



Hallgates to Elms Farm Pipeline Route

Leicester

Protected Species Report: Crayfish

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Drawing 1: Crayfish Survey Results Plan (Drawing Ref: G10480.019)

Executive Summary

Site Details	▶ The proposed pipeline route is approximately 13 km in length and is located between Bradgate Park, near Cropston (approximate grid reference SK 53423 11450) and fields to the west of the A46 road near Groby (approximate grid reference SK 52651 05979).
Survey Details	▶ Crayfish surveys were undertaken at three watercourse crossings (WC2, WC3 and WC4) on 12th and 30th September 2024. The surveys extended 250m upstream and downstream of each watercourse crossing location. ▶ WC1 was not subject to survey as access had not been granted by the land owner.
Summary	▶ White-clawed crayfish were confirmed absent from WC2, WC3 and WC4. There are no implications to the works in relation to white-clawed crayfish. ▶ Signal crayfish were confirmed present in each of WC2, WC3 and WC4. ▶ Crayfish plague was confirmed absent from WC2, WC3 and WC4.
Recommendations	▶ As signal crayfish is an invasive species, all works within each watercourse crossing must be supervised by a suitably qualified ecologist. The ecologist will humanely capture and kill any signal crayfish encountered during the works. ▶ Biosecurity measures are recommended during the works within each watercourse to prevent the spread of zoonotic diseases and accidental spread of signal crayfish. ▶ A crayfish survey of WC1 will be required once access can be arranged.

This Executive Summary is not a substitute for the full report. Refer to the full text of this report for further detail.

1.0 Introduction

- 1.1 The Environment Partnership (TEP) was commissioned by Severn Trent Water (STW) in May 2024 to undertake crayfish surveys to support proposals for the construction of a new water pipeline to the north-west of Leicester.
- 1.2 Surveys were undertaken at three watercourse crossings (WC2, WC3 and WC4), where the watercourses are crossed by the proposed pipeline route. The watercourse crossings were identified as potentially suitable for crayfish during the preliminary habitat survey conducted by TEP in May 2024 to inform the Preliminary Ecological Assessment (PEA) report (TEP Report Ref: 10480.001, June 2024).
- 1.3 This technical report includes details of the methods employed and any limitations of the surveys undertaken. Results are provided with supporting maps, together with an evaluation of the ecological features within the site, including an assessment of the potential impacts associated with the development proposals in relation to crayfish, and requirements for mitigation.

Site Location

- 1.4 The proposed pipeline route is approximately 13 km in length and is located between Bradgate Park, near Cropston (approximate grid reference SK 53423 11450) and fields to the west of the A46 road near Groby (approximate grid reference SK 52651 05979) (Figure 1).

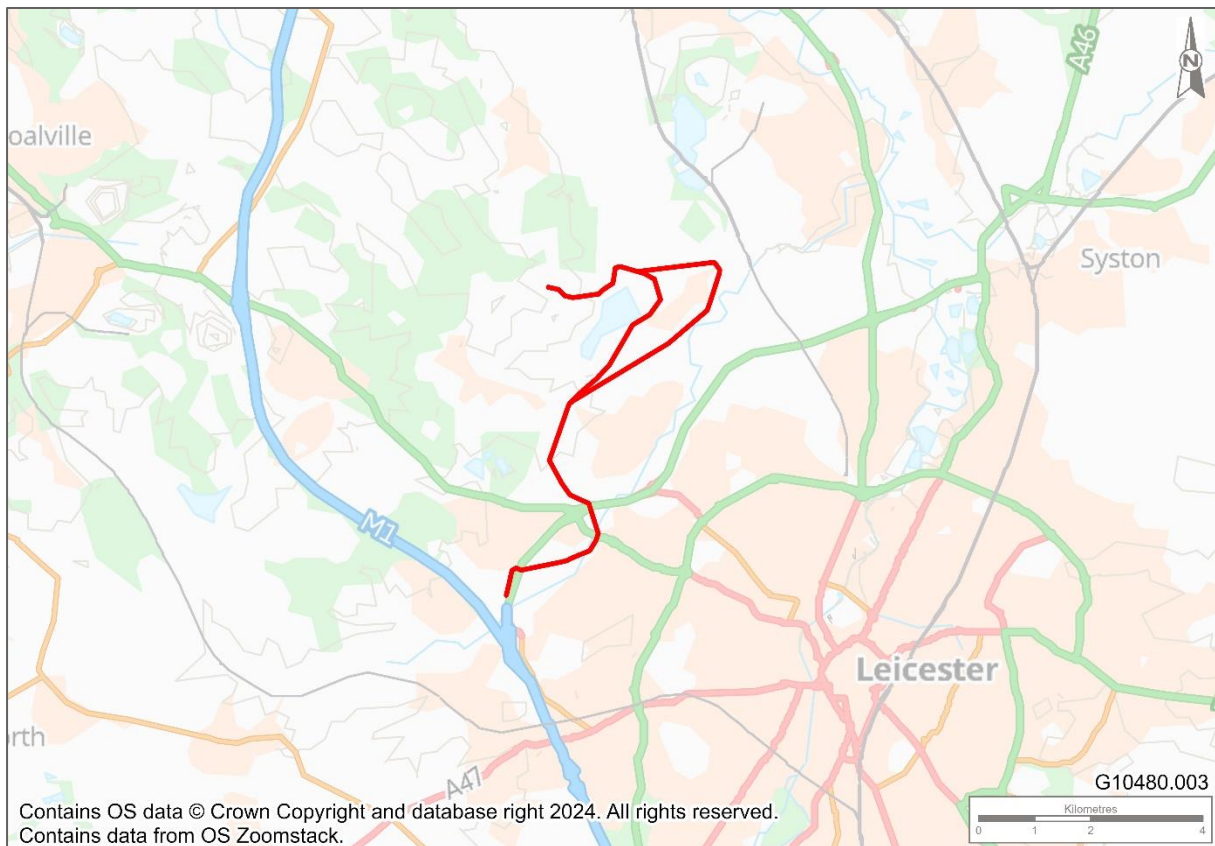


Figure 1: Site location and local context

Legislation

- 1.5 White-clawed crayfish is the only native UK freshwater crayfish species. It is fully protected as a European Protected Species (EPS) listed under Annex II and V of the EU Habitats Directive and Appendix II of the Bern Convention. It is also protected under Schedule 5 (WCA5) of the Wildlife and Countryside Act 1981 (as amended) and is listed as a Species of Principal Importance (SPI) under Section 41 of the Natural Environment and Rural Communities Act (2006). The legislation lists the following as offences:

- It is an offence to intentionally take or sell (live or dead), trap, handle, or harm white-clawed crayfish.
- In the UK, it is an offence to keep any crayfish without a licence, except in some parts of southern England.
- It is an offence to trap or remove native crayfish without a licence from the Environment Agency. Trapping must only be undertaken for scientific purposes.

- 1.6 Signal crayfish *Pacifastacus leniusculus* is an invasive species listed under Schedule 9 (WCA9) of the Wildlife and Countryside Act 1981 (as amended). The legislation lists the following as offences:
- It is an offence to release or allow the escape of this species into the wild.
 - In the UK, it is an offence to keep any crayfish without a licence, except in some parts of southern England.
 - It is an offence to trap or remove non-native crayfish without a licence from the Environment Agency.

Survey Rationale

- 1.7 The white-clawed crayfish survey is designed to establish the current status of white-clawed crayfish in the survey area and to evaluate the importance of the survey area for the species. The surveys were also designed to establish if non-native signal crayfish and the water mould crayfish plague *Aphanomyces astaci* are present. This information will be used to assess potential constraints in relation to crayfish to the proposed pipeline installation works.

2.0 Methods

Data Search

- 2.1 As part of the PEA report, species records for the last 10 years within 1km of the site were requested from Leicestershire and Rutland Environmental Records Centre (LRERC).

Field Survey

- 2.2 The preliminary habitat survey undertaken as part of the PEA identified that the watercourse crossings (WC2, WC3 and WC4) and associated upstream and downstream riparian habitats had the potential to support crayfish.
- 2.3 WC2 is located within an un-named stream which flows east to connect to Rothley Brook. WC3 and WC4 are both located within Rothley Brook.
- 2.4 WC1 was not subject to survey as access was not granted by the landowner.

Habitat Suitability Assessment and Manual Search

- 2.5 Habitat suitability assessment and manual search surveys for crayfish were undertaken at three watercourse crossings (WC2, WC3 and WC4) by TEP on 30th September 2024.
- 2.6 The manual search surveys were undertaken during the correct survey season for white-clawed crayfish surveys (July to September inclusive).
- 2.7 The crayfish surveys were undertaken by a suitably qualified Ecologist, who holds a white-clawed crayfish licence, accompanied by a health and safety assistant.
- 2.8 The survey included the watercourse crossing points, and extended 250m upstream and downstream of the watercourse crossing points. The location of the watercourse crossing points and survey area extents are shown on Drawing G10480.019 Crayfish Survey Results Plan.
- 2.9 Walking upstream, ecologists assessed the suitability of the tributary to support crayfish. This involved recording physical characteristics of the watercourse (such as channel width and depth, substrate, bank profile and flow types) and the vegetation structure within the channel and along the banks. Features offering suitable refuge habitat were recorded and inspected for crayfish. Suitable features include undercut banks, submerged tree roots, cobbles, rocks / stones, boulders and dams.

- 2.10 Invertebrate species were also recorded in order to further ascertain the suitability of the tributary for crayfish and to provide an indication of water quality levels.
- 2.11 Manual search surveys, including kick-sampling, sweep netting and stone turning were undertaken in line with current best practice guidance (Peay, 2003)¹. Kick sampling and sweep netting of vegetation was undertaken at regular intervals within each watercourse survey area extent. Manual survey was undertaken of individual refuges, which included turning of 300 fist-sized rocks within each watercourse.

Environmental DNA (eDNA)

- 2.12 Environmental DNA (eDNA) sampling was undertaken at WC2 on 12th September 2024. eDNA sampling was undertaken at WC3 and WC4 on 30th September 2024. White-clawed crayfish, signal crayfish and crayfish plague were tested for.
- 2.13 The eDNA surveys were undertaken during the correct survey season for white-clawed crayfish surveys (April to October inclusive).
- 2.14 Sample collection was undertaken by a suitably qualified Ecologist, accompanied by a health and safety assistant. Sample filter kits and analysis were provided by SureScreen Scientifics. In summary the sampling protocol is as follows:
- 20 water samples were taken from the watercourse perimeter. The location of the samples were spaced as evenly as possible. Samples were taken against the flow of the stream, working upstream in a diagonal pattern where possible.
 - The surveyor stayed out of the water while taking the samples (extension poles were used in situations where open/sufficiently deep water was at a distance from the dry banks) to avoid any disruption of sediment.
 - The water sample was taken from the middle of the water column (at least 10cm from the bottom where possible).
 - Once 20 water samples were collected, the bag was closed securely and shaken to mix the water sample. 50ml water samples were passed into and through the filter kit until a minimum of 250ml of water had passed through the filter. Preservative solution was added into the filter unit to prevent sample degradation during transport and caps were fitted to both ends of the filter unit.
 - At all times the surveyor ensured that the risk of contaminating the sampling equipment was minimised by avoiding the placement of the syringe or filter unit on the ground or on

¹ Peay S (2003). Monitoring the White-clawed Crayfish *Austropotamobius pallipes*. Conserving Natura 2000 Rivers Monitoring Series No. 1, English Nature, Peterborough.

any otherwise potentially contaminated surfaces and by changing gloves between the initial sampling stage and the syringing stages of the method.

Chain of Custody

- 2.15 On receipt from SureScreen Scientifics, the sampling kit was registered on a central database using the unique bar code. Immediately prior to survey, sampling kit was issued to the surveyor with an individual Sample Form using the unique bar code as identification. The site name and date of issue was also recorded on this form (and on the central database). Once in the field and at the watercourse, the surveyor confirmed that the appropriate field survey sheet was being completed by checking the bar code on the box and double checking the corresponding bar code on the sample tube. The surveyor then filled in the date of survey and the watercourse ID number (as well as other information relating to survey conditions) on the Sample Form.
- 2.16 On returning to the office the Sample Form was signed to confirm who received and checked the sample into the fridge, and the temperature of the fridge. The watercourse ID on the form was checked against a site map to confirm which watercourse had been sampled and this map was stored with the Sample Form. All this information was also recorded on the central database. The sample preserving tube was stored in a fridge until the morning of collection by the courier. The Sample Form and the central database were updated to confirm the date of collection by the courier.
- 2.17 A unique bar code was used by SureScreen Scientifics to report results. All results were recorded in the central database by one member of staff and cross checked by a second member of staff before issuing to the project leader for review.
- 2.18 The recommended period for carrying out white-clawed crayfish eDNA and other non-intrusive surveys is May to October (inclusive). The recommended period for manual search surveys is July to September, once the crayfish have released their young and are no longer as vulnerable to disturbance. The survey was carried out during the recommended survey period in late-July.

Assumptions

- 2.19 Information provided by third parties, including publicly available information, is assumed to be correct at the time of publication.

3.0 Results

Data Search

- 3.1 There are two records of white-clawed crayfish (EPS, WCA5, SPI, LBAP²) within 1km of the site; the closest record to site is associated with Rothley Brook and is located approximately 0.59 km to the south of the site near Ratby. These records are all from 2014.
- 3.2 Three records of the invasive non-native species signal crayfish (WCA9) were also returned within Rothley Brook within 1km of the site, dated 2015, 2017 and 2019. The nearest record is located approximately 0.26km east of the site boundary.

Field Survey

Habitat Suitability Assessment and Manual Search

Watercourse Crossing WC2

- 3.3 The water level was shallow, with a water depth up to 1m, at the time of survey. The watercourse was flowing, indicating that it is oxygenated. The flow was varied, with some static pools, slow flowing sections and faster flowing sections which would contain more oxygen.
- 3.4 The banks were vegetated by occasional trees and scrub, with frequent herbs, occasional grasses and rarely occurring sedges. No aquatic in-channel vegetation was present.
- 3.5 The base of the watercourse comprises gravel with stones and rocks. Dead wood and debris was also present within the channel. The stones, rocks, dead wood and debris provide potential refugia and sheltering opportunities for crayfish.
- 3.6 The banks are largely shallow, however, some vertical sections comprising soft, bare earth are present which are suitable for crayfish to burrow.
- 3.7 Fish were identified within the tributary at the time of survey. Fish decrease the suitability of the watercourse as they can predate on juvenile and small adult crayfish. Stickleback (Gasterosteidae) and bullhead fish *Cottus gobio* were observed.

² Local Biodiversity Action Plan Species

3.8 Moderate invertebrate numbers and diversity were noted, indicating moderate water quality. The following species were recorded during the survey:

- Mayfly nymph Baetidae sp.
- Freshwater shrimp *Gammarus pulex/fossarum agg.*
- Water snail Lymnaeidae sp.
- Blood worm *Glycera sp.*
- Pea mussel *Pisidium pulchellum*
- Burrowing mayfly Ephemera sp.
- Diving beetle Dytiscidae sp.
- Water beetle Dytiscidae sp.
- Cased caddis-fly *Trichoptera sp.*
- Water cricket Veliidae sp.

3.9 The habitat was assessed as being suitable for crayfish, with flowing oxygenated water of a moderate water quality, rocks and debris providing suitable cover, and earth banks suitable for burrowing.

3.10 No white-clawed crayfish or signal crayfish were identified during the survey.

Watercourse Crossing WC3

3.11 The water level was shallow, with a water depth up to 1m, at the time of survey. The watercourse had a fast flow, indicating oxygenated water.

3.12 The banks were vegetated by frequent trees, scrub and herbs, with limited sedges and grasses. Limited in-channel aquatic vegetation was present.

3.13 The base of the watercourse comprises gravel with stones and rocks. Dead wood and debris was also present within the channel. The stones, rocks, dead wood and debris provide potential refugia and sheltering opportunities for crayfish.

3.14 The banks are largely shallow, however, some vertical sections comprising soft, bare earth are present which are suitable for crayfish to burrow.

3.15 No fish were identified.

3.16 Moderate invertebrate numbers and diversity were noted, indicating a moderate water quality. The following species were recorded during the survey:

- Mayfly nymph Baetidae sp.

- Burrowing mayfly nymph Ephemeridae sp.
- Freshwater shrimp *Gammarus pulex/fossarum agg.*
- Pea mussel *Pisidium pulchellum*
- Pond skater Gerridae sp.
- Blackfly larvae Simuliidae sp.
- Non-biting midges Chironomidae sp.
- Midge larvae Chironomidae sp.
- Aquatic worm Oligochaeta sp.
- Leech Hirudinea sp.
- Flatworm Platyhelminthes sp.
- Water slater *Asellus aquaticus*

3.17 The habitat was assessed as being suitable for crayfish, with flowing oxygenated water of a moderate water quality, multiple rocks suitable for cover and vegetated banks suitable for burrowing.

3.18 No white-clawed crayfish or signal crayfish were identified during the manual search survey. However, an incidental sighting of a predated upon signal crayfish was noted at the watercourse crossing during an otter and water vole survey undertaken by TEP on 18th September 2024.

Watercourse Crossing WC4

3.19 Watercourse crossing WC4 has the same geomorphic structure as watercourse crossing WC3, with the shallow water levels, a fast flow, a mixture of shallow and steep banks, a base comprising gravel, stones and rocks, and debris within the channel.

3.20 The banks were vegetated by abundant trees and scrub, frequent herbs, and rarely occurring sedges and grasses. Limited aquatic vegetation was present.

3.21 Moderate invertebrate numbers and diversity were noted, indicating a moderate water quality. The invertebrate species recorded were the same as within watercourse crossing WC3.

3.22 The habitat was assessed as being suitable for crayfish, with flowing oxygenated water of a moderate water quality, multiple rocks suitable for cover and earth banks suitable for burrowing.

3.23 Four signal crayfish were found, all at approximately the same location within the watercourse. These are detailed in table 1 below.

Table 1: Biometric data from signal crayfish caught at WC4

ID	Grid reference	Sex	Carapace length (mm)	Damage / disease
1	SK 5404 0672	Male	39	Right claw missing
2	SK 5404 0672	Male	29	Left claw regenerating
3	SK 5404 0672	Female	20	Left claw missing
4	SK 5404 0672	Female	24	No damage / disease

Environmental DNA (eDNA) Survey

- 3.24 The eDNA results confirmed white-clawed crayfish to be absent from WC2, WC3 and WC4.
- 3.25 The results confirmed signal crayfish to be present within WC2, WC3 and WC4.
- 3.26 The results confirmed crayfish plague to be absent from WC2, WC3 and WC4.
- 3.27 Detailed results of the eDNA surveys are provided at Annex A.

4.0 Photographs

Watercourse Crossing WC2

Figure 2: WC2 – Overview (a)



Figure 3: WC2 – Overview (b)



Figure 4: WC2 – Overview (c)



Watercourse Crossing WC3

Figure 5: WC3 – Overview (a)



Figure 6: WC3 – Overview (b)



Figure 7: WC3 – Signal crayfish remains (a)



Figure 8: WC3 – Signal Crayfish remains (b)



Watercourse Crossing WC4

Figure 9: WC4 – Overview (a)



Figure 10: WC4 – Overview (b)



Figure 11: WC4 – Signal crayfish 1 (a)



Figure 12: WC4 – Signal Crayfish 1 (b)



5.0 Evaluation and Recommendations

- 5.1 A habitat assessment and manual search survey, including kick-sampling and netting, and eDNA sampling have been undertaken at watercourse crossings WC2, WC3 and WC4.
- 5.2 All watercourse crossings were assessed as being suitable habitat for crayfish due to the presence of flowing, oxygenated water, earth banks for burrowing, stones, rocks and debris which provide refugia, and a moderate water quality.
- 5.3 No evidence of white-clawed crayfish was recorded during the manual search survey. The eDNA analysis confirmed white-clawed crayfish to be absent from WC2, WC3 and WC4. White-clawed crayfish are not a constraint in respect to the development. No further action is required in respect to white-clawed crayfish.
- 5.4 Signal crayfish were recorded at WC3 and WC4 during the manual search survey. Signal crayfish were confirmed to be present at WC2, WC3 and WC4 through eDNA analysis. Signal crayfish is a non-native invasive species (WCA9). It is an offence to release or allow the escape of this species into the wild. All works within each watercourse crossing must therefore be supervised by a suitably qualified ecologist. The ecologist will humanely capture and kill any signal crayfish encountered during the works. The dead signal crayfish will then be left on site.
- 5.5 Crayfish plague was confirmed to be absent at WC2, WC3 and WC4 through eDNA analysis. Although the fungus is currently absent from the watercourses, there is potential that the fungus may be transmitted to the watercourses in the future, through expansion of any infected signal crayfish populations into the watercourses, transmission of infected crayfish into the watercourses by wildlife (e.g. otter), and transmission of viable zoospores through water or on fish skin.
- 5.6 Biosecurity measures are recommended when undertaking works within each watercourse to prevent the spread of zoonotic diseases and the accidental spread of signal crayfish, as follows:
 - All equipment, machinery and footwear (wellie boots, waders etc) must be washed and disinfected prior to their use within any on-site watercourse.
 - All equipment, machinery and footwear subsequently used within any on-site watercourse must then be washed and disinfected on site before its use within any other on-site or off-site watercourse. All mud must be removed. The item must then be thoroughly disinfected and left to dry out.
 - Disinfectants which can be used include a hypochlorite solution such as domestic bleach, or an iodophore-type disinfectant at 100ppm available iodine used for at least five minutes.

Disinfectants can be applied through a spray applicator or by washing within a bowl of disinfectant.

- Any sediment / silt removed from a watercourse crossing should ideally be returned to the same watercourse crossing following completion of works. Silt / sediment must not be transferred to any other watercourse on site or off-site site. If the sediment / silt needs to be removed from site, it should be taken to an appropriate waste disposal facility.

- 5.7 A habitat suitability assessment to assess the suitability of WC1 for crayfish is required. If the watercourse is suitable, a field survey to confirm the presence / absence of white-clawed crayfish, signal crayfish and crayfish plague will be required.
- 5.8 Crayfish surveys are valid for two years from the survey date. The surveys are therefore valid until September 2026. Repeat surveys will be required if works have not commenced by this date.

Annex A: SureScreen Scientifics eDNA Results

Folio No: 3588-2024
Purchase Order: PO 36413/10480
Contact: The Environment Partnership (TEP)
Issue Date: 24.10.2024
Received Date: 10.10.2024

eDNA Report

Technical Report



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
FK2711	Hallgate to Elms farm - 10480 WC2	SK 54012 07659	Crayfish plague	Pass	Negative	0
			Signal crayfish	Pass	Positive	1
			White-clawed crayfish	Pass	Negative	0
FK2712	Hallgate to Elms farm - 10480 WC4	SK 54045 06720	Crayfish plague	Pass	Negative	0
			Signal crayfish	Pass	Positive	11
			White-clawed crayfish	Pass	Negative	0
FK2713	Hallgate to Elms farm - 10480 WC34	SK 54297 07176	Crayfish plague	Pass	Negative	0
			Signal crayfish	Pass	Positive	11
			White-clawed crayfish	Pass	Negative	0



Folio No: 3588-2024
Purchase Order: PO 36413/10480
Contact: The Environment Partnership (TEP)
Issue Date: 24.10.2024
Received Date: 10.10.2024

Matters affecting result: none

Reported by: Chelsea Warner

Approved by: Lauryn Jewkes

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.

Drawings

Drawing 1: Crayfish Survey Results Plan (Drawing Ref: G10480.019)



KEY

- Pipeline route
- Watercourse surveyed (250m upstream and 250m downstream of crossing)

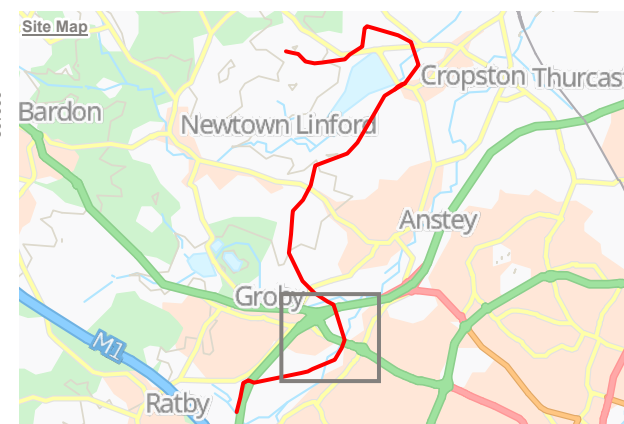
Crayfish Evidence

- Signal crayfish
- No evidence of white-clawed crayfish was recorded during the surveys

Note:
The locations of species, habitats and habitat features are indicative.

No evidence of crayfish was recorded during the survey of watercourse crossing 2.

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Rev	Description	Drawn	Approved	Date

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Project
Hallgates to Elms Farm Pipeline, Leicestershire

Title
Crayfish Survey Results Plan

Drawing Number
G10480.019

Drawn	Checked	Approved	Scale	Date
IJ	SP	KM	1:5,000 @ A3	17/10/2024

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