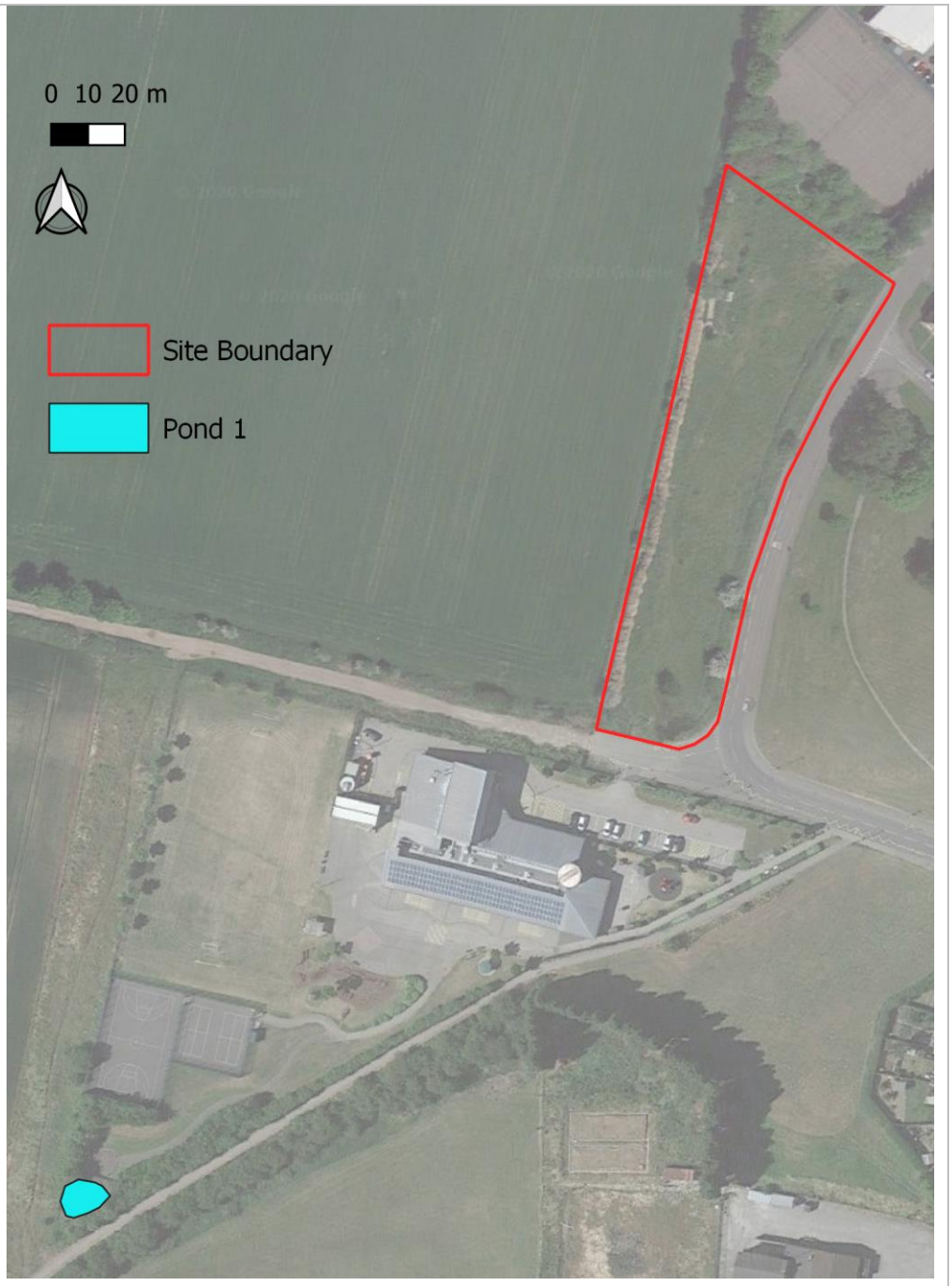
Site Inspection Report
Billingley View, Bolton upon Dearne

Project:	Billingley View, Bolton up Dearne	**Site Inspection reference:**	SI-4001-02
Date of inspection:	15.04.20	**No. of Pages:**	3
Inspection carried out by:	Sam Kitching BSc (Hons) GradCIEEM Ecologist		
Task description:	<u>eDNA survey</u> The presence of a single offsite pond, referred to as Pond 1, was flagged up by Barnsley Metropolitan Borough Council. The pond, which is not clearly visible on aerial photography, is located 190m southwest of the Site within the grounds of Lacewood Primary School (Fig 1). A water sample was collected from the Pond and set off to SureScreen for eDNA analysis to confirm the presence or likely absence of great crested newt (GCN). Access was provided by the school's onsite maintenance and management team. The eDNA survey involved taking water samples from 20 different locations around the pond's margin, focusing on areas where newts are most likely to gather. This involved using 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 15th April 2020 and the results returned on the 16th April 2020 - see Appendix 1 for full report. A negative result was returned, meaning that GCN DNA was <u>not</u> detected within the water sampled collected. This result can be used to demonstrate the likely absence of GCN from this pond.		
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.		

Unit A, 1 Station Road, Guiseley,
Leeds, LS20 8BX
Phone: **01943 884451**
Email:admin@brooks-ecological.co.uk
www.brooks-ecological.co.uk
Registered in England Number 5351418

Figure 1

Pond 1 in relation to Site



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Contact: Sam Kitching
Contact Details: sak@brooks-ecological.co.uk
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TECHNICAL REPORT

ANALYSIS OF ENVIRONMENTAL DNA IN POND WATER FOR THE DETECTION OF GREAT CRESTED NEWTS

Date sample received at Laboratory: 15/04/2020
Date Reported: 16/04/2020
Matters Affecting Results: None

RESULTS

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
0506	Billingley View Pond 1	SE 44462 02794	Pass	Pass	Pass	Negative	0

SUMMARY

When Great Crested Newts (GCN); Triturus cristatus inhabit a pond, they deposit traces of their DNA in the water as evidence of their presence. By sampling the water, we can analyse these small environmental DNA (eDNA) traces to confirm GCN habitation, or establish GCN absence.

The water samples detailed below were submitted for eDNA analysis to the protocol stated in DEFRA WC1067 (Latest Amendments). Details on the sample submission form were used as the unique sample identity.

RESULTS INTERPRETATION

Lab Sample No.- When a kit is made it is given a unique sample number. When the pond samples have been taken and the kit has been received back in to the laboratory, this sample number is tracked throughout the laboratory.

Site Name- Information on the pond.

O/S Reference – Location/co-ordinates of pond.

SIC- Sample Integrity Check. Refers to quality of packaging, absence of tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to results errors. Inspection upon receipt of sample at the laboratory. To check if the Sample is of adequate integrity when received. Pass or Fail.

DC- Degradation Check. Analysis of the spiked DNA marker to see if there has been degradation of the kit since made in the laboratory to sampling to analysis. Pass or Fail.

IC- Inhibition Check- PCR inhibitors can cause false results. Inhibitors are analysed to check the quality of the result. Every effort is made to clean the sample pre-analysis however some inhibitors cannot be extracted. An unacceptable inhibition check will cause an indeterminate sample and must be sampled again.

Result- NEGATIVE means that GCN eDNA was not detected or is below the threshold detection level and the test result should be considered as no evidence of GCN presence. POSITIVE means that GCN eDNA was found at or above the threshold level and the presence of GCN at this location at the time of sampling or in the recent past is confirmed. Positive or Negative.

Positive Replicates- To generate the results all of the tubes from each pond are combined to produce one eDNA extract. Then twelve separate analyses are undertaken. If one or more of these analyses are positive the pond is declared positive for the presence of GCN. 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.

METHODOLOGY

The laboratory testing adheres to strict guidelines laid down in WC1067 Analytical and Methodological Development for Improved Surveillance of The Great Crested Newt, Version 1.1

The analysis is conducted in two phases. The sample first goes through an extraction process where all six tubes are pooled together to acquire as much eDNA as possible. The pooled sample is then tested via real time PCR (also called q-PCR). This process amplifies select part of DNA allowing it to be detected and measured in 'real time' as the analytical process develops. qPCR combines PCR amplification and detection into a single step. This eliminates the need to detect products using gel electrophoresis. With qPCR, fluorescent dyes specific to the target sequence are used to label PCR products during thermal cycling. The accumulation of fluorescent signals during the exponential phase of the reaction is measured for fast and objective data analysis. The point at which amplification begins (the Ct value) is an indicator of the quality of the sample. True positive controls, negatives and blanks as well as spiked synthetic DNA are included in every analysis and these have to be correct before any result is declared so they act as additional quality control measures.

The primers used in this process are specific to a part of mitochondrial DNA only found in GCN ensuring no DNA from other species present in the water is amplified. The unique sequence appropriate for GCN analysis is quoted in DEFRA WC 1067 and means there should be no detection of closely related species. We have tested our system exhaustively to ensure this is the case in our laboratory. We can offer eDNA analysis for most other species including other newts.

Analysis of eDNA requires scrupulous attention to detail to prevent risk of contamination. Kits are manufactured by SureScreen Scientifics to strict quality procedures in a separate building and with separate staff, adopting best practice from WC1067 and WC1067 Appendix 5. Kits contain a 'spiked' DNA marker used as a quality control tracer (SureScreen patent pending) to ensure any DNA contained in the sampled water has not deteriorated in transit. Stages of the DNA analysis are also conducted in different buildings at our premises for added security.

SureScreen Scientifics Ltd also participate in Natural England's proficiency testing scheme and we also carry out inter-laboratory checks on accuracy of results as part of our quality procedures.

Reported by: Chris Troth **Approved by:** Chris Troth

End Of Report