

# Forestry England

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## Hole Farm Woodland Creation Project

### Great Crested Newt Survey (July 2022)

The findings of the Great Crested Newt (GCN) Survey were published by SureScreen Scientifics in July 2022 prior to the design of the Project being finalised. However, the findings remain valid as they would not be affected by the design changes; the sites requiring testing remain the same and the survey area included a 250m buffer around the site boundary. The findings of the GCN survey therefore remain valid.



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## 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:** 24/06/2022  
**Date Reported:** 06/07/2022  
**Matters Affecting Results:** None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
0757	G1 Bradwins, Fire Pond	TL 0326 1484	Pass	Pass	Pass	Negative	0
0758	Pond 1 Deadmansey Wood	TL 0334 1677	Pass	Pass	Pass	Negative	0
0763	HF1 Hole Farm	TQ 58096 89977	Pass	Pass	Pass	Negative	0
0764	Great Baldwins 2	TL 03826 15281	Pass	Pass	Pass	Negative	0

If you have any questions regarding results, please contact us: [ForensicEcology@surescreen.com](mailto:ForensicEcology@surescreen.com)

**Reported by:** Chris Troth

**Approved by:** Jennifer Higginbottom



## **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**

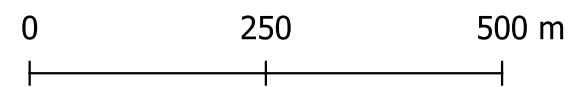
- SIC:**            **Sample Integrity Check** [Pass/Fail]  
When samples are received in the laboratory, they are inspected for any tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to inconclusive results.
- DC:**            **Degradation 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.






## Legend

- Hole Farm Site Boundary
- 250 m Buffer from Site Boundary



Atkins Limited © Woodcote Grove Ashley Road Epsom England KT18 5BW	Project: Hole Farm	Title: Figure to show Site boundary plus a 250 m buffer from Site boundary	Original scale: 1:8000	Drawn by: GH	Checked by:	Reviewed by:
	Client: 	Drawing number: N/A		Date: 09/09/2022	Date:	Date: