

TECHNICAL
NOTE

Cambridge South East Transport
Phase 2

SUBJECT	PROJECT NO.	DATE
CSET2 White-clawed crayfish environmental DNA survey	100102211.004	09 February 2026

Notice

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This document does not purport to provide legal advice.

This document has 9 pages including the cover.

Document history

Revision	Purpose description	Originated	Checked	Reviewed	Authorised	Date
1.0	Issue	AW	NC	LMG	EK	09/02/2026

Client signoff

Client	Cambridgeshire County Council		
Project	Cambridge South East Transport Phase 2	Project No.	100102211.004
Client signature/ date			

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1. Introduction

1.1 Terms of Reference

AtkinsRéalis UK Limited was commissioned by the Greater Cambridgeshire Partnership (GCP) to undertake a white-clawed crayfish (WCC) environmental DNA (eDNA) survey at the River Granta and a pond at Babraham for the Cambridge South East Transport route (CSET2) scheme (herein referred to as the Proposed Development).

WCC are protected under the Wildlife and Countryside Act 1981 (as amended) which makes it an offence to intentionally take from the wild. It is also an offence to intentionally or recklessly: possess or transport them (or any part, alive or dead) or sell, offer, or publish an advert to sell them.

1.2 The Site and Proposed Scheme

The GCP is the local delivery body for a City Deal signed by central Government to bring powers and investment to the region, including improvements to critical infrastructure. The GCP comprises five partners:

- Cambridge City Council;
- Cambridgeshire County Council;
- Cambridgeshire & Peterborough Combined Authority Business Board
- South Cambridgeshire District Council; and
- University of Cambridge.

The Proposed Development is located approximately 3.3 km southeast of Cambridge City Centre.

The Proposed Development is a mostly new, segregated, guided bus route, travel hub, and active travel path located to the south east of the city. The site comprises all of the land within the red line boundary which coincides with the proposed Order Limits as defined in the draft Transport and Works Act Order (TWAO) accompanying the application.

The Proposed Development begins at Ordnance Survey National Grid Reference (OSNGR) TL 45981 55041 and ends at OSNGR TL 52090 50137 (hereafter referred to as the Application Site).

1.3 Background and Scope of the Assessment

eDNA surveys for WCC were originally undertaken for the Proposed Development in 2022 which returned negative results for all watercourses and waterbodies sampled. Due to the time that had elapsed since the previous survey, an updated WCC eDNA survey was undertaken in 2025 of the River Granta at Staplegord and a large pond at Babraham.

2. Survey Methodology

WCC release DNA into the water (eDNA) which can be detected by analysing water samples to confirm their presence or likely absence within watercourses or waterbodies. The eDNA surveys involved the collection of water samples from

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the River Granta at the downstream limit of the Proposed Development and a pond at Babraham. This was conducted by suitably experienced ecologists on the 27th and 28th October 2025. Sampling followed a scientifically-rigorous methodology to minimise cross-contamination, with sterile kits supplied by SureScreen Scientifics. The use of eDNA sampling to confirm WCC presence is a survey method approved by Natural England¹.

The samples were then analysed at the laboratory using real-time Polymerase Chain Reaction (PCR) to test for WCC-specific DNA markers. SureScreen provided a number of positive qPCR replicates out of a series of 12 for each site. If one or more of these are found to be positive, the watercourse is declared positive for WCC 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.

This is due to an extremely high number of variable factors which can affect eDNA sampling and the distribution of eDNA across ecological sites. Such factors which can affect detection include differences in seasonal species-specific activity levels; how a species sheds DNA; size of site; population size; population locality to sample collection area; weather (including temperature and flow rate); and the presence of bacteria and inhibitory molecules. Without having an accurate picture of all of these factors, it can be difficult to link DNA concentrations within the sample to population sizes within the sample site².

3. Limitations

No limitations were encountered during the survey.

4. Results

The laboratory returned negative results (0/12 replicates) for WCC eDNA at the River Granta sampling location and at the pond at Babraham. Therefore, WCC are likely absent at these locations.

The River Granta tested positive for signal crayfish (12/12 replicates) which is an invasive species³ introduced into the UK in the 1960s and partly responsible for the decline of native WCC, which further reduces the potential for WCC to be present.

The River Granta also tested positive for crayfish plague which is a disease that affects WCC and has contributed to significant WCC population declines.

The SureScreen laboratory reports have been appended to this report (**Error! Reference source not found.**).

¹ Environment Agency (2024). Operationalisation of eDNA methods for Crayfish monitoring. Environment Agency, Bristol.

² Email communication with Forensic Ecology department of SureScreen Scientifics, received on 30/07/2025

³ Listed on Schedule 09 of the Wildlife and Countryside Act 1981 (As amended) which are species that are illegal to plant or allow to spread in the wild.

Appendix A. SureScreen Lab Reports

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A.1 River Granta

Folio No: 4352-2025
Purchase Order: 2410
Contact: Atkins Réalis
Issue Date: 24.11.2025
Received Date: 10.11.2025

eDNA Report

Technical Report



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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

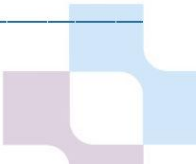
Lab ID	Site Name	OS Reference	Target Species	Sample Integrity Check	Result	Positive Replicates
FK3941	Stapleford River Granta	TL 48054 51244	White-clawed crayfish (Austropotamobius pallipes)	Pass	Negative	0
			Signal crayfish (Pacifastacus leniusculus)	Pass	Positive	12
			Crayfish plague (Aphanomyces astaci)	Pass	Positive	1

Matters affecting result: none

Reported by: Vanessa Hind

Approved by: Vanessa Hind

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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)

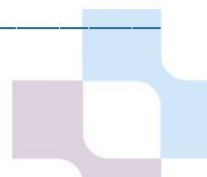
Positive: DNA was identified within the sample, indicative of species presence within the sampling location at the time the sample was taken or within the recent past.

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.

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A.2 Pond at Babraham

Folio No: 4354-2025
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