

FAO Greg Brabin
Barratt Homes Manchester,
4 Brindley Road,
City Park,
Manchester
M16 9HQ

Job No. UG1795
Date 23rd May 2023

Dear Greg,

R.E: White Clawed Crayfish eDNA Survey at Hogshaw Farm, Buxton

The following letter statement has been produced to provide the results of eDNA surveys carried out in relation to the proposed works at Hogshaw Farm in Buxton on behalf of Barratt Homes. The PEA of the site, conducted in October 2022, identified the watercourse Nun Brook present adjacent to the northern and southern boundaries of the site, culverting underneath the adjacent Railway Land Hogshaw Local Wildlife Site. eDNA surveys were therefore recommended to assess Nun Brook for the presence of white-clawed crayfish (*Austropotamobius pallipes*).

Survey Methodology

The methodology supplied by SureScreen Scientific for standardised contamination free eDNA sampling was followed. The sampling was completed by Assistant Ecologists Max Grindle QCIEEM and Barney Gardner QCIEEM. The samples were collected on 12th April 2023, which is within the acceptable eDNA sample period as defined by SureScreen Scientific. Weather conditions were sunny with scattered clouds (7°C, 4/8 oktas) and a slight breeze (2 Beaufort scale).

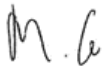
Survey Results

The eDNA sampling returned negative results for white-clawed crayfish with no indication of degradation or contamination. Please refer to Appendix 1 for the attached survey return sheet, supplied by SureScreen Scientific.

Conclusion

The eDNA sampling returned negative results for white-clawed crayfish within Nun Brook. It is deemed likely that white-clawed crayfish are absent from within the site based on these results.

Yours Sincerely,



Max Grindle BSc QCIEEM
Assistant Ecologist

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Appendix 1 - SureScreen Scientifics Results



Folio No: E16447
Report No: 1
Purchase Order: UG1108
Client: URBAN GREEN
Contact: Max Grindle

TECHNICAL REPORT

ANALYSIS OF ENVIRONMENTAL DNA SAMPLES FOR THE DETECTION OF CRAYFISH SPECIES AND CRAYFISH PLAGUE

SUMMARY

All organisms continuously release small amounts of environmental DNA (eDNA) into their habitat. By collecting and analysing this eDNA from water samples from lakes, ponds or rivers we can detect the presence or absence of crayfish species including: the white-clawed crayfish (*Austropotamobius pallipes*), signal crayfish (*Pacifastacus leniusculus*), the marbled crayfish (*Procambarus virginalis*) and the crayfish plague (*Aphanomyces astaci*).

RESULTS

Date sample received at Laboratory: 13/04/2023
Date Reported: 25/04/2023
Matters Affecting Results: None

Lab Sample ID.	Site Name	O/S Reference	Species	Result	SIC	DC	IC	Positive Replicates
FK1052	Nun Brook, Hogshaw Farm	SK06481 74361	White-Clawed Crayfish	Negative	Pass	Pass	Pass	0

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

Reported by: Gabriela Danickova

Approved by: Chris Troth



METHODOLOGY

The analysis is conducted in two phases. The sample first goes through an extraction process where the filter is incubated in order to obtain any DNA within the sample. The extracted sample is then tested via real time PCR (also called q-PCR) for each of the selected target species. This process uses species-specific molecular markers (known as primers) to amplify a select part of the DNA, allowing it to be detected and measured in 'real time' as the analytical process develops. qPCR combines amplification and detection of target DNA into a single step. With qPCR, fluorescent dyes specific to the target sequence are used to label targeted PCR products during thermal cycling. The accumulation of fluorescent signals during this reaction is measured for fast and objective data analysis. The primers used in this process are specific to a part of mitochondrial DNA only found in each individual species. Separate primers are used for each of the species: white-clawed crayfish, signal crayfish and crayfish plague, ensuring no DNA from any other species present in the water is amplified.

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. These methods have been extensively tested since 2015 in a number of different environments, habitats, conditions and ecological situations in order to successfully enable the full application of eDNA for the detection of crayfish species and the crayfish plague.

RESULTS INTERPRETATION

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 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 eDNA [Positive/Negative/Inconclusive]

Positive: DNA was identified within the sample, indicative of species 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 species 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 species presence.

Negative: eDNA was not detected or is below the threshold detection level and the test result should be considered as evidence of species absence, however, does not exclude the potential for species presence below the limit of detection.

Inconclusive: Controls indicate inhibition or degradation of the sample, resulting in the inability to provide conclusive evidence for species presence or absence.

