waterbody 1.
Conclusions and Recommendations	Based on this information, it is concluded that the risk of GCN being present on Site is negligible and no further survey or specific mitigation is considered necessary.

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Photo 1

Waterbody 1
25.04.2023

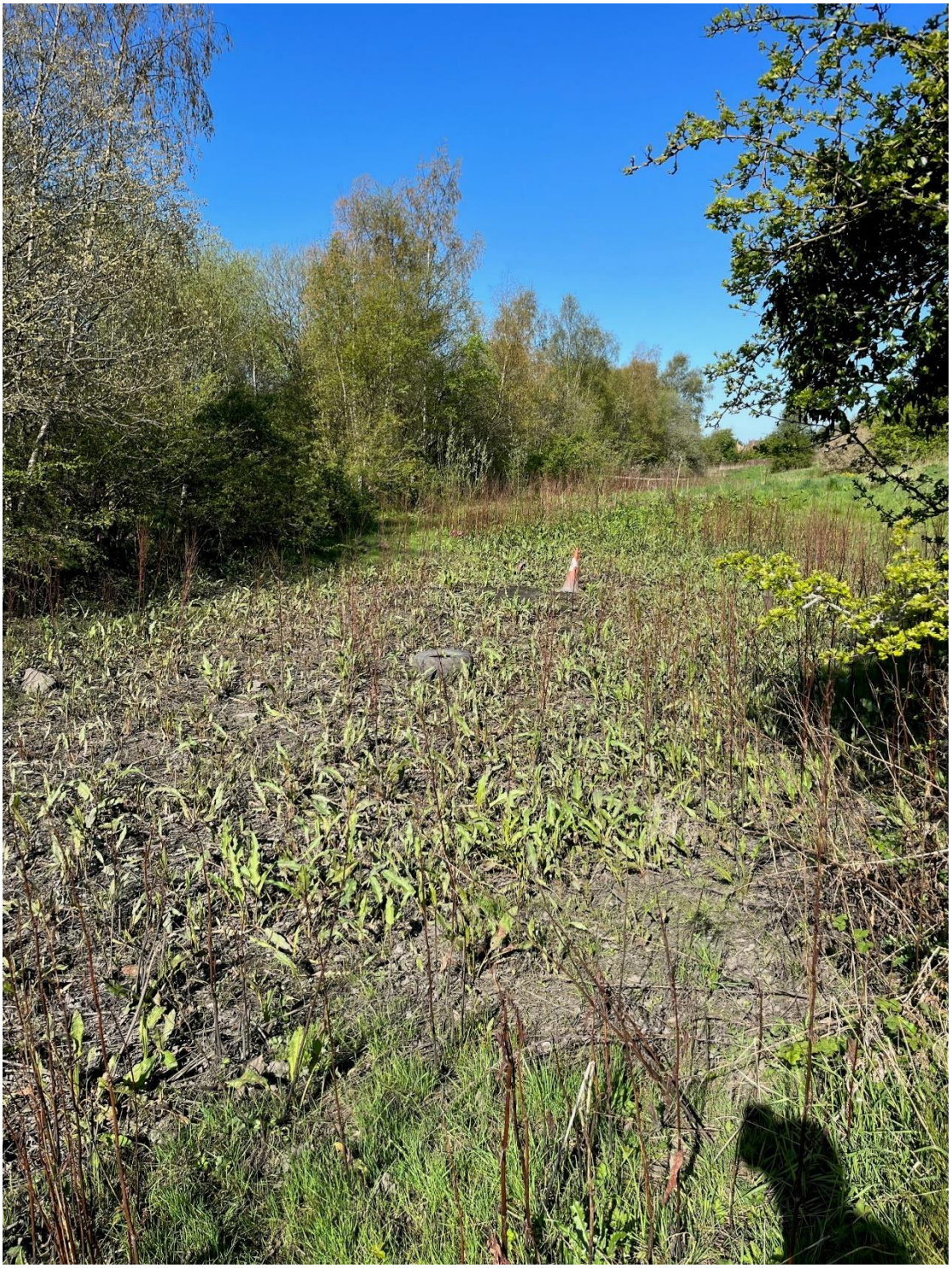

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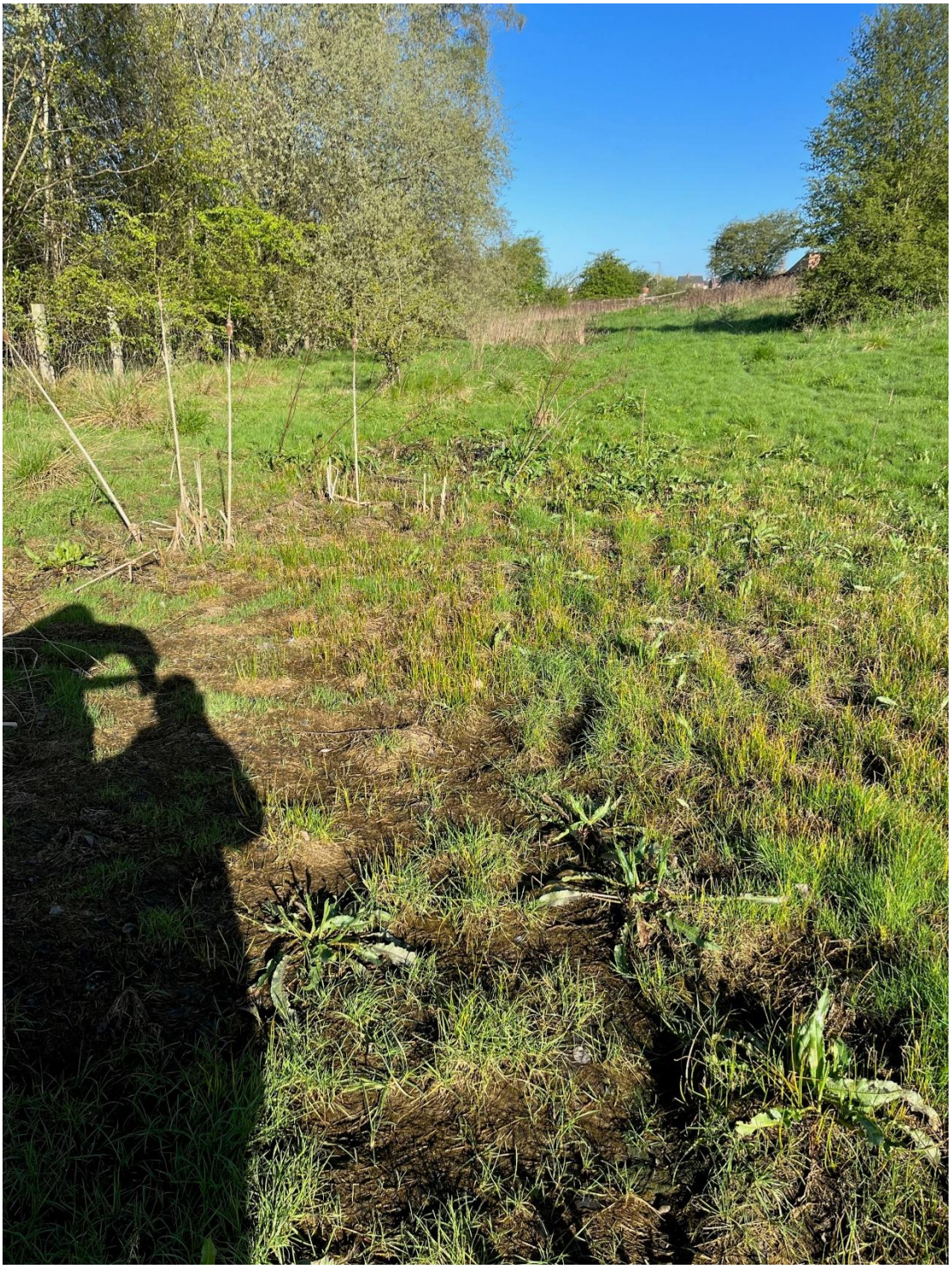
Photo 2

Waterbody 2
25.04.2023



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Appendix 1 – SureScreen Scientific Results

SureScreen Scientifics

Folio No: E16906
Report No: 1
Purchase Order: 6514
Client: BROOKS ECOLOGICAL
Contact: Vickie Baker

TECHNICAL REPORT

ANALYSIS OF ENVIRONMENTAL DNA IN POND WATER FOR THE DETECTION OF GREAT CRESTED NEWTS (TRITURUS CRISTATUS)

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

RESULTS

Date sample received at Laboratory: 26/04/2023
Date Reported: 08/05/2023
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
1658	Darton PX	SE 31885 09838	Pass	Pass	Pass	Negative	0

If you have any questions regarding results, please contact us: ForensicEcology@surescreen.com

Reported by: Chris Troth **Approved by:** Jennifer Higginbottom

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Company Registration No. 08950940

SureScreen Scientifics

METHODOLOGY

The samples detailed above have been analysed 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 which then undergoes DNA extraction. The extracted sample is then analysed 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 scrupulous attention to detail to prevent 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 security.

SureScreen Scientifics Ltd is ISO9001 accredited and participate in Natural England's proficiency testing scheme for GCN eDNA testing. We also carry out regular inter-laboratory checks on accuracy of results as part of our quality control procedures.

INTERPRETATION OF RESULTS

SIC: **Sample Integrity Check** [Pass/Fail]
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.

DC: **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.

IC: **Inhibition Check** [Pass/Fail]
The presence of inhibitors within a sample are assessed using a DNA marker. If inhibition is detected, samples are purified and re-analysed. 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 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.

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Folio No: E17689
Report No: 1
Purchase Order: 6343
Client: BROOKS ECOLOGICAL
Contact: Vickie Baker

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RESULTS

Date sample received at Laboratory: 30/05/2023
Date Reported: 01/06/2023
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
4007	Rossington - Pond 2	-	Pass	Pass	Pass	Negative	0

If you have any questions regarding results, please contact us: ForensicEcology@surescreen.com

Reported by: Jennifer Higginbottom **Approved by:** Jackson Young

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