TECHNICAL NOTE

DATE:	14 December 2022	**CONFIDENTIALITY:**	Confidential
SUBJECT:	Cambridge South East Transport (CSET) White-Clawed Crayfish Survey Technical Note		
PROJECT:	ED/000553-02	**AUTHOR:**	Lucy Gradwell, Assistant Ecologist
CHECKED:	Tabatha Boniface, Technical Director	**APPROVED:**	Tabatha Boniface, Technical Director

INTRODUCTION

WSP RE&I Ecologists were commissioned by the Greater Cambridgeshire Partnership in July 2022 to undertake white-clawed crayfish *Austropotamobius pallipes* eDNA surveys in relation to the development of Phase 2 of the Cambridge South East Transport (CSET) Project. The results of the survey will be used to inform the design of the scheme.

LEGISLATION

White-clawed crayfish are listed under Schedule 5 of the Wildlife & Countryside Act 1981 (as amended), making it illegal to take them from the wild or sell them. It is listed under Annex II and V of the European Union Habitats Directive for which sites can be designated due to their presence and Appendix III of the Bern Convention. It is also a recognised UK Species of Principal Importance.

METHODS

The survey areas were tested for white-clawed crayfish using eDNA[1] sampling kits obtained from NatureMetrics Ltd. These kits were then sent back to NatureMetrics for laboratory analysis to determine the presence or absence of white-clawed crayfish within the sampled watercourses.

- The following four sites were sampled on 27 October 2022: River Granta at Cheveley (D1)
- River Granta at Deal (D2)
- Hobson's Conduit at Nine Wells (D4), and
- A pond at North Farm (WB45).

The site locations can be found on the map at Appendix A.

Samples were collected by Mark Johnson, Ecologist, and Tom Norman, Assistant Ecologist, following the protocol outlined by NatureMetrics, which is summarised below:

1. Using sampling bag and gloves provided, collect samples from the waterbody, minimising contact with the water during collection. Deposit the sample in the sampling bag, seal and shake for 20 to 30 seconds to ensure the water is well-mixed.
2. Draw up 100 ml of water from the sampling bag into a large syringe. Attach the syringe to the filter inlet and press the plunger to push the water through the filter.

[1] Short for 'environmental DNA'. Refers to DNA deposited in the environment through excretion, shedding, mucous secretions, saliva etc.

3. Repeat step two until all the water has been filtered or the filter clogs.
4. Detach the syringe from the filter and fill the syringe with air. Reattach the filter and push the air through to expel any water trapped inside the filter. Repeat several times to remove as much water as possible.
5. Transfer all of the preservative solution from the smaller syringe to the filter. Cap both ends of the filter using the Luer Lock caps.
6. Place the filter inside the specimen bag and seal. Return samples to lab within two weeks of sampling.

Laboratory testing of the samples was carried out. eDNA was extracted from NatureMetrics eDNA disk filters using commercially available DNA extraction kits, and further purified to remove inhibitors[2]. qPCR[3] amplification targeting white-clawed crayfish were carried out in 12 replicates per sample per target, using species-specific primers[4] and probes[5], in the presence of both positive and negative controls (template and extraction). A score is given for the number of positive replicates out of 12.

Constraints

One of the syringes in an eDNA kit provided by NatureMetrics was broken, meaning that this sample (Nine Wells) may have been compromised during the collection and transportation process.

A negative result does not preclude the presence of white-clawed crayfish at a level below the limits of detection.

RESULTS

The results returned from laboratory analysis of the samples are outlined in the table below.

Site Name	Result
River Granta at Cheveley (D1)	Negative
River Granta at Deal (D2)	Negative
Hobson's Conduit at Nine Wells (D4)	Negative
North Farm (WB45)	Negative

A negative result means that white-clawed crayfish eDNA was not detected in any of the collected samples. It can therefore be assumed that white-clawed crayfish are not present, or are present in extremely limited numbers, at the four sites sampled.

[2] Naturally-occurring chemicals/compounds that cause DNA amplification to fail, potentially resulting in false-negative results.

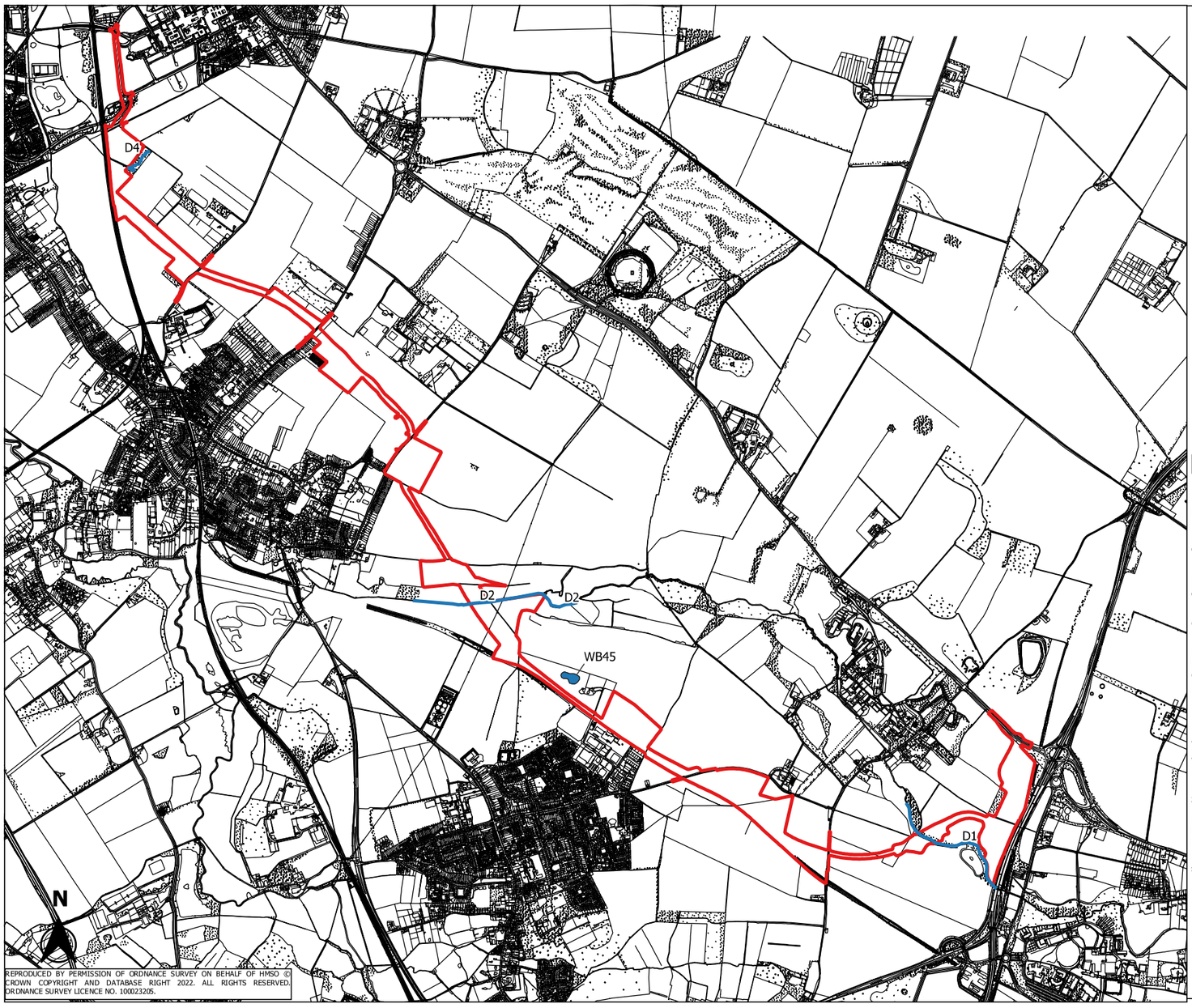
[3] Stands for 'quantitative PCR'. A PCR reaction incorporating a coloured dye that fluoresces during amplification, allowing a machine to track the progress of the reaction. If the species is not present in the sample, no fluorescence will be detected.

[4] Short sections of synthesised DNA that bind to either end of the DNA segment to be amplified by PCR.

[5] A short section of synthesised DNA that binds to a specific section of the target species' DNA within the section flanked by the primers. Designed to be totally specific to that species. It fluoresces during amplification, which is used to infer the presence of the target species' DNA in the sample.

This is consistent with the findings of surveys carried out in 2020 by Ecologylink through walkover survey and an assessment of the habitat suitability of this species.

APPENDIX A – SAMPLE LOCATIONS MAP



Red line boundary on 24.11.22

Pond sampled for white-clawed crayfish in 2022

Watercourses sampled for white-clawed crayfish in 2022

Rev	Drwn	Chkd	Appd	Date
P01.1	TR	LG	TB	5/12/2022

Purpose of Issue

S2 - Issued for Information

Classification

Commercial in Confidence

Client

Greater Cambridgeshire Partnership

Project

Cambridge South East Transport (CSET) Phase 2

Drawing

Waterbodies/watercourses sampled for white-clawed crayfish eDNA in 2022

Scale at A3	Drawn	Checked	Approved
1:20000	TR	LG	TB

Project No	Date
ED/000553	14/12/2022

Drawing Identifier	Revision
STND-WSP-EBD-XX-GS-LE-1064	P01.1