keystone
making ecology our business

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Mr S Kidley
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Your Ref:
Our Ref: 223340/5

27 September 2022

Dear Stephen,

Re: Quakers Yard, Treharris– White-clawed Crayfish eDNA Survey

Following Keystone Ecology's appointment to carry out a White-Clawed Crayfish eDNA survey of the River Bargoed Taf on site, water samples were collected from 20 locations along the watercourse in accordance with the technical white paper and filtration sample collection guidance produced by Surescreen Scientifics. Samples were taken on 15th August 2022 and sent for analysis by Surescreen Scientifics.

Please find appended the results which confirm absence of White-clawed Crayfish in the surveyed section of the River Bargoed Taf passing through the site.

Yours sincerely

Tas Adcock
Senior Ecologist

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SureScreen Scientifics

Folio No: E15274
Report No: 1
Purchase Order: 22/12269
Client: KEYSTONE ENVIRONMENTAL
Contact: Ron Thomas

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: 17/08/2022
Date Reported: 30/08/2022
Matters Affecting Results: None

Lab Sample ID.	Site Name	O/S Reference	Species	Result	SIC	DC	IC	Positive Replicates
FK847	Treharris		*White-Clawed Crayfish*	Negative	Pass	Pass	Pass	0

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

Reported by: Chelsea Warner **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.