

**FAO Adam McNally
Story Homes Ltd
Story House,
Lords Way,
Kingmoor Business Park,
Carlisle,
CA6 4SL**

Job No. UG1415
Date 4th May 2022

Dear Adam,

The following letter statement has been produced to provide the results of eDNA surveys carried out in relation to the proposed works at Edgehill, Gameriggs Road in Whitehaven on behalf of Story Homes. The PEA of the site, conducted in January 2022, identified six ponds present within 250m of the site. eDNA surveys were therefore recommended to assess the ponds for the presence of great crested newts (*Triturus cristatus*). A map of the ponds surveyed is provided in Appendix 1.

Survey Methodology

The methodology supplied by SureScreen Scientific for standardised contamination free eDNA sampling was followed for all ponds surveyed. The sampling was completed by Assistant Ecologist Jake Healy and Assistant Ecologist Max Grindle. The samples were collected on 19th April 2022, which is within the acceptable eDNA sample period as defined by Natural England. Weather conditions were sunny with sparse passing clouds (13°C, 2/8 oktas) and a slight breeze (2 Beaufort scale).

Pond 1 was not surveyed as, during the time of surveying, it had dried over and no longer featured a body of standing water. Pond 2 was not surveyed due to a lack of access available; a construction site had fenced the area off. However, this pond was very shallow, contained no emergent or bankside vegetation, and was present atop a lined sheet of construction material on an active construction site. It is anticipated that this would greatly limit the likelihood of great crested newt presence.

Survey Results

The eDNA sampling returned negative results for great crested newts on all samples with no indication of degradation or contamination. Please refer to Appendix 2 for the attached survey return sheet, supplied by SureScreen Scientific.



Photograph 1 – Pond 1



Photograph 2 – Pond 2



Photograph 3 – Pond 3



Photograph 4 – Pond 4



Photograph 5 – Pond 5



Photograph 6 – Pond 6



Conclusion

The eDNA sampling returned negative results for great crested newts within Pond 3, Pond 4, Pond 5, and Pond 6; whilst Pond 1 and Pond 2 were assessed as unsuitable. It is deemed likely that great crested newts are absent from within the site based on previous survey data and the updated survey completed.

Yours Sincerely,

Max Grindle

Assistant Ecologist, BSc



Appendix 1 - Pond Locations Plan

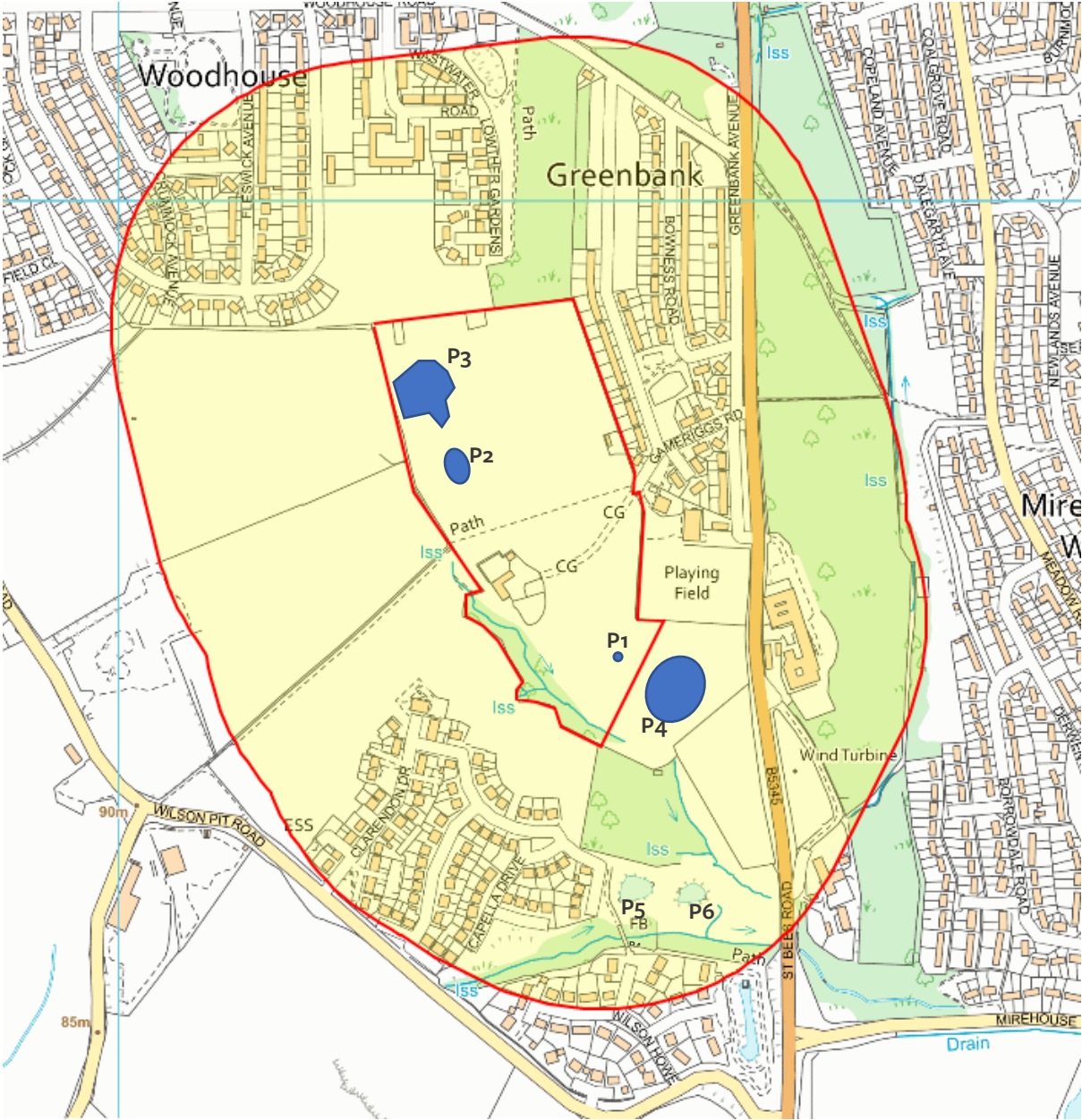


Figure 1. Map of ponds within 250m of the site boundary.

Appendix 2 - SureScreen Scientifics Results



Folio No: E12796
Report No: 1
Purchase Order: UG809
Client: URBAN GREEN
Contact: Jake Healy

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: 21/04/2022
Date Reported: 26/04/2022
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
1342	Pond 4	NX 97497 15513	Pass	Pass	Pass	Negative	0
1343	Pond 6	NX 97518 15346	Pass	Pass	Pass	Negative	0
1344	Pond 5	NX 97510 15350	Pass	Pass	Pass	Negative	0
1345	Pond 3	NX 97296 15786	Pass	Pass	Pass	Negative	0

If you have any questions regarding results, please contact us:

Reported by: Chris Troth

Approved by: Esther Strafford



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.

