Sheringham Shoal and Dudgeon Offshore Wind Farm Extension Projects

Environmental Statement

Volume 3

Appendix 20.9 – White Clawed Crayfish Survey Report (Revision B) (Tracked)

Revision B
Deadline 3
May 2023
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The data which we have prepared and provided is accurate, and has been prepared and provided in accordance with the CIEEM's Code of Professional Conduct. We confirm that any opinions expressed are our best and professional bona fide opinions.

This report conforms to the British Standard 42020:2013 Biodiversity – Code of practice for planning and development.

CONTENTS

LIST OF ACRONYMS

ASC	American Signal Crayfish
DCO	Development Consent Order
DEP	Dudgeon Offshore Wind Farm Extension Project
eDNA	Environmental DNA
EP1HS	Extended Phase 1 Habitat Survey
EPS	European Protected Species
ETG	Expert Topic Group
NBIS	Norfolk Biodiversity Information Service
NCG	Norfolk Crayfish Group
NRT	Norfolk Rivers Trust
OS	Ordnance Survey
PEIR	Preliminary Environmental Information Report
SEP	Sheringham Shoal Offshore Wind Farm Extension Project
SSL	SureScreen Scientifics Ltd.
WCC	White-clawed Crayfish
WFE	Wild Frontier Ecology Ltd.

GLOSSARY OF DEFINITIONS

Term	Definition
Order Limits / DCO boundary	The area subject to the application for development consent, including all permanent and temporary works for SEP and DEP.
Dudgeon Offshore Wind Farm Extension Project (DEP)	The Dudgeon Offshore Wind Farm Extension onshore and offshore sites including all onshore and offshore infrastructure.
DEP onshore site	The Dudgeon Offshore Wind Farm Extension onshore area consisting of the DEP onshore substation site, onshore cable corridor, construction compounds, temporary working areas and onshore landfall area.
European site	Sites designated for nature conservation under the Habitats Directive and Birds Directive. This includes candidate Special Areas of Conservation, Sites of Community Importance, Special Areas of Conservation and Special Protection Areas, and is defined in regulation 8 of the Conservation of Habitats and Species Regulations 2017.
Evidence Plan Process (EPP)	A voluntary consultation process with specialist stakeholders to agree the approach, and information to support, the EIA and HRA for certain topics.
Expert Topic Group (ETG)	A forum for targeted engagement with regulators and interested stakeholders through the EPP.
Horizontal directional drilling (HDD) zones	The areas within the onshore cable route which would house HDD entry or exit points.
Jointing bays	Underground structures constructed at regular intervals along the onshore cable route to join sections of cable and facilitate installation of the cables into the buried ducts.
Landfall	The point at the coastline at which the offshore export cables are brought onshore, connecting to the onshore cables at the transition joint bay above mean high water
Onshore cable corridor	The area between the landfall and the onshore substation sites, within which the onshore cable circuits will be installed along with other temporary works for construction.
Onshore export cables	The cables which would bring electricity from the landfall to the onshore substation. 220 – 230kV.
Onshore Substation	Compound containing electrical equipment to enable connection to the National Grid.
PEIR boundary	The area subject to survey and preliminary impact assessment to inform the PEIR.
Sheringham Shoal Offshore Wind Farm Extension Project (SEP)	The Sheringham Shoal Offshore Wind Farm Extension onshore and offshore sites including all onshore and offshore infrastructure.
SEP onshore site	The Sheringham Shoal Wind Farm Extension onshore area consisting of the SEP onshore substation site, onshore cable corridor, construction compounds, temporary working areas and onshore landfall area.
Study area	Area where potential impacts from the project could occur, as defined for each individual Environmental Impact Assessment (EIA) topic.
The Applicant	Equinor New Energy Limited

EXECUTIVE SUMMARY

Wild Frontier Ecology Ltd. was commissioned by Equinor New Energy Ltd. to undertake white-clawed crayfish (WCC) surveys of all suitable watercourses within the Development Consent Order (DCO) boundary associated with the proposed and Sheringham Shoal Offshore Wind Farm Extension Project and Dudgeon Offshore Wind Farm Extension Project. The 2021 WCC surveys were preceded by a screening exercise during which watercourses within the DCO boundary which were assessed as being suitable for WCC were screened in as requiring further surveys. In total, seven watercourses were screened in and were subsequently surveyed in 2021. The screening exercise was based on information obtained on watercourses during the Extended Phase 1 Habitat Surveys completed between March 2020 and January 2021.

The 2021 WCC survey comprised an environmental DNA (eDNA) test of water samples collected from each watercourse. Sample collection was completed by Wild Frontier Ecology Ltd. ecologists within the accepted WCC eDNA survey season, on 28th July 2021. Sample kits were sourced from SureScreen Scientifics Ltd., which also completed the laboratory analysis of the samples.

The survey results were received from the laboratory in August 2021. They indicated the likely absence of WCC from six of the seven surveyed watercourses and confirmed presence in one: namely the River Tiffey.

The laboratory also tested the water samples for the eDNA of the non-native American signal crayfish (ASC). The two species do not typically co-exist in the same watercourses because ASC can outcompete WCC for resources, and they carry crayfish plague which is lethal to WCC; therefore, the presence of ASC will typically indicate the absence of WCC. Laboratory results confirmed the presence of ASC in five of the seven surveyed watercourses. The only watercourses in which ASC was not detected were the River Tiffey (which returned a positive result for WCC) and an unnamed stream (a tributary of the River Yare) near the village of Ketteringham, which tested negative for both species.

A biological records search was also undertaken with the Norfolk Biodiversity Information Service. This returned four records of WCC within 2 kilometres (km) of the DCO boundary. Three of these records are from the River Glaven (which is outside the DCO boundary) and date from 2006; the other record is from the River Wensum at Attlebridge dating from 2009. A survey completed by Wild Frontier Ecology Ltd. in 2018 found evidence of WCC in otter feeding remains alongside the River Wensum, near the river crossing of the Marriott's Way, approximately 1.3km upstream of Attlebridge.

The 2020 report on WCC presence in selected watercourses in Norfolk, produced by the Norfolk Crayfish Group (NCG)[1] revealed that no watercourses within the DCO boundary had been surveyed. However, WCC were confirmed present nearby in the River Glaven and at the Beach Road outfall of Weybourne Stream (which is directly east of the DCO boundary at the landfall location). The report also notes that WCC are 'presumed absent' from the River Bure, where ASC are 'assumed present', although the NCG did not complete targeted surveys here in 2020. The author of the 2020 NCG report also confirmed that WCC had been recorded in the River Yare at Marlingford, but that ASC are present upstream and downstream of this population; the DCO boundary crosses the River Yare upstream of Marlingford.

[1] Juta, U. (2020). *Norfolk Crayfish Group Actions 2020*. Norfolk Rivers Trust, Holt, Norfolk

No further WCC surveys will be undertaken as the onshore construction works for SEP and DEP will adopt trenchless installation techniques (i.e. no open-cutting through river channels) across all main watercourses. A suite of best-practice and industry accepted measures will be adhered to during construction works to ensure the risks to WCC are minimised, including risks from the possible spread of ASC. There is no requirement for licensed mitigation for the species.

1. BACKGROUND

Equinor New Energy Limited (hereafter Equinor) is proposing to extend the existing operational Sheringham Shoal Offshore Wind Farm and Dudgeon Offshore Wind Farm, named the Sheringham Shoal Offshore Wind Farm Extension Project (SEP) and Dudgeon Offshore Wind Farm Extension Project (DEP). SEP and DEP will consist of a number of offshore and onshore elements including the offshore wind turbines, offshore export cables and offshore substation. The offshore export cables will connect to shore on the North Norfolk coast, with onshore infrastructure connecting the offshore wind farms to the National Grid, which will comprise underground cables from landfall at Weybourne to an onshore substation and National Grid connection at Norwich Main. A full description of SEP and DEP is provided within the **ES Chapter 4 Project Description** (document reference 6.1.4).

In 2021, Wild Frontier Ecology Ltd. (WFE) was commissioned by Equinor to undertake surveys to establish the presence and/or likely absence of WCC *Austropotamobius pallipes* in watercourses within the DCO boundary to inform an ecological impact assessment of the proposed onshore grid connection for SEP and DEP. The onshore components comprise a c.60km corridor with landfall location around Weybourne on the North Norfolk coast, with the onshore cable corridor then running southwards and eventually eastwards around the west and south sides of Norwich, where it is to connect with a proposed onshore electricity substation, feeding into the National Grid near Norwich Main Substation.

Maps showing the survey area (i.e. the DCO boundary and watercourses within it which were assessed as providing suitable habitat for WCC and therefore surveyed for WCC) are provided in **Figure 1** to **Figure 4**, below.

This report outlines the aims, methods and results of the WCC eDNA surveys for which have been completed in July 2021.

2. RELEVANT LEGISLATION AND POLICY BACKGROUND

WCC are listed on Schedule 5 of the Wildlife and Countryside Act 1981 (as amended) but only receive protection under Sections 9(1) and 9(5). This makes it an offence to take or sell WCC. Section 9 applies to all stages in their life cycle.

Schedule 9 of the Wildlife and Countryside Act 1981 (as amended) also applies to the ASC *Pacifastacus leniusculus*, which makes it illegal to distribute or allow distribution of ASC, unless under special licensed conditions. The rule applies to any accidental removal or capture of ASC from watercourses (e.g. during fishing), and requires that ASC are humanely dispatched in-situ and carcasses not returned to the watercourse.

Although not directly related to legislation or policy, WCC is listed as an 'Endangered' species by the International Union for Conservation of Nature.

3. SURVEY METHODS

3.1. Desk Study

During the Terrestrial Ecology and Ornithology Expert Topic Group (ETG) meeting on 28th January 2020, attended by Natural England, the Environment Agency, Broadland District Council, Norfolk County Council, North Norfolk District Council and South Norfolk District Council, it was agreed that suitable watercourses within the Preliminary Environmental Information Report (PEIR) boundary (which was an earlier and wider iteration of the Order Limits, which preceded the DCO boundary) would be surveyed to determine the presence or likely absence of WCC.

Watercourses within the PEIR boundary were identified from a desk-based review of Ordnance Survey (OS) maps and other freely available mapping software such as Google Earth. Between March 2020 and January 2021, an Extended Phase 1 Habitat Survey (EP1HS) of all accessible parts of the PEIR boundary was completed; this included appraisals of the suitability for WCC of all accessible watercourses. The appraisal of watercourses for WCC was based on size/permanence (seasonal watercourses which are dry for much of the year were considered unsuitable), water quality (watercourses with poor quality, stagnant water were considered unsuitable) and suitable substrate. WCC require gravelly or stony substrates in watercourses, as this provides shelter and often supports small invertebrates which WCC prey on. Watercourses found to have muddy or sandy substrates, with an absence of any gravel or stony sections, were considered unsuitable for WCC.

3.1.1. White-Clawed Crayfish and American Signal Crayfish Survey Data Provided by Norfolk Crayfish Group/Norfolk Rivers Trust

In 2020, the NCG in association with the Norfolk Rivers Trust (NRT) conducted targeted WCC eDNA surveys on a number of watercourses in Norfolk, including some within and near the DCO boundary. Watercourses included in the NCG report which are relevant to this assessment are:

- Weybourne Stream (approximately 40m east of the DCO boundary at the landfall, although Spring Beck, which feeds into Weybourne Stream, intersects with the DCO boundary south of Weybourne);
- River Glaven (the headwaters of which are approximately 100m west of the DCO boundary near Bodham); and,
- River Bure (which intersects with the DCO boundary near Saxthorpe).

The NCG report also includes information on the Rivers Stiffkey, Tat and Wissey, and Sheringham Stream, but these are all well separated from the DCO boundary (by at least 3km) so the NCG information on those watercourses is not included in this report.

The NCG's 2020 report on their surveys was reviewed for relevant information on watercourses within and near to the DCO boundary. The report confirms that the methodology used in the surveys was the same as the eDNA surveys completed by WFE in 2021; further detail on the eDNA survey methodology is provided below.

The lead author of the report was contacted in November 2020 to obtain any additional information the NCG may have held on other watercourses in Norfolk within or near to the DCO boundary. This information was provided to WFE in November 2020.

3.1.2. Norfolk Biodiversity Information Service White-Clawed Crayfish and American Signal Crayfish Records

A data search was completed with the Norfolk Biodiversity Information Service (NBIS) in January 2021 for all biological records (including of WCC and ASC) within the PEIR boundary and the surrounding 2km area.

3.1.3. WFE Records

A review of WFE's past surveys (completed for other projects) of watercourses within the DCO boundary was also completed to obtain any relevant records of WCC, ASC or other relevant information.

3.2. Presence/Likely Absence Survey using eDNA Testing

By the time of the WCC eDNA surveys in summer 2021, the site selection process had refined the onshore cable corridor from the PEIR boundary to the DCO boundary, meaning it was only suitable watercourses within the DCO boundary which warranted surveys.

Each screened-in watercourse (i.e. watercourses which provided suitable habitat for WCC) within the DCO boundary was subject to an eDNA survey to determine the presence or likely absence of WCC. The presence of WCC eDNA in a watercourse confirms that the species is present, and the absence of any eDNA indicates likely absence of this species.

The surveys used water sample collection and eDNA processing kits sourced from SureScreen Scientifics Ltd. (SSL). The survey technique was undertaken in accordance with the instructions provided by SSL[2]. Twenty water samples were taken from across each watercourse using sterile equipment: samples were taken using gloves and a ladle, working upstream (against the flow) in order that any disturbed sediment from the surveyor was not accidentally collected in the samples. The surveyor walked a diagonal pattern across the watercourse while collecting samples, thereby sampling across the whole width of the watercourse (from bank to bank). The surveyor did not collect water from the bottom of the river or amongst the sediment, to minimise the risk of recording historic (rather than recent/present) eDNA.

For each watercourse, the water samples collected with a ladle were all poured into a mixing bag and combined. A syringe was then used to extract 50 millilitres (ml) of water from this mixing bag, and a filter was then attached to the end of the syringe and the water pushed out of the syringe, through the filter. The filter collected sediment from the water flowing through it. This process was repeated until 500ml of water from the mixing bag had been pushed through the filter, or until the filter was completely full/blocked by sediment. The filter was then filled with preservative, sealed at both ends, and packaged for sending to the SSL laboratory. This process was completed for each surveyed watercourse.

All surveys were completed on 28th July 2021 by the following WFE staff (working in pairs):

[2] [redacted]

- Ptolemy McKinnon BSc MSc
- Alice Petherick BA MA
- William Riddett BA ACIEEM
- Graham Riley BSc ACIEEM

Weather conditions at the time of sample collection were suitable for the survey; air temperature was approximately 19-23ºC, wind was estimated to Beaufort scale 1-2, cloud cover was variable, and there was no precipitation for the majority of the survey (aside from a very light shower which lasted for roughly 10 minutes when surveyors were driving between sample sites).

4. RESULTS

4.1. Desk Study

The following seven watercourses as shown on **Figure 1** to **Figure 4** were screened in for eDNA surveys based on the presence of suitable habitat for WCC:

- River Bure;
- Unnamed tributary of the River Wensum east of the village of Swannington;
- River Wensum (this is designated as a Special Area of Conservation and Site of Special Scientific Interest, with WCC listed as one of the site's reasons for its designation);
- River Tud;
- River Yare;
- River Tiffey; and,
- Unnamed tributary of the River Yare at Furze Meadow east of the village of Ketteringham.

The sample collection points were targeted at the points the DCO boundary crosses these watercourses.

4.1.1. White-Clawed Crayfish and American Signal Crayfish Survey Data Provided by Norfolk Crayfish Group/Norfolk Rivers Trust

The 2020 NCG report confirmed that WCC are present in the River Glaven and in Weybourne Stream. However, the sampling locations on the River Glaven are all beyond 6km from the DCO boundary; the headwaters of the river, which are the closest part of it to the DCO boundary (within approximately 100m), were not among the sampling locations. The sampling location for Weybourne Stream is Beach Road outfall, which is within 50m of the DCO boundary at the landfall location.

The report also makes reference to the River Bure (which the DCO boundary does cross, but which was not directly surveyed by NCG in 2020), stating that WCC are 'assumed absent', and ASC are 'known present'.

The lead author of the NCG 2020 report, separately confirmed that WCC are present in the River Yare at Marlingford, but ASC are present upstream and downstream of this population. The River Yare flows past Marlingford approximately 2.9km (as measured following the course of the river) east (downstream) of the location at which the DCO boundary crosses the River Yare. It is relevant to note that the confluence of the River Tiffey and the River Yare is downstream (east) of the DCO crossing point of the River Yare, but upstream (west) of Marlingford; see **Figure 3**.

4.1.2. Norfolk Biodiversity Information Service White-Clawed Crayfish and American Signal Crayfish Records

The NBIS data search returned four records of WCC within the DCO boundary and the surrounding 2km buffer. Three of these records are located in the River Glaven around Bodham (dating from 2006), which is outside the DCO boundary. The other record is from the River Wensum at Attlebridge (dating from 2009). The precise locations of

these records are all outside of the DCO boundary. However, the DCO boundary does cross the River Wensum near Attlebridge (see **Figure 2**), approximately 1.6km west (upstream) of the location of this record. This record suggests WCC would have been present in the vicinity of the section of River Wensum crossed by the DCO boundary in 2009.

4.1.3. WFE Records

A Phase 1 Habitat survey and protected species appraisal of the section of the River Wensum between the Marriott's Way river crossing and the A1067 Fakenham Road river crossing (between the villages of Lenwade and Attlebridge respectively) was completed by WFE in September 2018. This found evidence of WCC (i.e. a WCC claw) in otter *Lutra lutra* feeding remains close to the river crossing of the Marriott's Way. The location of the record is approximately 1.3km north-west (upstream) of the point the DCO boundary crosses the River Wensum. Full detail is provided in the WFE report[3].

4.2. Presence/Likely Absence Survey using eDNA Testing

Maps showing the sampling positions are provided in **Figure 1** to **Figure 4** and results are provided in **Table 1**, below. The report provided by SSL is provided in Annex 1 to this report.

Table 1: eDNA Results (to be read in conjunction with Figure 1 to Figure 4)

WCC and ASC Watercourse Survey Site	Approximate Sampling Location Description	Approximate Sampling Location National Grid Reference	WCC eDNA Detected	ASC eDNA Detected
River Bure	At Saxthorpe Hall, east of Saxthorpe	TG 1308 2987	No	Yes
Unnamed tributary of River Wensum	South of Church Lane, east of Swannington	TG 1410 1893	No	Yes
River Wensum	South of the A1067 Fakenham Road at Attlebridge	TG 1288 1650	No	Yes
River Tud	Unnamed plantation woodland north-west of Easton	TG 1245 1154	No	Yes
River Yare	South-east of Colton Wood	TG 1186 0847	No	Yes
River Tiffey	East of Barford	TG 1195 0757	Yes	No
Unnamed tributary of River Yare	Furze Meadow, east of Ketteringham	TG 1772 0304	No	No

In summary, the eDNA surveys confirmed the presence of WCC in one of the seven sampled watercourses, the River Tiffey. ASC were detected in five other watercourses,

[3] Wild Frontier Ecology Ltd. (2018). *River Wensum Reach 9, Attlebridge: Phase 1 Habitat & Protected Species Surveys.* Wild Frontier Ecology Ltd., Fakenham, Norfolk.

all of which returned negative (likely absent) results for WCC. One watercourse (the unnamed tributary of the River Yare at Furze Meadow near Ketteringham) returned negative results for both species, indicating likely absence of both crayfish species from this watercourse.

Figure 1: White-Clawed Crayfish Survey Results Map (River Bure)

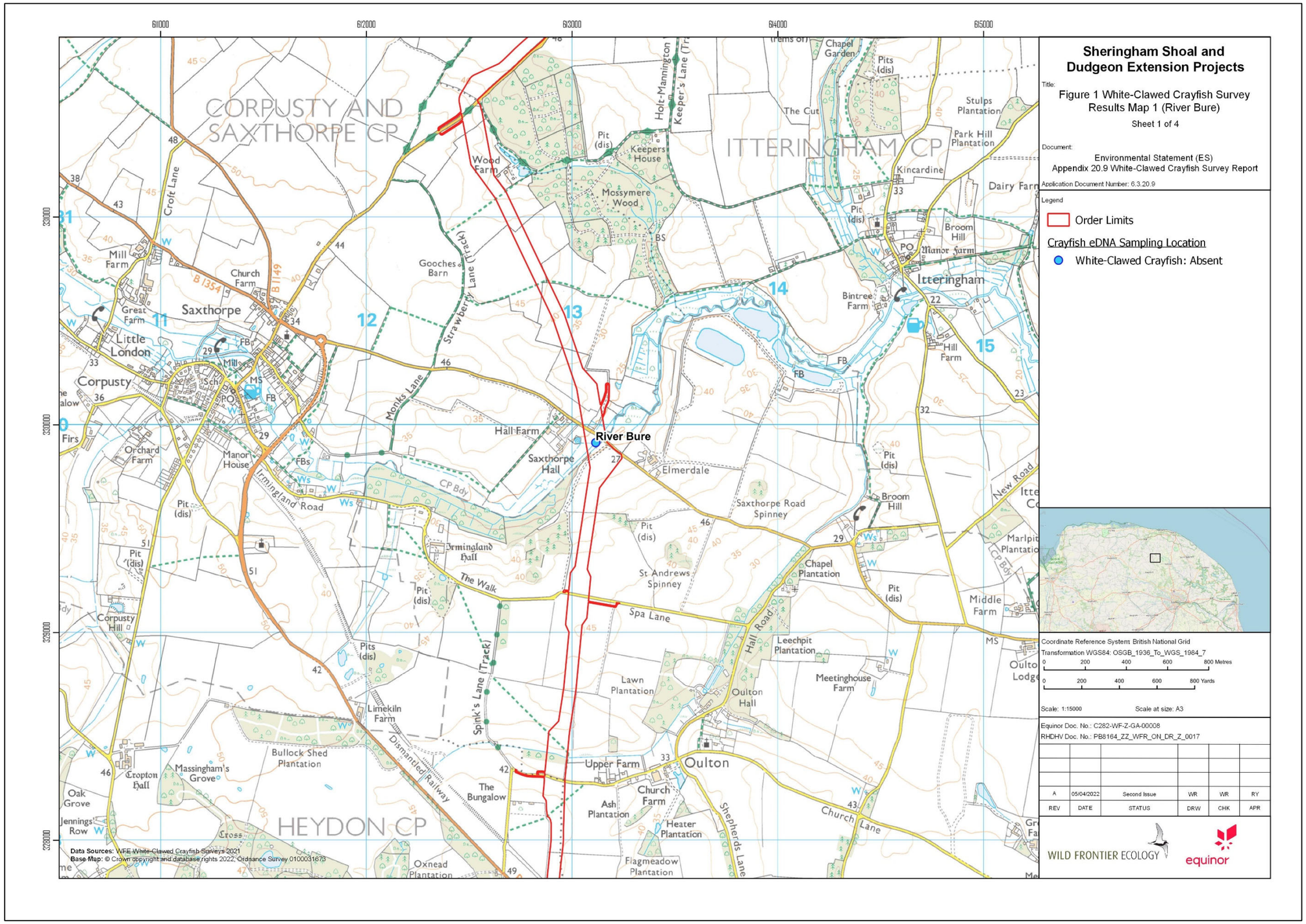


Figure 2: White-Clawed Crayfish Survey Results Map (River Wensum and Unnamed Tributary of River Wensum at Swannington)

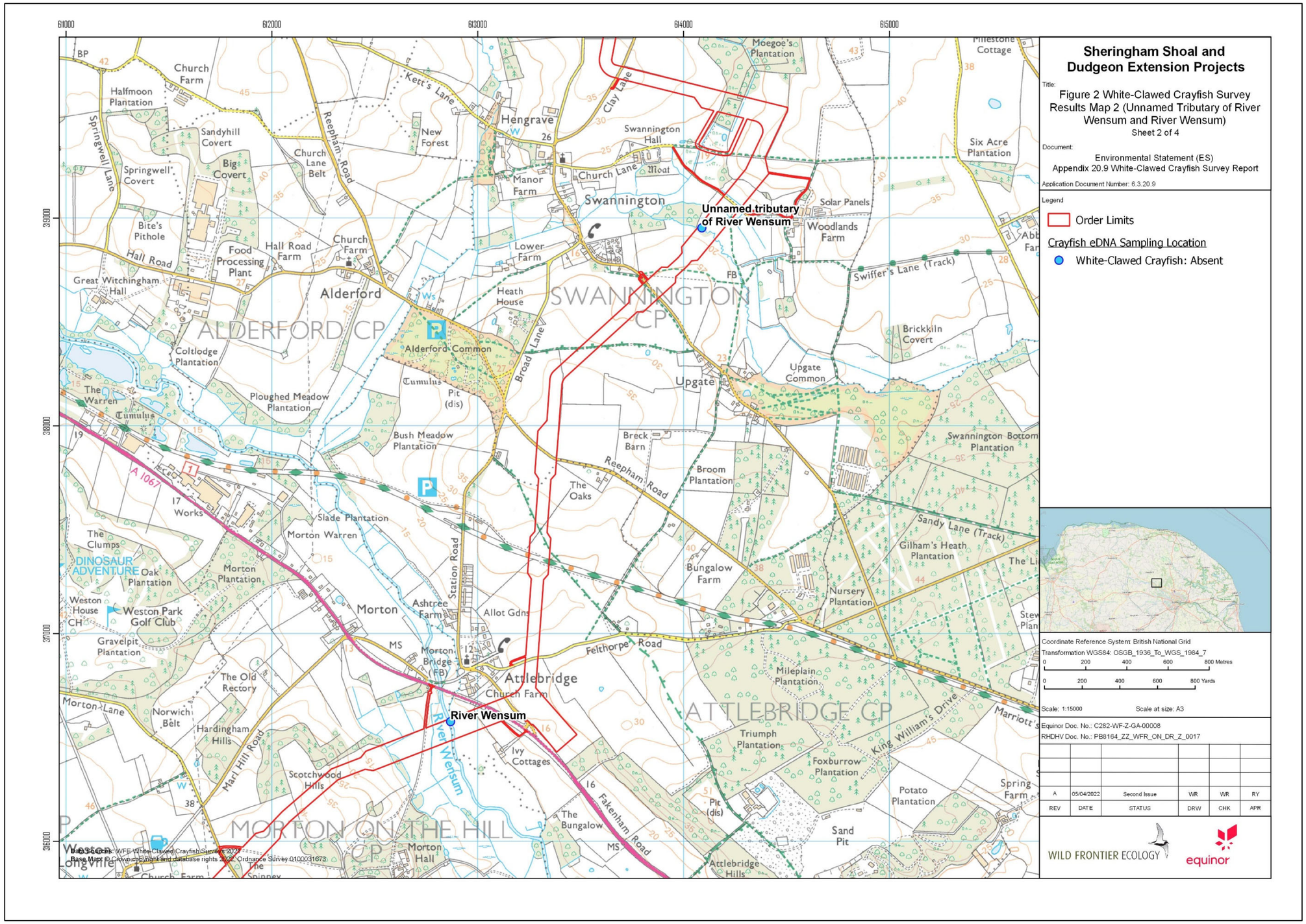
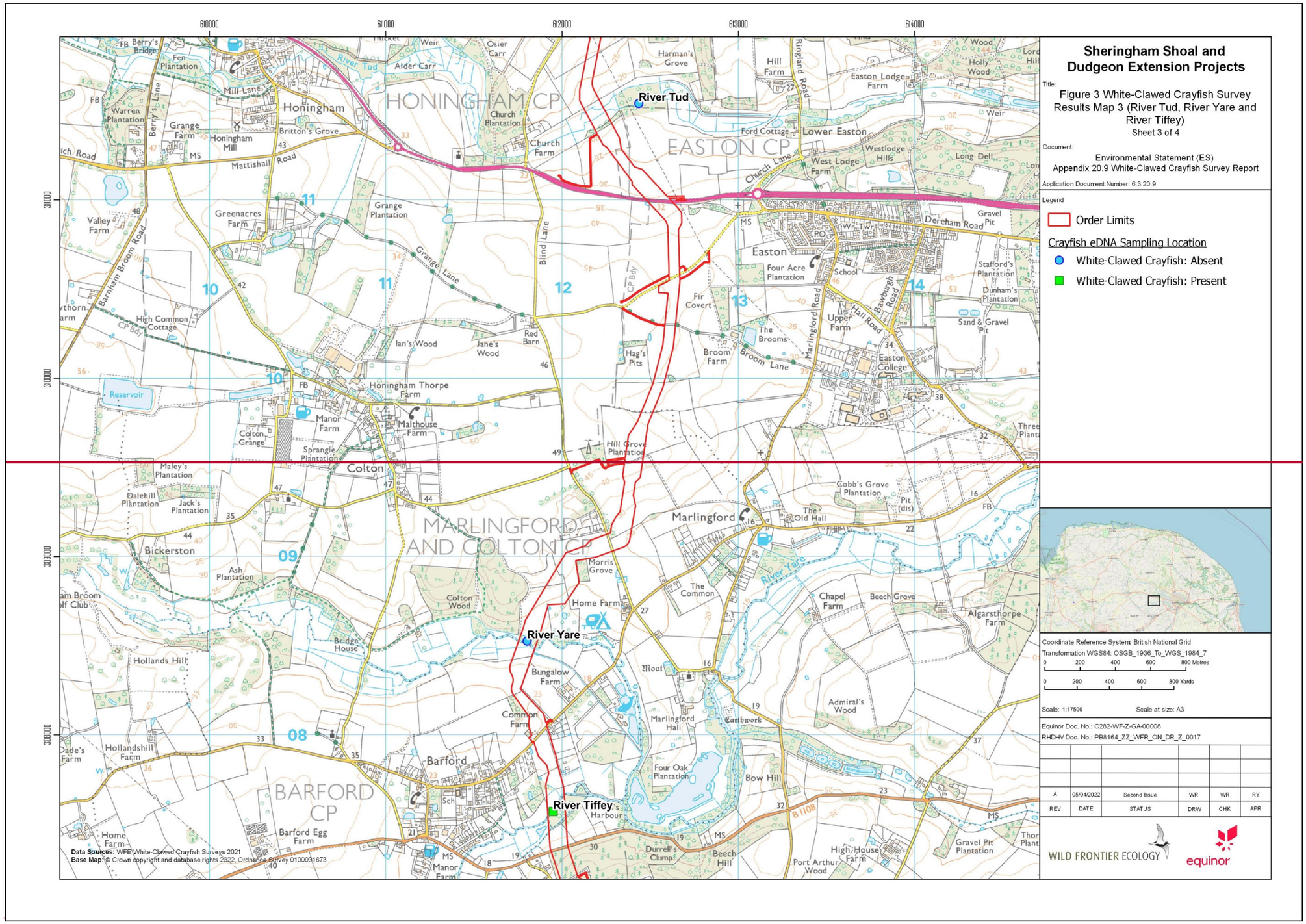


Figure 3: White-Clawed Crayfish Survey Results Map (River Tud, River Yare and River Tiffey)


610000
611000
612000
613000
614000
309000
308000
River Tud
River Yare
River Tiffey
HONINGHAM CP
EASTON CP
MARLINGFORD AND COLTON CP
BARFORD CP
Honingham
Easton
Lower Easton
Colton
Marlingford
Barford
Bickerston
Mattishall Road
Church Lane
Dereham Road
Grange Lane
Blind Lane
Barnham Broom Road
Marlingford Road
Broom Lane
Hall Road
Bawburgh Road
Ringland Road
B 1108
Data Sources: WFE White-Clawed Crayfish Surveys 2021
Base Map: © Crown copyright and database rights 2022, Ordnance Survey 0100031673
Sheringham Shoal and Dudgeon Extension Projects
Title:
Figure 3 White-Clawed Crayfish Survey Results Map 3 (River Tud, River Yare and River Tiffey)
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Document:
Environmental Statement (ES)
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Application Document Number: 6.3.20.9
Legend
Order Limits
Crayfish eDNA Sampling Location
White-Clawed Crayfish: Absent
White-Clawed Crayfish: Present
Coordinate Reference System: British National Grid
Transformation WGS84: OSGB_1936_To_WGS_1984_7
0 200 400 600 800 Metres
0 200 400 600 800 Yards
Scale: 1:17500
Scale at size: A3
Equinor Doc. No.: C282-WF-Z-GA-00008
RHDHV Doc. No.: PB8164_ZZ_WFR_ON_DR_Z_0017
A 05/04/2022 Second Issue WR WR RY
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WILD FRONTIER ECOLOGY
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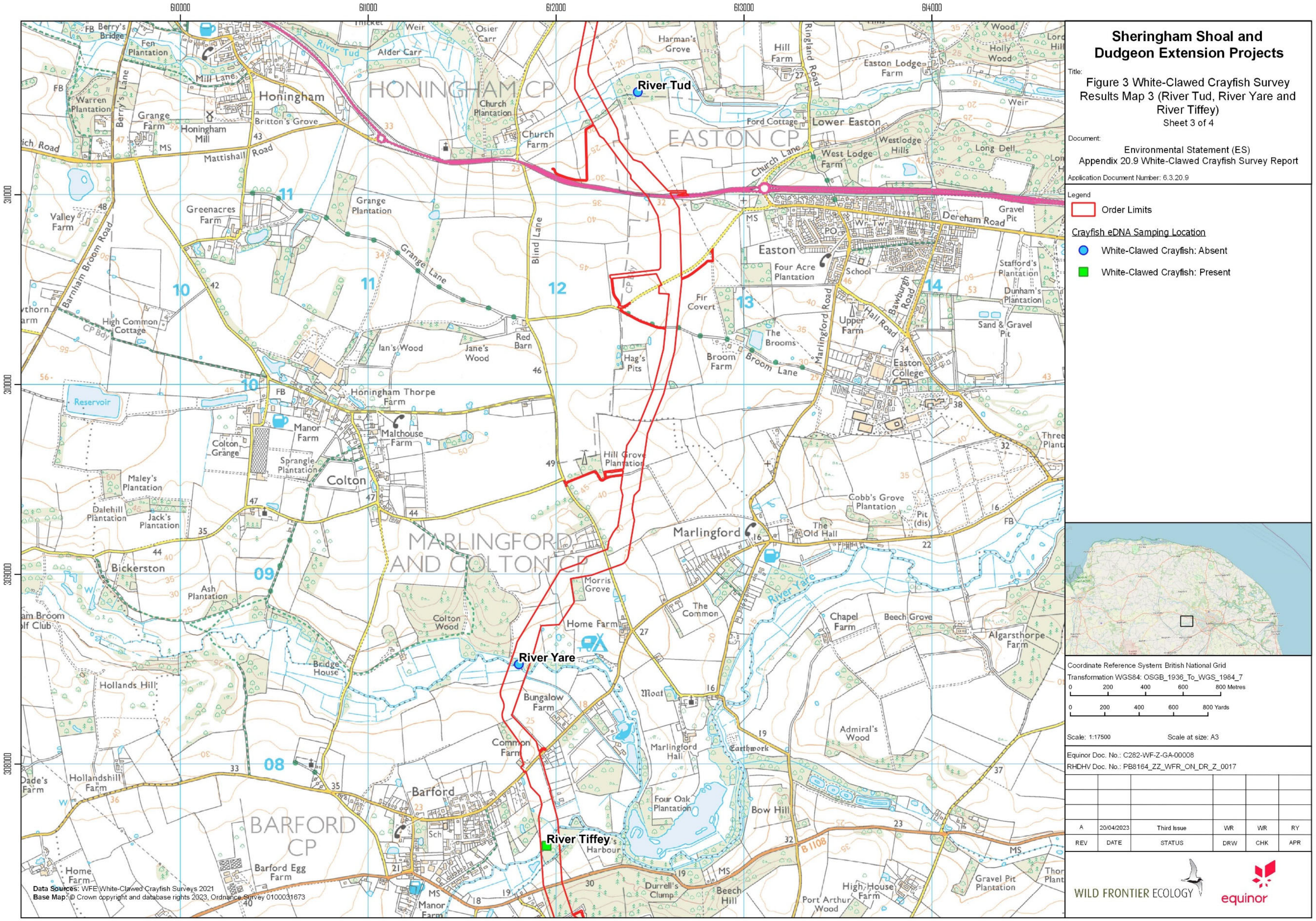
Sheringham Shoal and Dudgeon Extension Projects
Title:
Figure 3 White-Clawed Crayfish Survey Results Map 3 (River Tud, River Yare and River Tiffey)
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Environmental Statement (ES)
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Application Document Number: 6.3.20.9
Legend
Order Limits
Crayfish eDNA Samping Location
White-Clawed Crayfish: Absent
White-Clawed Crayfish: Present
River Tud
River Yare
River Tiffey
Coordinate Reference System: British National Grid
Transformation WGS84: OSGB_1936_To_WGS_1984_7
0 200 400 600 800 Metres
0 200 400 600 800 Yards
Scale: 1:17500
Scale at size: A3
Equinor Doc. No.: C282-WF-Z-GA-00008
RHDHV Doc. No.: PB8164_ZZ_WFR_ON_DR_Z_0017
A 20/04/2023 Third Issue WR WR RY
REV DATE STATUS DRW CHK APR
WILD FRONTIER ECOLOGY
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Data Sources: WFE White-Clawed Crayfish Surveys 2021
Base Map: © Crown copyright and database rights 2023, Ordnance Survey 0100031673
610000
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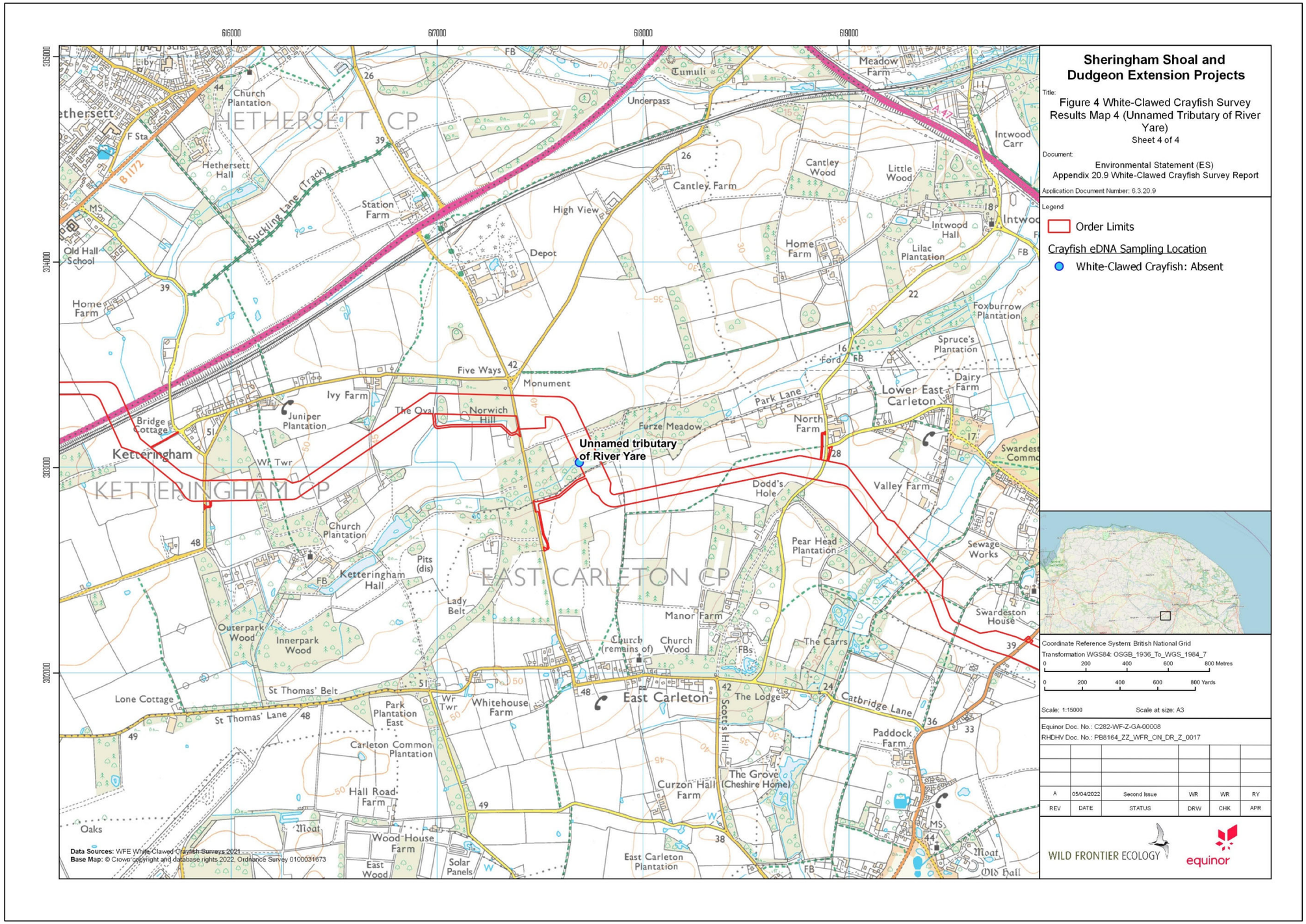
614000
310000
309000
308000
HONINGHAM CP
EASTON CP
MARLINGFORD AND COLTON CP
BARFORD CP
Honingham
Easton
Colton
Marlingford
Barford

Figure 4: White-Clawed Crayfish Survey Results Map (Unnamed Tributary of the River Yare)



4.3. Constraints and Limitations of Survey

There are inherent constraints to the eDNA surveys, such as potential natural contamination, such as from birds or other wildlife transferring eDNA between waterbodies, which could lead to false Positives. Conversely, there is also the potential for false Negatives for various reasons, such as sampling points not aligning with specific stretches of a watercourse where a species' eDNA is detectable. However, by conducting simultaneous testing for two competing species (WCC and ASC, which do not tend to co-exist), the presence of one species will typically indicate that the other is absent. This clearly appears to have been borne out in the results for the five sampled watercourses where ASC were detected present but no eDNA of WCC was detected. Similarly, the one watercourse where WCC eDNA was detected returned no detected eDNA of ASC. This contrast increases confidence in the results and minimises the possibility of eDNA detection errors for each particular species.

The sampling location on the River Tud is located approximately 200m downstream (east) of the intersection of the DCO boundary with this river, because at the time of sampling this location was within the alignment of the DCO boundary as it was at that time. Given that negative WCC and positive ASC results were returned for the sampling location, it is extremely unlikely that WCC would be present 200m upstream.

These constraints are not considered to have a substantial impact on the reliability of the survey results; the results are therefore considered to be sufficiently accurate and reliable to inform the ecological impact assessment and in turn identify any required mitigation requirements.

4.4. Further Survey Requirements, Expiry Dates and Mitigation/Licensing Options

Government guidelines[4] do not specify expiry dates for WCC surveys but suggest that it is the responsibility of the ecologist to assess whether survey data provides a sufficiently up-to-date and reliable source on which to base an assessment of the impacts of a particular scheme. For the SEP and DEP, it is considered, as a precaution, that the data will remain valid for two years (meaning it will have 'expired' by the end of July 2023).

However, SEP and DEP will adopt trenchless installation techniques using Horizontal Directional Drilling (HDD) beneath all seven of the sampled watercourses. Accordingly, the minimal risk posed to WCC during construction is considered to negate any requirement for updated surveys. Residual risks to WCC can be suitably addressed by adopting precautionary mitigation measures during construction.

Possible risks include bentonite breakouts (during HDD) or accidental contamination/spread of invasive species (namely ASC), potentially introducing crayfish plague *Aphanomyces astaci*. Detail of appropriate precautionary best-practice measures are provided within the **Outline Ecological Management Plan**. Best-practice measures will include:

- Prohibition of any entry by machinery, materials or personnel into any watercourse known to support WCC or ASC. Specifically, this includes: Weybourne Stream, River Glaven, River Bure, unnamed tributary of the River

[4] https://www.gov.uk/guidance/white-clawed-crayfish-protection-surveys-and-licences

Wensum at Swannington, River Wensum, River Tud, River Yare and River Tiffey. Although survey results indicate WCC are absent from five of these watercourses, the mitigation measures will still need to be apply because ASC has been detected in them; the main purpose of mitigation at these watercourses is therefore to minimise the risk of construction works spreading ASC or associated crayfish plague. In addition, information obtained during the desk study suggests there could be remaining WCC populations (which have not been detected by the eDNA surveys) in parts of these river systems. For example, NBIS and NCG data suggests that WCC populations either have recently existed or may still exist in the Rivers Wensum and Yare. Indeed, the NCG reported that WCC are present in the River Yare at Marlingford, and the eDNA surveys confirmed the presence of the species at the River Tiffey (a tributary of the Yare). It is therefore possible that WCC are present in sections of the River Yare downstream from the DCO crossing point of this river; this WCC population in the River Yare may be connected to the population detected in the River Tiffey. These issues mean that precautionary mitigation measures are warranted for all seven watercourse crossing points.

- Monitoring for bentonite breakouts throughout HDD beneath the relevant watercourses, with a commitment to cease drilling and enact remedial measures immediately upon discovery of a breakout. Further detail on remedial measures is provided in the **Outline Code of Construction Practice**.

Given the commitment to adopt HDD beneath all seven relevant watercourses, there is no requirement for a WCC licence from Natural England to legally permit the onshore works associated with SEP and DEP.

5. CONCLUSIONS

The 2021 WCC surveys have confirmed that the species is present in one watercourse crossed by the DCO boundary, the River Tiffey near Barford. Desk study results also indicate WCC are present in two nearby watercourses (Weybourne Stream and the River Glaven) and have been present in the past at (or may still be present at certain stretches of) the Rivers Wensum and Yare. However, the confirmed presence of ASC along with negative eDNA results for WCC in five sampled watercourses within the DCO boundary strongly suggest that WCC are absent from these watercourses, or at least from the stretches of these watercourses which were sampled.

No further surveys for WCC are expected to be necessary, given the commitment to adopt HDD beneath all of the surveyed watercourses. All other (non-surveyed) watercourses within the DCO boundary are considered unsuitable for WCC and so there is no requirement for HDD beneath them to mitigate risks to WCC. Best practice mitigation measures are advised for all seven watercourses which were sampled for WCC in order to minimise risk to the species from construction works, but also from possible spread of ASC and crayfish plague. There is no requirement for licensed mitigation measures.

6. REFERENCES

https://www.gov.uk/guidance/white-clawed-crayfish-protection-surveys-and-licences accessed on 04/10/21

[redacted]

Juta, U. (2020). *Norfolk Crayfish Group Actions 2020*. Norfolk Rivers Trust, Holt, Norfolk

Wild Frontier Ecology Ltd. (2018). *River Wensum Reach 9, Attlebridge: Phase 1 Habitat & Protected Species Surveys*. Wild Frontier Ecology Ltd., Fakenham, Norfolk.

Annex 1: SureScreen Scientifics Ltd. Report

SureScreen Scientifics

Folio No: E11906
Report No: 1
Purchase Order: 2021/WCC
Client: WILD FRONTIER ECOLOGY
Contact: Will Riddett

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: 30/07/2021
Date Reported: 13/08/2021
Matters Affecting Results: None

Lab Sample ID.	Site Name	O/S Reference	Species	Result	SIC	DC	IC	Positive Replicates
FK127	EQUINOR RIVER BURE		*White-Clawed Crayfish*	Negative	Pass	Pass	Pass	0
			Signal Crayfish	Positive	Pass	Pass	Pass	12
FK128	EQUINOR SWANNINGTON STREAM		*White-Clawed Crayfish*	Negative	Pass	Pass	Pass	0
			Signal Crayfish	Positive	Pass	Pass	Pass	9
FK129	EQUINOR RIVER WENSUM		*White-Clawed Crayfish*	Negative	Pass	Pass	Pass	0
			Signal Crayfish	Positive	Pass	Pass	Pass	12
FK130	EQUINOR TUD		*White-Clawed Crayfish*	Negative	Pass	Pass	Pass	0

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			Signal Crayfish	Positive	Pass	Pass	Pass	12
FK131	EQUINOR YARE		*White-Clawed Crayfish*	Negative	Pass	Pass	Pass	0
			Signal Crayfish	Positive	Pass	Pass	Pass	12
FK132	EQUINOR TIFFEN		*White-Clawed Crayfish*	Positive	Pass	Pass	Pass	1
			Signal Crayfish	Negative	Pass	Pass	Pass	0
FK133	EQUINOR MEADOW STREAM		*White-Clawed Crayfish*	Negative	Pass	Pass	Pass	0
			Signal Crayfish	Negative	Pass	Pass	Pass	0

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

Reported by: Chris Troth **Approved by:** Gabriela Danickova

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

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*SSL has inaccurately transcribed some of the sample names (for example, River Tiffen instead of River Tiffey).

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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.

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Annex 2: Watercourse Photographs

Photo 1: Approximate location of eDNA sample on the River Bure

Photo 2: Approximate location of eDNA sampling on the unnamed stream (tributary of the River Wensum) near Swannington

Photo 3: Approximate location of eDNA sampling on the River Wensum

Photo 4: Approximate location of eDNA sampling on the River Tud

Photo 5: Approximate location of eDNA sampling on the River Yare

Photo 6: Approximate location of eDNA sampling on the River Tiffey

Photo 7: Approximate location of eDNA sampling on the unnamed stream in Furze Meadow near Ketteringham