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Ref: 81-303-L1-1
Date: 19th May 2023

Hallmark Developments Ltd

BY Email

GREAT CRESTED NEWT SURVEYS OAKENROD SCHOOL, ROCHDALE

BACKGROUND

E3P were instructed to undertake great crested newt (*Triturus cristatus*) eDNA surveys at Oakenrod School, Rochdale, hereafter referred to as "the site".

An Extended Phase 1 Habitat Survey was undertaken by Rachel Hacking Ecology in December 2022. During the survey, three waterbodies were identified within the site.

GREAT CRESTED NEWT EDNA SURVEYS

The eDNA survey was undertaken on 05th May 2023 by Celia Barlow BSc (Hons), MCIEEM who holds a Natural England Great Crested Newt Licence (2016-26721-CLS-CLS). The survey comprised a water sample extraction and lab analysis for GCN eDNA following protocol set out in Appendix 5 of the DEFRA WC1067 Technical Advice Note.

Water samples were retrieved from Waterbody 1 (WB1), WB2 and WB3. No constraints were noted during the sample collection.

RESULTS

The results of the survey were received on 18th May 2023. All three waterbodies tested **NEGATIVE** for the presence of great crested newts. Please see Figure 1 detailing the results.



FIGURE 1 EDNA RESULTS

RESULTS

Date sample received at Laboratory: 09/05/2023
Date Reported: 18/05/2023
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
3113	Oakenrod School - P3	SD 88720 13020	Pass	Pass	Pass	Negative	0
3114	Oakenrod School - P1	SD 88764 13091	Pass	Pass	Pass	Negative	0
3115	Oakenrod School - P2	SD 88727 13054	Pass	Pass	Pass	Negative	0

CONCLUSIONS AND RECOMMENDATIONS

Great crested newts have been confirmed as absent from all waterbodies on site. No further surveys or precautionary working methods with regards to great crested newts are required for development to proceed.

Yours sincerely,
For and on behalf of E3P Ltd

Celia Barlow
Associate Director- Ecology



Folio No: E17214
Report No: 1
Purchase Order: 004758
Client: E3P
Contact: C. Barlow

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: 09/05/2023
Date Reported: 18/05/2023
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
3113	Oakenrod School - P3	SD 88720 13020	Pass	Pass	Pass	Negative	0
3114	Oakenrod School - P1	SD 88764 13091	Pass	Pass	Pass	Negative	0
3115	Oakenrod School - P2	SD 88727 13054	Pass	Pass	Pass	Negative	0

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

Reported by: Gabriela Danickova

Approved by: Jackson Young



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.

