



Immingham Green Energy Terminal

TR030008

Volume 6

6.4 Environmental Statement Appendices

Appendix 8.E: Great Crested Newt Survey Report

Planning Act 2008

Regulation 5(2)(a) and 5(2)(l)

Infrastructure Planning (Applications: Prescribed
Forms and Procedure) Regulations 2009 (as
amended)

September 2023

Infrastructure Planning

Planning Act 2008

The Infrastructure Planning
(Applications: Prescribed Forms and
Procedure) Regulations 2009 (as amended)

Immingham Green Energy Terminal

Development Consent Order 2023

6.4 Environmental Statement Appendices

Appendix 8.E: Great Crested Newt Survey Report

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1. Introduction

1.1. Background

1.1.1 This great crested newt survey report has been prepared by AECOM on behalf of Associated British Ports (the Applicant), to assess the ecological constraints, specifically pertaining to great crested newt, in connection with the proposed Immingham Green Energy Terminal (“IGET”) (hereafter referred to as the Project). The Project is located adjacent to Kings Road, Immingham, as shown by the Site Boundary on Figure 1 in Annex A. All land situated within this Site Boundary is hereafter referred to as the Site.

1.1.2 The assessment of ecological constraints pertaining to great crested newt has been undertaken with reference to current good practice (standing advice published by Natural England in 2022) (Ref 1-3) and forms part of the technical information commissioned by the Applicant in connection with the Project.

1.2. Purpose of Report

1.1.3 This great crested newt survey report presents ecological information obtained during the following studies and surveys:

- a. Desk-study undertaken during March 2022 and updated in May 2023 to obtain records of great crested newt within 2km of the Site (the area covered by the desk study is hereafter referred to as the Study Area).
- b. Review of 1:25,000 Ordnance Survey mapping to identify waterbodies within 250m¹ of the Site Boundary
- c. A Habitat Suitability Index (“HSI”) assessment (where accessible) of all waterbodies within 250m of the Site Boundary with the potential to support great crested newt; and
- d. Environmental DNA (“eDNA”) survey of all waterbodies (where accessible) within 250m of the Site Boundary with the potential to support great crested newt.

¹ This is the typical terrestrial range of great crested newts from their breeding ponds and is considered a sufficient survey area for this species where there are no obvious barriers to newts dispersing into the wider environment (Ref 1-3).

2. Legislation and Planning Policy

- 2.1.1 Great crested newt is listed under Schedule 5 of the Wildlife and Countryside Act 1981 (as amended) (Ref 1-5) and Schedule 2 of the Conservation of Habitats and Species Regulations 2019 (as amended) (Ref 1-6). This legislation, when taken together, results in a level of protection that prohibits the intentional, deliberate or reckless:
- a. Killing, injuring, taking or disturbance of great crested newt.
 - b. Damaging, destroying or obstructing any place used by great crested newt for the purposes of breeding, sheltering or protection.
 - c. Selling and/or advertising for sale a great crested newt or any part thereof.
- 2.1.2 Great crested newt is listed as a species of principal importance for nature conservation in England in Section 41 of the Natural Environment and Rural Communities (“NERC”) Act 2006 (Ref 1-7). Section 40 of the same Act requires that all public authorities have regard to the conservation of biodiversity in England, when carrying out their normal functions.

3. Methods

3.1. Desk Study

3.1.1 The following sources of information were used to gain background information on the status and distribution of great crested newt relevant to the zone of influence of potential impacts of the Project:

- a. Lincolnshire Environmental Records Centre (“LERC”) for great crested newt records within 2km of the Project (Ref 1-8); and
- b. MAGIC website (www.magic.gov.uk) (Ref 1-9) - information on granted European Protected Species Mitigation (“EPSM”) licences for great crested newt issued by Natural England within 2km of the Project.

3.1.2 In addition, planning applications published on the North East Lincolnshire Council (“NELC”) planning portal in the vicinity of the Project were examined for relevant survey information pertaining to the status of great crested newt.

3.2. Identification of Waterbodies within 250m

3.2.1 There are no waterbodies within the Site Boundary.

3.2.2 Four potential waterbodies were identified from 1:25,000 Ordnance Survey mapping within 250m of the Site Boundary as identified in **Figure 1 in Annex A**.

3.2.3 In addition, a drainage ditch was present at the landward toe of the flood embankment, which will be crossed by the Pipe Rack and Jetty Access Road. Although clearly fulfilling a drainage function, the section of the ditch adjacent to the Associated Petroleum Terminal (“APT”) site appeared relatively stagnant with little/no flow. This ditch was therefore scoped into the eDNA survey, although was not subject to HSI assessment as this tool is not applicable to running water or ditches.

3.2.4 The other drainage ditches within the Site Boundary are subject to seasonal fluctuations in water levels and have been observed during the course of other surveys to regularly dry out in the spring/early summer. They are therefore unsuitable for breeding great crested newt because they do not regularly hold sufficient water or aquatic vegetation to enable successful breeding activity (the larvae of this species are entirely aquatic until late summer).

3.3. Habitat Suitability Index (“HSI”) Assessment

3.3.1 Where accessible for the purposes of survey, waterbodies were assessed for their potential to support great crested newt using the HSI in accordance with standard methodology (Ref 1-4).

3.3.2 The HSI assessment considers the following ten habitat attributes that are considered to influence the suitability of a pond for breeding great crested newt:

- a. Location – within a UK-wide context reflecting the differences in national distribution of this species;

- b. Area – waterbodies between 100 and 300m² in size are considered to represent the most suitable habitat for great crested newt;
- c. Drying – the number of years in which a waterbody dries over a ten year period. Occasional drying kills fish which is beneficial for great crested newt, but the species predominantly favours waterbodies that do not dry out every year;
- d. Water quality – qualitative evidence-based assessment to infer good (diverse aquatic invertebrate assemblage), moderate (moderate invertebrate diversity), poor (low invertebrate diversity, few submerged plants) or bad (clearly polluted) water quality;
- e. Shade – percentage of pond perimeter shaded to at least 1m from the shore. great crested newt favours lightly shaded waterbodies;
- f. Waterfowl – qualitative evidence-based assessment of presence or absence and numbers is made. Large numbers of waterfowl can result in nutrient enrichment of the water and habitat damage, which is less favourable for great crested newt;
- g. Fish – qualitative evidence-based assessment of likely presence or absence is made. great crested newt favour breeding ponds that do not support fish because their open-water swimming larvae are vulnerable to fish predation;
- h. Number of waterbodies within 1km – great crested newt populations are typically best developed where they have access to a network of waterbodies, and therefore the species is more likely to be found where there are several waterbodies within 1km that are linked by suitable terrestrial habitat; and
- i. Macrophyte cover – percentage of pond surface area occupied by macrophyte cover. Female great crested newt require aquatic vegetation for egg-laying.

3.4. Environmental DNA (“eDNA”) Survey

- 3.4.1 An eDNA sample of the flood embankment drainage ditch was collected on 30 June 2022 and sent to ADAS for analysis in accordance with approved field and laboratory protocols (Ref 1-2).
- 3.4.2 An eDNA sample was collected from Pond 1 on 23 May 2023 and sent to Surescreen for analysis in accordance with approved field and laboratory protocols (Ref 1-2).
- 3.4.3 The waterbodies were not entered by the surveyor during sample collection to ensure compliance with the survey methodology. The samples were collected by a suitably qualified ecologist holding a Natural England great crested newt survey licence.
- 3.4.4 The presence or absence of great crested newt was determined based on the results of the eDNA analysis. If eDNA is detected this provides confirmation of presence and the relevant waterbodies are likely to represent a development constraint that requires further consideration. If eDNA is not detected then this provides high confidence that there is no reasonable likelihood of great crested

newt being present in the relevant waterbodies, and they require no further assessment with regard to this species.

3.5. Limitations

- 3.5.1 It was not possible to safely access the entire perimeter of Pond 1 for the purpose of collecting water samples (as is recommended by the protocol for eDNA sampling); however it is considered that the samples were collected from a sufficient diversity of marginal habitats to meet the survey requirements, with approximately 25% of the banks safely accessible. This is therefore not considered a significant limitation to the eDNA survey.

4. Results

4.1. Desk Study

- 4.1.1 The desk study returned no recent records of great crested newt within the Desk Study Area.
- 4.1.2 Surveys of the wetland complex in the adjacent landfill site (Pond 3) conducted in 2011 and 2013 for a planning application (NELC planning reference DM/1027/13/OUT) did not record great crested newt. Environmental DNA surveys of these ponds in 2017 for the landfill permission condition variation (Planning Ref: DM/0968/19/FUL) did not return any positive great crested newt eDNA records and the species was considered likely absent.

4.2. Survey

- 4.2.1 A total of four waterbodies were initially identified within 250m of the Site Boundary from the 1:25,000 Ordnance Survey mapping. These waterbodies were subject to an initial habitat suitability index assessment appraisal using HSI methodology (where the waterbody was accessible and considered potentially suitable for great crested newt), and/or evaluation of desk study data and information regarding the function and location of the waterbody.
- 4.2.2 In addition, one ditch with an area of stagnant water at the landward toe of the flood embankment was scoped into the survey.
- 4.2.3 Survey results are presented in Error! Reference source not found.

Pond 1

- 4.2.4 This is a large firewater storage lagoon within the APT site, which is adjacent to the Pipe Rack and Jetty Access Road and East Road – Hydrogen Production sites, and is located approximately 10m from the nearest part of the Site Boundary.
- 4.2.5 The pond is partially shaded by woodland along the southern bank and supports stands of common reed (*Phragmites australis*) to its margins. Macrophyte cover is limited to small areas of water milfoil (*Myriophyllum* sp.) in the water column. The pond has concrete headwall structures at the site of a piped outfall at its western end. The pond scored 'average' on the HSI assessment (see **Table 1**), with the absence of additional ponds within a 1km radius a substantial factor in reducing its suitability for the species.
- 4.2.6 Samples were taken from Pond 1 on 5 May 2023 and sent to Surescreen for analysis, where a negative result for great crested newt eDNA was returned. The species is therefore concluded to be likely absent from the pond. The laboratory results are provided in **Annex B**.

Photograph 1: Pond 1



Table 1: Pond 1 HSI Assessment

Habitat Attribute	Field Score	HSI Score
Location	A	1.00
Pond area	750m ²	1.00
Pond drying	Never	0.90
Water quality	Moderate ²	0.67
Shoreline shade	20%	1.00
Fowl	Minor	0.67
Fish	Possible	0.67
Pond count within 1km	0	0.1
Terrestrial habitat	Poor	0.67
Macrophytes	10%	0.4
SCORE		0.61 (Average)

² Based on visual assessment; pond was not subject to specific survey for aquatic invertebrates

Pond 2

- 4.2.7 On Ordnance Survey mapping this waterbody appears to be a square process lagoon within the APT site, which is approximately 95m from the nearest part of the Site Boundary (Pipe Rack and Jetty Access Road). However, when inspected by an ecologist in May 2023, it was confirmed that this structure is an emergency storage bund that provides capacity for spillages from the APT site. The structure does not hold water (other than a very small amount of rainwater on occasion) and therefore is concluded to be unsuitable for breeding amphibians and scoped out from any further survey.

Pond 3

- 4.2.8 This is a complex of ponds used for drainage within the landfill site that lies to the approximately 100m south of the West Site. It is assumed that the waterbodies are relatively transient due to the nature of the site, resulting in change/disturbance to their location and extent. The ponds were not accessible for survey.

- 4.2.9 Previous surveys of the ponds (most recently in 2017) have not recorded great crested newt. There are no other ponds within 500m, and there are no known great crested newt populations within 1km that could potentially provide a donor population. There are also major barriers to great crested newt dispersal onto the landfill site in the form of roads (Kings Road, Queens Road and the A1173), extensive industrial development to the south, and North Beck Drain to the immediate south. It is therefore concluded there is no reasonable likelihood that great crested newt would have colonised these ponds in the intervening period (i.e. since the most recent survey indicating absence in 2017). Based on the above, and the previous records of great crested newt absent stretching back over a 10+ year period, it is therefore concluded that great crested newt is likely absent from the ponds and scoped out from any further survey.

Pond 4

- 4.2.10 This is a process lagoon within the APT site that is regularly drained and therefore is not suitable to support breeding amphibians.

Flood Drainage Ditch

- 4.2.11 This is a large drainage ditch at the landward toe of the flood embankment that is linked to the local drainage network; the drain runs south-easterly along the flood embankment and connects to North Beck Drain, from where there is a storm water outfall into the estuary. The ditch is culverted for a short section at the northern end of Long Strip Woodland (within the Pipe Rack and Jetty Access Road site) where the public right of way crosses it. The surveyed section was limited to the area of stagnant water upstream of the culvert (adjacent to the APT site boundary); downstream of the culvert there was very little water in the ditch.
- 4.2.12 The survey section of the ditch supported extensive stands of common reed, with little standing open water, and was considered to represent potentially suitable habitat for great crested newt, although saline influences could not be ruled out given its proximity to the estuary and its potential interactions with the marine environment.

- 4.2.13 Water samples were taken from the ditch on 30 June 2023 and sent to ADAS for analysis. An 'inconclusive' result for great crested newt eDNA was returned, which is often a result of chemical contamination within the water affecting the ability of the process to detect any eDNA within a watercourse. The laboratory results are provided in **Annex B**.
- 4.2.14 It is concluded that this habitat is unsuitable for great crested newt given its likely contamination and saline influence, and therefore it is concluded that the species is likely absent. This conclusion is further strengthened by the negative eDNA result from Pond 1, which is very close (within 10m) to the ditch. If great crested newts had been present in Pond 1 then it would have been more likely that they could also be present in the ditch.

Photograph 2: Flood Drainage Ditch (Pond 1 visible in the background)



Table 2: Waterbody Survey Results

Pond Ref	Location	Distance from Nearest Part of Site Boundary	Review of Great crested newt Habitat Suitability	Scoped into eDNA survey?	Survey Result
1	TA 211 155	10 m	<p>Fire water storage lagoon within APT site.</p> <p>May be suitable for great crested newt as holds water permanently; Scores 'average' on HSI assessment.</p>	Yes	<p>Negative</p> <p>Great crested newt likely absent</p>
2	TA 210 154	95 m	<p>Emergency storage bund providing capacity for containing spillages from APT site.</p> <p>Does not hold water other than occasional small amounts of rainwater; concluded unsuitable for breeding amphibians.</p>	No	
3	TA 198 141	100 m	<p>Complex of waterbodies within landfill site south of West Site.</p> <p>Previous assessments in 2011, 2013 and 2017 have concluded that they are unsuitable for great crested newt.</p> <p>Surveys in 2017 did not record the species.</p> <p>No other ponds within 500 m with known great crested newt populations that could have colonised in the intervening period.</p>	No	
4	TA 209 156	10 m	<p>Process lagoon within APT site.</p> <p>Regularly drained down as part of its function within the APT site; concluded unsuitable for breeding amphibians.</p>	No	

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Pond Ref	Location	Distance from Nearest Part of Site Boundary	Review of Great crested newt Habitat Suitability	Scoped into eDNA survey?	Survey Result
Flood Drainage Ditch	TA 211 155	Adjacent	Drainage ditch linked to North Beck Drain. May have saline influence, although areas of standing water provide potentially suitable breeding habitat.	Yes	Inconclusive Great crested newt likely absent due to habitat unsuitability and water quality

5. Conclusions

5.1.1 There are no waterbodies within the Site Boundary.







5.1.2 Great crested newt is concluded likely absent from waterbodies located within 250m of the Site Boundary. This species can therefore be scoped out of the ecological impact assessment as there is no impact pathway by which the Project could result in direct (killing, injury) of great crested newt or effects would which be deemed to have a significant impact upon the conservation status of great crested newt.

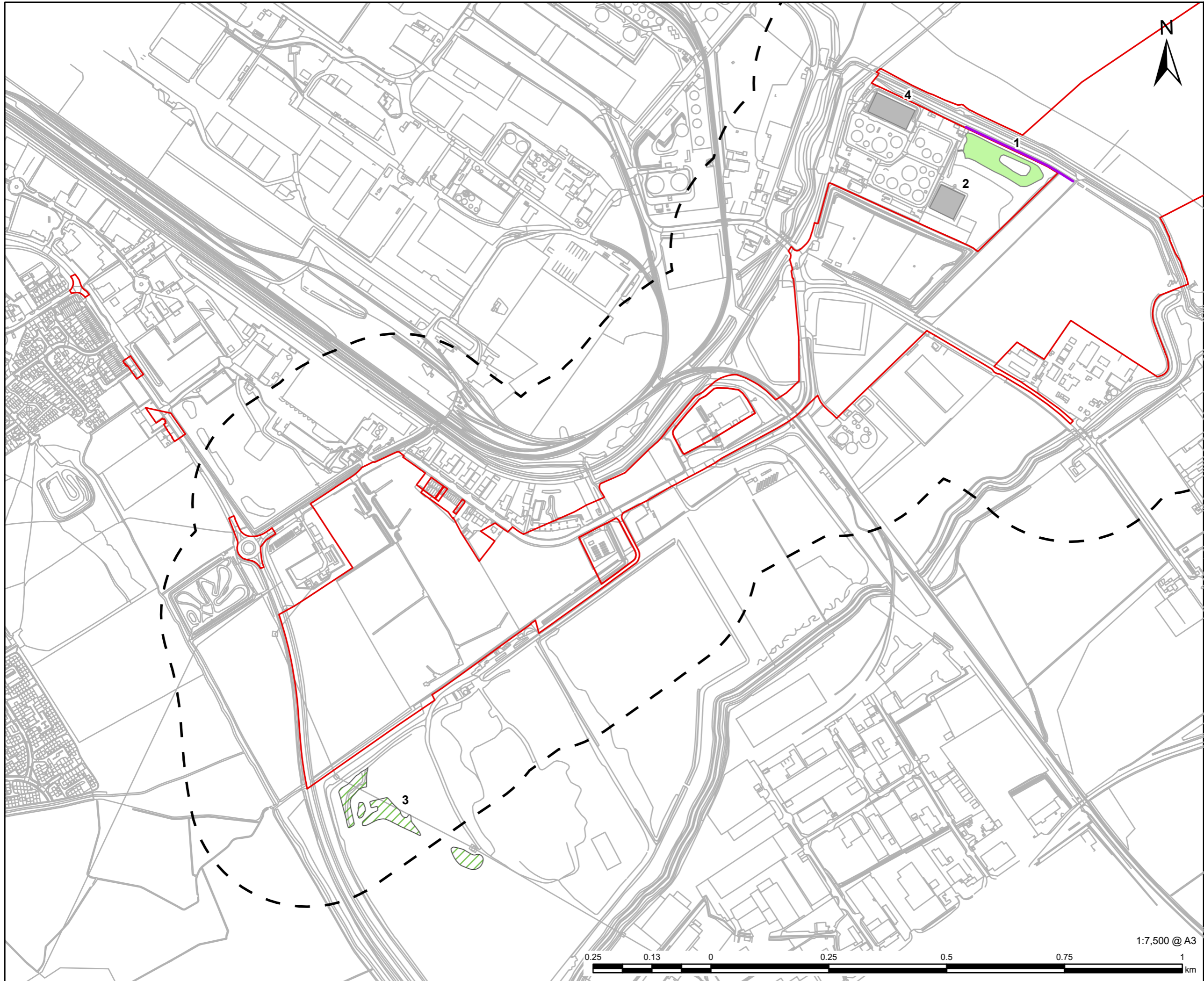
6. References

- Ref 1-1 ARG UK (2010). ARG Advice Note 5: *Great crested newt Habitat Suitability Index*. Amphibian and Reptile Groups of the United Kingdom.
- Ref 1-2 Biggs, J., Ewald, N., Valentini, A., Gaboriaud, C., Griffiths, R.A., Foster, J., Wilkinson, J., Arnett, A., Williams, P. and Dunn, F. (2014). *Analytical and methodological development for improved surveillance of the Great crested newt*. Defra Project WC1067. Freshwater Habitats Trust, Oxford.
- Ref 1-3 Natural England (2022). Natural England Standing Advice (Great crested newts).
- Ref 1-4 Oldham R.S., Keeble J., Swan M.J.S. & Jeffcote M. (2000). *Evaluating the suitability of habitat for the Great crested newt (Triturus cristatus)*. Herpetological Journal 10(4), 143-155.
- Ref 1-5 The Stationary Office Limited (1981). Wildlife and Countryside Act (as amended).
- Ref 1-6 The Stationary Office Limited (2019). Conservation of Habitats and Species Regulations (as amended).
- Ref 1-7 The Stationary Office Limited (2006). Natural Environment and Rural Communities Act.
- Ref 1-8 Lincolnshire Environmental Records Centre (2023). Great crested newt records within 2 km of the Project.
- Ref 1-9 Natural England (2022). Multi-Agency Geographic Information for the Countryside (MAGIC) website.

Annex A: Figure 1

LEGEND

	Site Boundary
	250m Study Area
	Inconclusive GCN eDNA Survey Result – AECOM 2023
	Negative GCN eDNA Survey Result – AECOM 2023
	Negative GCN Survey Results – Others
	Unsuitable for Breeding Amphibians



NOTES
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ISSUE PURPOSE
Environmental Statement

PROJECT NUMBER
60673509

DEVELOPMENT CONSENT ORDER NO
TR030008

FIGURE TITLE
Location of Ponds within 250m

FIGURE NUMBER
Figure 1



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Annex B: Laboratory eDNA Result Sheets

Client: [REDACTED]
AECOM



ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

Tel: [REDACTED]

www.adas.uk

Sample ID: ADAS-4842 Condition on Receipt: High Sediment Volume: Passed
Client Identifier: CLEE Description: pond water samples in preservative
Date of Receipt: 07/07/2022 Material Tested: eDNA from pond water samples

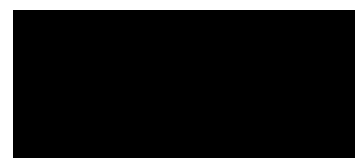
Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	12/07/2022
Degradation Control [§]	Evidence of degradation	Real Time PCR	12/07/2022
Great Crested Newt*	Indeterminate	Real Time PCR	12/07/2022
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

Signed:



Signed:



Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 13/07/2022 Date of issue: 13/07/2022

eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.*

[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#] Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

Appendix 1: Interpretation of results

Sample Condition

Upon sample receipt we score your samples according to quality: good, low sediment, medium sediment, high sediment, white precipitate, and presence of algae.

There are three reasons as to why sediment should be avoided:

1. It is possible for DNA to persist within the sediment for longer than it would if it was floating in the water which could lead to a false positive result i.e. in this case GCN not recently present but present a long time ago
2. In some cases sediment can cause inhibition of the PCR analysis used to detect GCN eDNA within samples which could lead to an indeterminate result.
3. In some cases sediment can interfere with the DNA extraction procedure resulting in poor recovery of the eDNA which in turn can lead to an indeterminate result.

Algae can make the DNA extraction more difficult to perform so if it can be avoided then this is helpful.

Sometimes samples contain a white precipitate which we have found makes the recovery of eDNA very difficult. This precipitate can be present in such high amounts that it interferes with the eDNA extraction process meaning that we cannot recover the degradation control (nor most likely the eDNA itself) at sufficient levels for the control to be within the acceptable limits for the assay, therefore we have to classify these type of samples as indeterminate.

What do my results mean?

A positive result means that great crested newts are present in the water or have been present in the water in the recent past (eDNA degrades over around 7-21 days).

A negative result means that DNA from the great crested newt has not been detected in your sample.

On occasion an inconclusive result will be issued. This occurs where the DNA from the great crested newt has not been detected but the controls have indicated that either: the sample has been degraded and/or the eDNA was not fully extracted (poor recovery); or the PCR inhibited in some way. This may be due to the water chemistry or may be due to the presence of high levels of sediment in samples which can interfere with the DNA extraction process. A re-test could be performed but a fresh sample would need to be obtained. We have successfully performed re-tests on samples which have had high sediment content on the first collection and low sediment content (through improved sample collection) on the re-test. If water chemistry was the cause of the indeterminate then a re-test would most likely also return an inconclusive result.

The results will be recorded as indeterminate if the GCN result is negative and the degradation result is recorded as:

1. evidence of decay - meaning that the degradation control was outside of accepted limits
2. evidence of degradation or residual inhibition - meaning that the degradation control was outside of accepted limits but that this could have been due to inhibitors not being removed sufficiently by the dilution of inhibited samples (according to the technical advice note)

Folio No: [REDACTED]
Report No: 1
Purchase Order: [REDACTED]
Client: AECOM INFRASTRUCTURE
AND ENVIRONMENT
Contact: [REDACTED]

TECHNICAL REPORT

ANALYSIS OF ENVIRONMENTAL DNA IN POND WATER FOR THE DETECTION OF GREAT CRESTED NEWTS (*TRITURUS CRISTATUS*)

SUMMARY

When great crested newts (GCN), *Triturus cristatus*, inhabit a pond, 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 GCN habitation or establish GCN absence.

RESULTS

Date sample received at Laboratory: 12/05/2023
Date Reported: 22/05/2023
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
3453	Immingham - Firepond	TA 21142 15546	Pass	Pass	Pass	Negative	0

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

Reported by: [REDACTED]

Approved by: [REDACTED]



METHODOLOGY

The samples detailed above have been analysed for the presence of GCN eDNA following the protocol stated in DEFRA WC1067 'Analytical and methodological development for improved surveillance of the Great Crested Newt, Appendix 5.' (Biggs et al. 2014). Each of the 6 sub-sample tubes are first centrifuged and pooled together into a single sample which then undergoes DNA extraction. The extracted sample is then analysed using real time PCR (qPCR), which uses species-specific molecular markers to amplify GCN DNA within a sample. These markers are unique to GCN DNA, meaning that there should be no detection of closely related species.

If GCN DNA is present, the DNA is amplified up to a detectable level, resulting in positive species detection. If GCN 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 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.

SureScreen Scientifics Ltd is ISO9001 accredited and participate in Natural England's proficiency testing scheme for GCN eDNA testing. We also carry out regular inter-laboratory checks on accuracy of results as part of our quality control procedures.

INTERPRETATION OF RESULTS

- 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 lead indicate a risk of false 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 GCN eDNA** [Positive/Negative/Inconclusive]
Positive: GCN DNA was identified within the sample, indicative of GCN 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 GCN 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 GCN presence.
Negative: GCN eDNA was not detected or is below the threshold detection level and the test result should be considered as evidence of GCN absence, however, does not exclude the potential for GCN presence below the limit of detection.

