URBAN GREEN

Taylor Wimpey

Date	23/07/2024
Project Ref	UG1451

Dear Cheryl,

Re: Standen Phase 5 and 6 – eDNA Surveys for White-Clawed Crayfish (*Austropotamobius pallipes*)

An eDNA survey of Pendleton Brook at Standen (hereafter referred to as "the site") was completed on 6th June 2024 by Jake Healy, Ecologist at Urban Green. The weather conditions were 15°C, passing clouds (3/8 oktas), with a windspeed of 4 on the Beaufort scale.

This report has been produced to demonstrate the results of environmental DNA (eDNA) of white clawed crayfish at the Site. Specifically, the survey of Pendleton Brook, which runs along the southern boundary of the site, was deemed suitable to support white-clawed crayfish. The purpose of the survey was to establish the presence or likely absence of white clawed crayfish on site. This report should be provided in support of the forthcoming planning application for the Standen residential housing development proposal.

Ecology Background

Pendleton Brook is located along the southern boundary; and qualifies as a habitat of principle importance, as determined by the Preliminary Ecological Appraisal (Urban Green 2021). The waterbody was found to have calm water, debris for refuges and good connectivity which provide suitable conditions to support white-clawed crayfish. This, combined with anecdotal evidence of suitable white-clawed crayfish habitat resulted in a recommendation to Taylor Wimpy to commission an eDNA survey for this species.

Relevant UK legislation for the protection of white-clawed crayfish is detailed in Appendix 1.

Methodology

A biosecurity friendly eDNA kit (which uses single use components to reduce risk of plague transmission) was used for sampling purposes.

Water samples are collected from the brook during a period of summer when water levels are lower with stable water temperatures (6th June) in line with government guidance. Twenty samples were collected from the brook, with the surveyor travelling in a zig-zag formation up stream, as to ensure no ancient sediment (which may contain historical DNA) becomes suspended in the water column.

The eDNA samples were sent on 6th June 2024 to a certified laboratory to be analysed.

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Results

On the 24th June, the eDNA analysis returned as 'negative' for white-clawed crayfish (see Appendix 2). A negative result indicates no white-clawed crayfish eDNA was detected or is below the threshold detection level. It is therefore assessed that white-clawed crayfish are likely absent from the waterbody.

See Appendix 2 for the full eDNA laboratory results and methodology.

Recommendations

No further surveys or mitigation are required for white-clawed crayfish for the works to lawfully proceed.

Notwithstanding this, indirect impacts to the brook are possible (as part of the proposed works) due to the proximity between the site and the development footprint. As this is a habitat of principle importance, it is recommended a minimum 10m buffer is implemented throughout the construction phase to reduce the potential of negative impacts (accidental spills, pollutant entering the watercourse).

Please let me know if you require any further information.

Yours sincerely,

[redacted]

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Appendix 1 – Relevant Legislation

Legislation for white-clawed crayfish

White-clawed crayfish are protected under Schedule 5 of the Wildlife and Countryside Act 1981 (as amended). Under this act it is an offence to:

- Intentionally take white-clawed crayfish from the wild; and,
- Sell or attempt to sell, any part of a white-clawed crayfish, alive or dead, or advertise that one buys or sells, or intends to buy or sell any part of a white-clawed crayfish.

The white-clawed crayfish is listed under Annex II and V of the EC Habitats Directive. The Conservation of Habitats and Species Regulations 2010 implements the European Union's 'Habitats Directive' (Council Directive 92/43/EEC (a) on the Conservation of Natural Habitats and of Wild Fauna and Flora) in Great Britain. Annex II requires that Special Areas of Conservation (SAC) are established specifically to conserve this and other listed species. In a SAC designated for white-clawed crayfish a precautionary principle must be applied when considering the potential impacts of any operations that may affect white-clawed crayfish and their habitat.

White clawed crayfish are listed as a SPI under Section 41 of NERC Act 2006.

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eDNA Report

Technical Report

SureScreen Scientifics

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Purchase Order: UG1320
Contact: Urban Green
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SureScreen Scientifics

eDNA Analysis

Summary

When aquatic organisms inhabit a waterbody such as a pond, lake or river 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 the presence or absence of the target species within the waterbody.

Results

Lab ID	Site Name	OS Reference	Target Species	Sample Integrity Check	Result	Positive Replicates
FK2136	Pend eton Brook	SD7435240536	White-c awed crayfish	Pass	Negative	0

Matters affecting result: none

Reported by:Lauryn Jewkes

Approved by: Chelsea Warner

SureScreen Scientifics

Methodology

Samples have been analyzed for the presence of target species eDNA following readily available and scientifically published eDNA assays and protocols.

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, ensuring no DNA from any other species present in the water is amplified. If target species DNA is present, the DNA is amplified up to a detectable level, resulting in positive species detection. If target 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 the risk of false positive and false negative results. 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. Stages of the analysis are also conducted in different buildings at our premises for added security. SureScreen Scientifics Ltd is ISO9001 accredited and participates in Natural England's proficiency testing scheme for GCN eDNA testing.

Interpretation of Results

Sample Integrity Check:

Laboratory Arrival:

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. Any samples which fail this test are rejected and eliminated before analysis.

Degradation and Inhibition check:

Analysis of the spiked DNA marker to see if there has been degradation or inhibition 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. If inhibition is detected, samples are purified and re-analyzed. 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.

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. Even a score as low as 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.