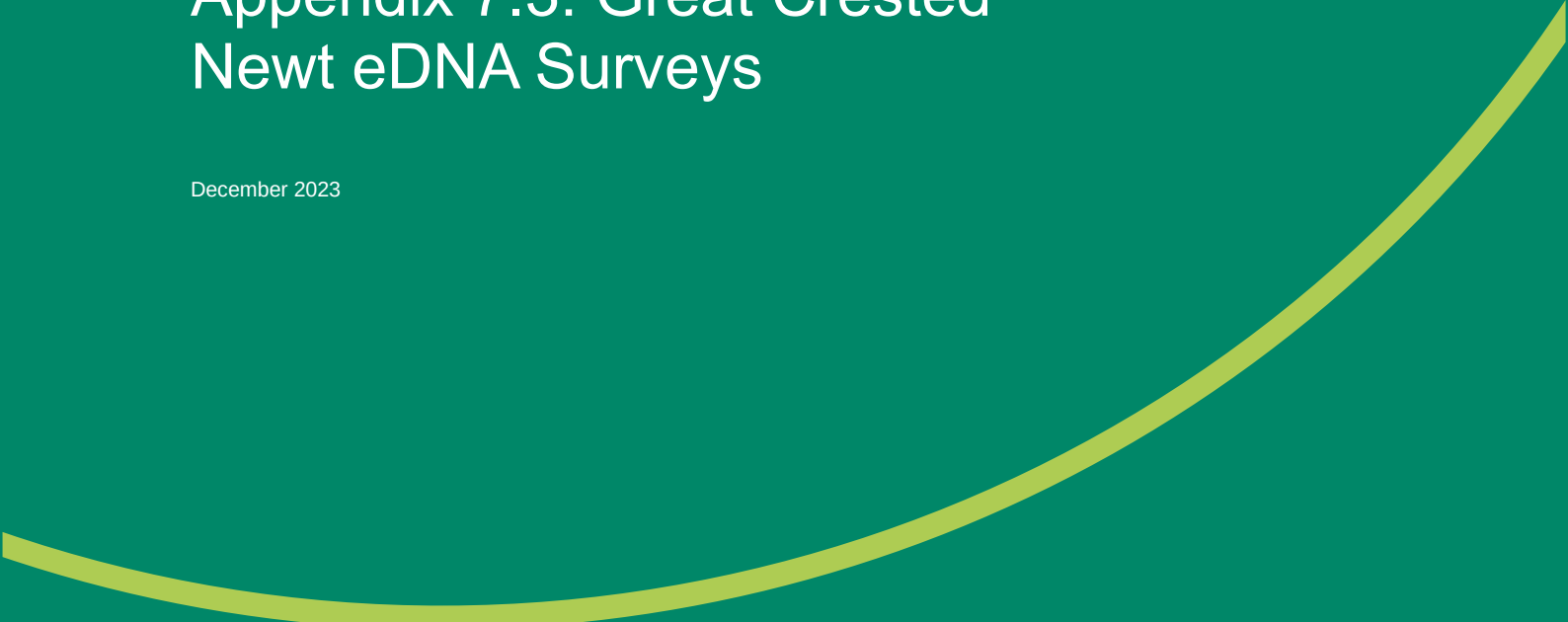


National Grid Cable Tunnel Replacement Project

Environmental Statement - Volume VI

Appendix 7.3: Great Crested Newt eDNA Surveys

December 2023



Quality information

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1 Introduction

1.1 Background

1.1.1 AECOM Ltd (hereafter referred to as AECOM) was commissioned by National Grid to undertake an ecological assessment to support the Environmental Statement for the Tilbury to Grain and Tilbury to Kingsnorth (TKRE) Cable Tunnel Replacement Project (hereafter referred to as the Proposed Development). The Proposed Development relates to the replacement of the below ground cabled section of the TKRE 400 kV circuits with the construction of a new bored tunnel under the River Thames to carry new XLPE cables, together with following above-ground components at both ends of the new tunnel:

- **A new cable sealing end compound:** consisting of:
 - **a new tunnel headhouse** which will cover the shaft into the tunnel;
 - **a new overhead line gantry structure** which will connect the overhead line downlead conductors and transfer them to six sealing end structures; and
- **Modifications to the existing overhead line (OHL):** The new OHL conductors will be connected to the existing TKRE 400kV OHL conductors via new terminal pylons. The old OHL conductors and existing pylons will be either replaced, refurbished or removed.

1.1.2 The required headhouses and SECs would fall within the jurisdiction of Thurrock Council (north of the River Thames) and Gravesham Borough Council (south of the River Thames).

1.2 Scope

1.2.1 A Preliminary Ecological Appraisal (PEA)¹ was completed in November 2022 in support of the Proposed Development, covering a wider area than the Red Line Boundary shown in Figure 1. The data search within the PEA (ES Volume VI Appendix 7.1) identified a single historic record of great crested newt located 520m north-east of the Tilbury Site. In addition, records of great crested newts were recorded 900m to the south-west of the Gravesend Site. In both cases the desk study records were more than ten years old.

1.2.2 Given that great crested newt is known to be widespread in both Essex and Kent, the PEA incorporated a Habitat Suitability Index (HSI) assessment of a total of six waterbodies within 250m of the Tilbury and Gravesend sites to gauge their suitability for this species. This assessment was based on the standard method outlined in the advice note produced by the Amphibian and Reptile Groups of the UK (ARG)². The HSI results for each of the six water bodies are listed in Table 1 and the locations are shown in Figure 1.

Table 1. Water Body Locations and HSI Results

Water Body	Location	HSI Result
Waterbody 1	Tilbury - TQ 66362 75557	0.78 (Good Quality)
Waterbody 2	Tilbury - TQ 66689 75623	0.74 (Good Quality)
Waterbody 3	Tilbury - TQ 66476 75830	0.76 (Good Quality)
Waterbody 4	Gravesend - TQ 680249 73979	0.75 (Good Quality)
Waterbody 5	Gravesend - TQ 67486 74190	0.75 (Good Quality)

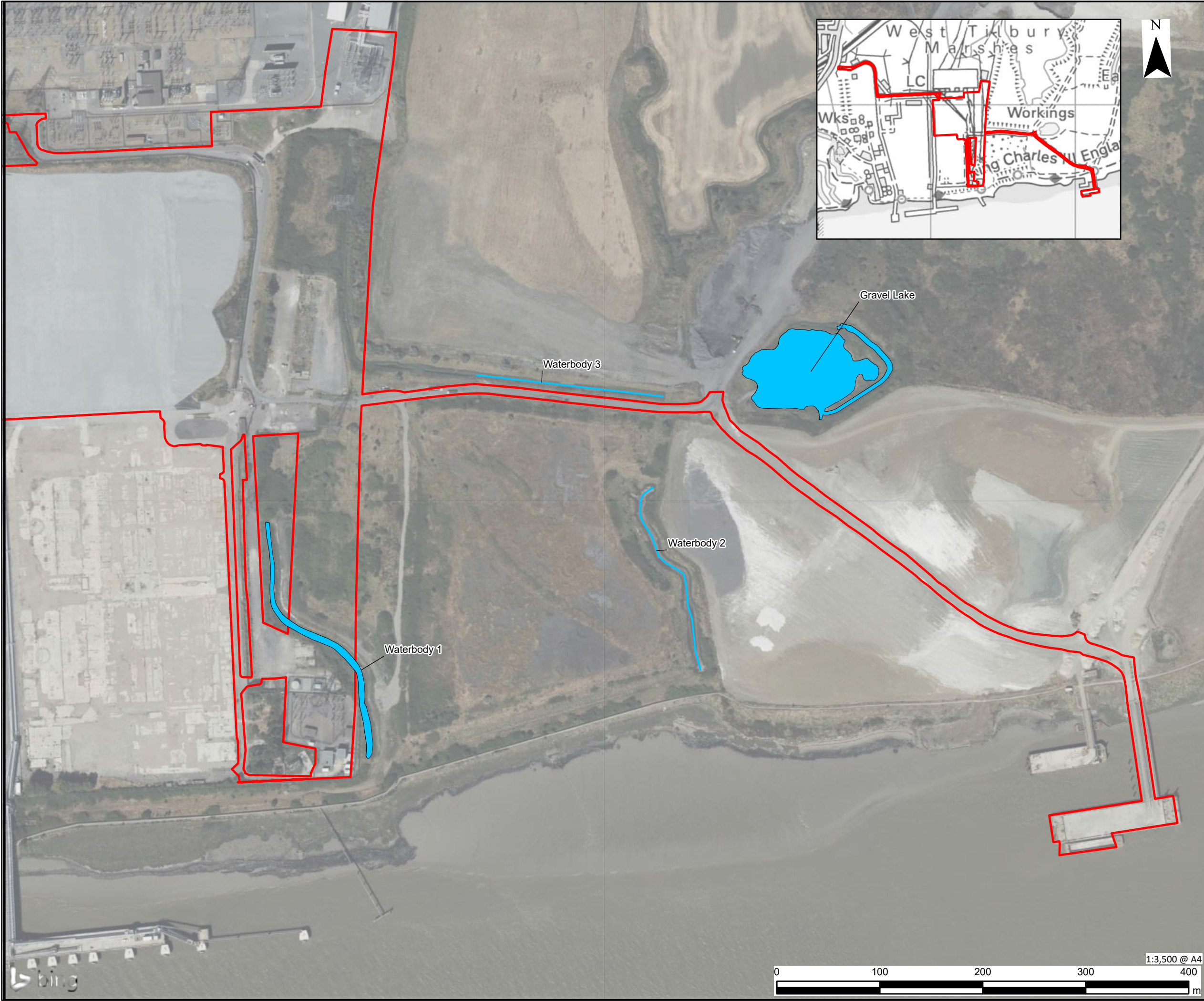
¹ AECOM Ltd (2023) TKRE Preliminary Ecological Appraisal.

² ² <https://www.arguk.org/info-advice/advice-notes/9-great-crested-newt-habitat-suitability-index-arg-advice-note-5/file>

Water Body	Location	HSI Result
Waterbody 6 - Gravel Lake	Tilbury - TQ 66790 75825	0.46 (Poor Quality)

1.3 Objective

- 1.3.1 The objective of the eDNA great crested newt survey was to investigate the presence or likely absence of great crested newts, which may constrain the works or influence the design and implementation of the Proposed Development.
- 1.3.2 If great crested newts were found to be present, results would be used to identify any mitigation or licensing requirements to facilitate the Proposed Works, while protecting the Favourable Conservation Status (FCS) of the local great crested newt population.



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TKRE National Grid

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National Grid

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LEGEND
 Red Line Boundary (Above Ground)
 Waterbody
 Waterbody

NOTES
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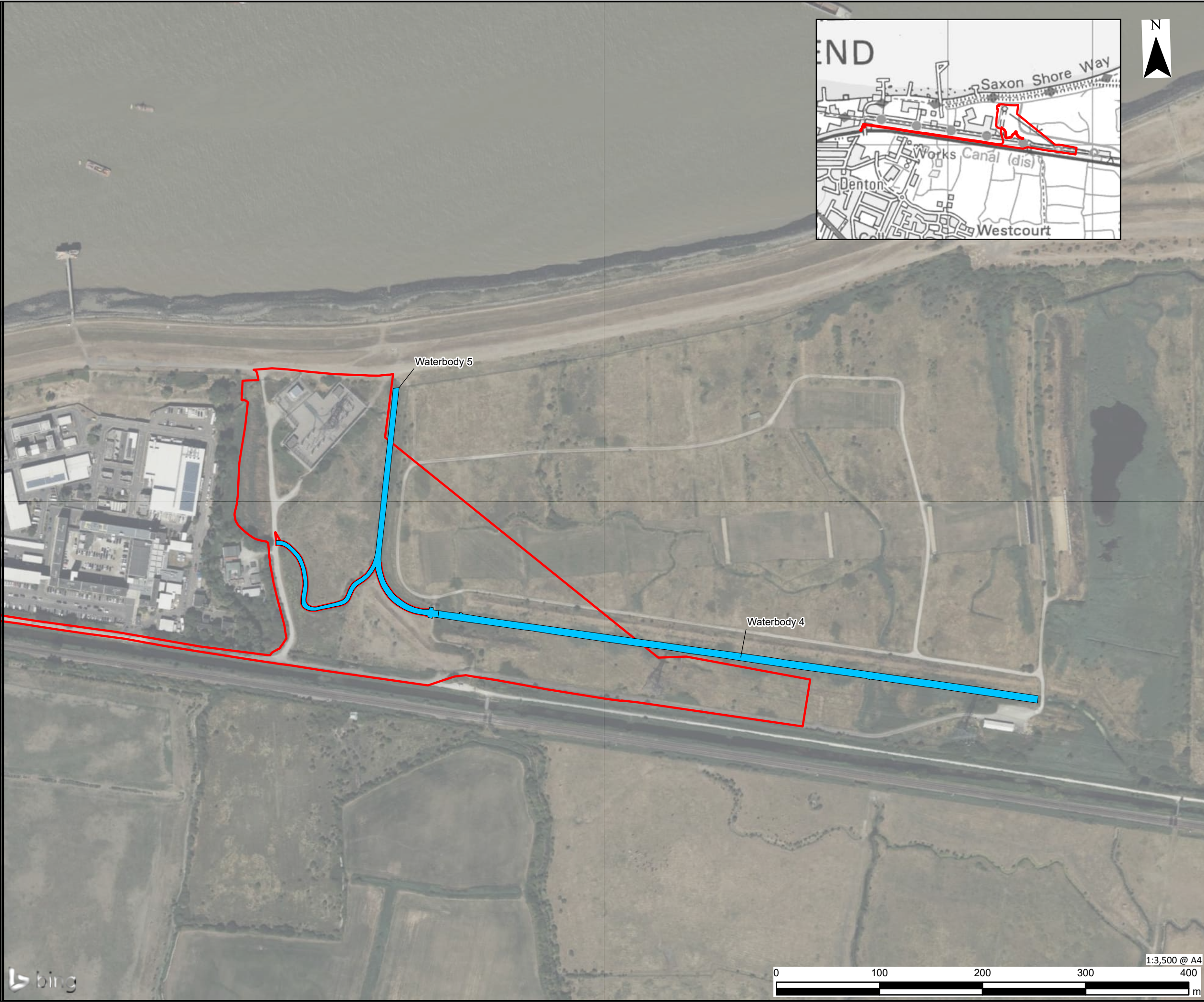
ISSUE PURPOSE
GCN Report

PROJECT NUMBER
60677311

FIGURE TITLE
Location of Waterbodies
Page 1 of 2 - Tilbury

FIGURE NUMBER
Figure 1

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LEGEND
 Red Line Boundary (Above Ground)
 Waterbody

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ISSUE PURPOSE
GCN Report

PROJECT NUMBER
60677311

FIGURE TITLE
Location of Waterbodies
Page 2 of 2 - Gravesend

FIGURE NUMBER
Figure 1

2 Legislation

- 2.1.1 Great crested newts are licenced under Schedule 5 of the Wildlife and Countryside Act 1981 (as amended), and Schedule 2 of the Conservation of Habitats and Species Regulations 2017 (as amended). This legislation, when taken together results in a level of protection that prohibits:
- Killing, injuring, taking or disturbance of the great crested newt;
 - Damaging, destroying or obstructing any place used by great crested newts for the purpose of breeding or refuge; and
 - Selling and/or advertising the sale of a great crested newt or any body part of this species.
- 2.1.2 Note that a Natural England (NE) European Protected Species (EPS) mitigation licence would be required to allow an otherwise unlawful act to proceed if great crested newts are present and work would impact their aquatic or terrestrial habitats i.e., impacting the breeding and/or resting site of an EPS.
- 2.1.3 Great crested newts are listed under Section 41 of the 'Natural Environment and Rural Communities (NERC) Act 2006 (as amended) as a Species of Principal Importance for Nature Conservation in England. Section 40 of the same Act requires that local and regional 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 Records of protected and/or notable species recorded within a 2km radius of Proposed Development were obtained from Essex Field Club and Kent³ and Medway Biological Records Centre (KMBRC)⁴ in support of the PEA⁵.
- 3.1.2 In addition, a review of publicly available information collected in support of nearby large scale infrastructure developments has been undertaken, and included a search for relevant data in planning documentation for the following developments:
- Lower Thames Crossing⁶;
 - Thurrock Flexible Generation Plant⁷; and
 - Tilbury 2⁸.

3.2 eDNA Sampling

- 3.2.1 Environmental DNA or eDNA refers to DNA that can be extended from environmental services such as water, soil or faeces, without first isolating any target organism. All living organisms leave traces of their DNA within the environment and this DNA enables the detection of species regardless of their life stage or gender.
- 3.2.2 eDNA surveys can prove presence or likely absence of great crested newts without the typical suite of nocturnal presence/absence surveys being undertaken. This survey technique was developed through research funded by Defra⁹ and can be carried out between the 15th of April and the 30th of June.
- 3.2.3 Two ecologists (one of whom holds a Natural England Class 1 licence to survey great crested newts) undertook eDNA sampling on the four waterbodies on the Tilbury section of the Site and the two waterbodies on the Gravesend section of the Site, to determine the presence/ likely absence of great crested newts within those waterbodies. The eDNA sampling was undertaken on the 4th and 9th of May 2023 and the results were returned by the laboratory on the 15th of May 2023.
- 3.2.4 Negative results for the eDNA surveys are accepted as evidence of the likely absence of great crested newts within a waterbody and further surveys are subsequently not required.
- 3.2.5 Positive results from the eDNA surveys indicate that great crested newts are present within a waterbody and, therefore, further surveys might be required to determine an estimate of the population size.

3.3 Laboratory Analysis

- 3.3.1 The method used by the laboratory is shown in the two letters detailed in Annex A of this report.

3.4 Survey Limitations

- 3.4.1 Two water bodies (WB2, WB3) located on the Tilbury section of the Site returned results as inconclusive. Follow up tests on the water were carried out to determine if the level of salinity of the water was suitable to support populations of great crested newts.

³ Essex Field Club (2022) Datasearch Report Ref: EFC5454 28-11-2022

⁴ Kent and Medway Biological Records Centre (2022) Records Search Ref: ENQ/22/506

⁵ AECOM Ltd (2023) TKRE Preliminary Ecological Appraisal.

⁶ [Lower Thames Crossing | National Infrastructure Planning \(planninginspectorate.gov.uk\)](#)

⁷ [Thurrock Flexible Generation Plant | National Infrastructure Planning \(planninginspectorate.gov.uk\)](#)

⁸ [Tilbury2 | National Infrastructure Planning \(planninginspectorate.gov.uk\)](#)

⁹ Biggs, J., et al (2014) Analytical and methodological development for improved surveillance of the Great Crested Newt

- 3.4.2 A single water body at Tilbury WB1 and a single water body on Gravesend WB5 were inaccessible to survey due to the steepness of the banks, and areas of bare mud that made it unsafe to access the full extent. The water body WB4 was connected to WB5 on Site, so it is likely that the results from WB5 would be applicable for WB4. It is considered likely that due to distance of WB1 from WB2 (280m) and WB3 (300m), the presence of suitable habitat between the waterbodies, and the brackish nature of WB2 and WB3, the results obtained from WB2 and WB3 can be extended to WB1.
- 3.4.3 An ecological survey represents a 'snapshot' in time of the ecological condition of a site. The ecological character of a site can change substantially throughout both the course of a year, as well as from year to year, impacting the extent and quality of habitats potential to support protected species.
- 3.4.4 The results of an eDNA survey remain valid for two years, after which, if works have not yet commenced, advice from an ecologist is recommended to confirm if an update survey is required to be undertaken.

3.5 Quality Assurance

- 3.5.1 All AECOM ecologists are members or are under appreciate for membership of the Chartered Institute of Ecology and Environmental Management (CIEEM) at the appropriate level and follow the code of professional conduct when undertaking work.
- 3.5.2 The eDNA sampling was undertaken by an ecologist with a Natural England great crested newt licence, as required by the methodology set out in regards to eDNA sampling.

4 Results

4.1 Tilbury Waterbodies

Desk Study

- 4.1.1 Records from the Essex Field Club contain a record of a single GCN at Grid Reference TQ658007 6000 from 2007. This is approximately 230m to the west of an area of existing hardstanding that will be utilised for storage, and approximately 515m from the tunnel shaft location. There is no waterbody at this location, and the site has undergone significant change since 2007 with the demolition of the power station. The closest potentially suitable waterbody still present after the power station demolition is a pond at TQ65592 76015. This pond is approximately 430m from the boundary of the Tilbury Site and is now separated from the Tilbury Site by large areas of hardstanding. It is considered unlikely GCN are present, and even if they were utilising this pond any linkage with the Tilbury Site is negligible such that they are assumed absent from any of the habitats within the Tilbury Site.
- 4.1.2 The four waterbodies at Tilbury identified for survey in 2023 (WB1, WB2, WB3 and WB6 – See Figure 1), are all identified within the Thurrock Flexible Power Generation Environmental Statement¹⁰ as having been subject to great crested newt survey in 2016/2017 and 2017/2018 by RWE, along with a range of other waterbodies more distant from the Tilbury Site. No great crested newts were identified within 500m of the Tilbury Site.
- 4.1.3 WB6 (gravel pit lake) was the only of the four waterbodies relevant to the Tilbury Site which were subject to survey by National Highways in support of the Lower Thames Crossing¹¹. The pond returned a negative result for eDNA and conventional survey in 2018 and GCN was assumed absent.

Field Survey

- 4.1.4 Four waterbodies of interest were recorded on Site in 2023, with samples taken for three of the waterbodies (WB2, WB3 and WB6) and analysed by the laboratory. Results are summarised in **Error! Reference source not found.**
- 4.1.5 As detailed in Section 3.4, waterbody WB1 could not be safely accessed for survey in 2023.
- 4.1.6 The gravel pit lake designated as WB6 returned a negative result from the laboratory analysis. Therefore, great crested newts are assumed to be absent from the lake.
- 4.1.7 Laboratory analysis of the samples from waterbodies WB2 and WB3 returned an ‘inconclusive’ result. An inconclusive result indicates that while no GCN eDNA was detected in the sample there may be some underlying degradation or inhibition which could have affected the result. Sediment, algae, plant matter or an unusually high concentration of inhibitory molecules can result in inconclusive results¹².
- 4.1.8 Brackish water within the ditches was identified as a potential reason for the inconclusive results, and in order to test this theory water samples were collected from waterbodies WB2 and WB3, and their conductivity measured to assess their salinity.
- 4.1.9 Analysis of the water samples from WB2 and WB3 revealed that the two waterbodies had a conductivity of 12,000 uS/cm. Freshwater typically ranges between 0 and 1,500 uS/cm, while ocean water has a conductivity of 50,000 uS/cm.

¹⁰ RPS (2020) Thurrock Flexible Generation Plant – Environmental Statement Volume 3 – Chapter 9: Onshore Ecology [EN010092-001012-Environmental Statement Volume 3 Chapter 9 - Onshore Ecology \(Dec 2020\).pdf \(planninginspectorate.gov.uk\)](#)

¹¹ National Highways (2022) Lower Thames Crossing 6.2 Environmental Statement Figures: Figure 8.8 – GCN Presence/Absence Results [TR010032-001769-6.2 Environmental Statement Figure 8.8 - GCN Presence_Absence Results.pdf \(planninginspectorate.gov.uk\)](#)

¹² [Detailed-GCN-Sample-Collection-Guidance-V4-1.pdf \(surescreenscientifics.com\)](#)

Table 2. Summary of eDNA survey results at Tilbury

Waterbody	Location	his	Laboratory eDNA Analysis Result	Conductivity
Waterbody 1 (WB1)	TQ 66362 75557	0.78 (Good Quality)	N/A – not accessible	N/A
Waterbody 2 (WB2)	TQ 66689 75623	0.74 (Good Quality)	Inconclusive	12,000 uS/cm
Waterbody 3 (WB3)	TQ 66476 75830	0.76 (Good Quality)	Inconclusive	12,000 uS/cm
Waterbody 6 (WB6) (gravel pit lake)	TQ 66790 75825	0.46 (Poor Quality)	Negative	N/A

4.2 Gravesend Waterbodies

Desk Study

- 4.2.1 The desk study records from Kent and Medway Biological Records Centre identified no records of great crested newt within 1km of the Gravesend Site.
- 4.2.2 No waterbodies within 500m of the Gravesend Site were found to support GCN during surveys in support of the Lower Thames Crossing¹³.

Field Survey

- 4.2.3 Two waterbodies were identified for survey (WB4 and WB5). Results are summarised in Table 3.
- 4.2.4 WB4 was sampled and analysis returned a negative result. Therefore, great crested newt are assumed absent.
- 4.2.5 WB5 could not be safely access to take water samples.

Table 3. Summary of eDNA survey at Gravesend Site

Waterbody	Location	HSI	Laboratory eDNA Analysis Result
Water Body 4 (WB4)	TQ 680249 73979	0.75 (Good Quality)	Negative
Water Body 5 (WB5)	TQ 67486 74190	0.75 (Good Quality)	N/A – not accessible

¹³ National Highways (2022) Lower Thames Crossing 6.2 Environmental Statement Figures: Figure 8.8 – GCN Presence/Absence Results [TR010032-001769-6.2 Environmental Statement Figure 8.8 - GCN Presence Absence Results.pdf \(planninginspectorate.gov.uk\)](#)

5 Discussion

5.1 Tilbury

- 5.1.1 eDNA survey undertaken in 2023 found that great crested newt were absent from WB6, the only fully freshwater waterbody in the vicinity of the Tilbury Site.
- 5.1.2 WB2 and WB3 gave 'inconclusive' eDNA results. The relatively high salinity readings returned from these samples are a likely reason for these results.
- 5.1.3 Amphibians, including great crested newts respire partially through their damp skin through osmosis, and high levels of salinity reduce the efficiency of respiration, with some chemicals found within saline waters suspected to be toxic to amphibians, especially in high concentrations¹⁴.
- 5.1.4 Due to the brackish conditions of the water samples from WB2 and WB3, both are considered unsuitable to support great crested newt populations.
- 5.1.5 While WB1 could not be safely accessed for survey, due to similar connectivity to the Thames foreshore salinity levels are likely to be similar, and as such WB1 is also considered likely to be unsuitable to support great crested newt.
- 5.1.6 In conclusion, considering the results obtained from the eDNA laboratory analysis, the conductivity measurements and the limitations identified in Section 3.4, great crested newt are considered likely to be absent from the Tilbury Site.

5.2 Gravesend

- 5.2.1 eDNA surveys undertaken in 2023 identified that great crested newt were absent from WB4. While it was not possible to access WB5 to obtain water samples, given it is connected to WB4 and similar in nature (steep sided slow flowing and brackish) it is considered very unlikely that great crested newt would utilise WB5.
- 5.2.2 Based on the above, no further surveys are considered to be necessary and great crested newt are assumed to be absent from WB4 and WB5.

¹⁴ Duff J.P., Colville K and Foster J (2011) Mass mortality of great crested newts (*Triturus cristatus*) on ground treated with road salt, Veterinary Record 2011 168: 282

Annex A Laboratory Results

Folio No: E17310
Report No: 1
Purchase Order: 679009
Client: AECOM INFRASTRUCTURE
AND ENVIRONMENT
Contact: John Cheese

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: 11/05/2023
Date Reported: 15/05/2023
Matters Affecting Results: R314 and R320: Inhibited

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
R313	TKRE Tilbury	TQ 66790 75825	Pass	Pass	Pass	Negative	0
R314	TKRE Tilbury 3	TQ 66689 75623	Pass	Pass	Fail	Inconclusive	0
R320	TKRE Tilbury 2	TQ 66476 75830	Pass	Pass	Fail	Inconclusive	0

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

Reported by: Chris Troth

Approved by: Chelsea Warner



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.



Folio No: E17309
Report No: 1
Purchase Order: 1586976
Client: AECOM INFRASTRUCTURE
AND ENVIRONMENT
Contact: John Cheese

TECHNICAL REPORT

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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: 11/05/2023
Date Reported: 15/05/2023
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
R317	TKRE Gravesend 1	TQ 680249 73979	Pass	Pass	Pass	Negative	0

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

Reported by: Chris Troth

Approved by: Chelsea Warner



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.

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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.



