Annex E: GCN eDNA Results

DNA Analysis Report - Commercial in Confidence

Customer:	AECOM
Address:	2 City Walk Leeds LS11 9AR
Contact:	Jo Atkinson
Email:	jo.atkinson@aecom.com
Tel:	07881511261
Report date:	15-Jun-2018
Order Number:	GCN18-0909
Samples:	Pond Water
Analysis requested:	Detection of Great Crested Newt eDNA from pond water.

Thank you for submitting your samples for analysis with the Fera eDNA testing service. The details of the analysis are as follows:

Method:

The method detects pond occupancy from great crested newts (GCN) using traces of DNA shed into the pond environment (eDNA). The detection of GCN eDNA is carried out using real time PCR to amplify part of the cytochrome 1 gene found in mitochondrial DNA. The method followed is detailed in Biggs J., et al, (2014). Analytical and methodological development for improved surveillance of the Great Crested Newt. Appendix 5. Technical advice note for field and laboratory sampling of great crested newt (Triturus cristatus) environmental DNA. Freshwater Habitats Trust, Oxford.

The limits of this method are as follows: 1) the results are based on analyses of the samples supplied by the client and as received by the laboratory, 2) any variation between the characteristics of this sample and a batch will depend on the sampling procedure used. 3) the method is qualitative and therefore the levels given in the score are for information only, they do not constitute the quantification of GCN DNA against a calibration curve, 4) a 'not detected' result does not exclude presence at levels below the limit of detection.

The results are defined as follows:

Positive:	DNA from the species was detected.
eDNA Score:	Number of positive replicates from a series of twelve.
Negative:	DNA from the species was not detected; in the case of negative samples the DNA extract is further tested for PCR inhibitors and degradation of the sample.
Inconclusive:	Controls indicate degradation or inhibition of the sample, therefore the lack of detection of GCN DNA is not conclusive evidence for determining the absence of the species in the sample provided.

DNA Analysis Report - Commercial in Confidence

CustomerReference	Fera Reference	GCN Detection	eDNA Score	Inhibition	Degradation
-	S18-015120	Negative	0	No	No

The results indicate that eDNA for great crested newts was not detected in the sample submitted. Analysis was conducted in the presence of the following controls: 1) extraction blank, 2) appropriate positive and negative PCR controls for each of the TaqMan assays (GCN, Inhibition, and Degradation). All controls performed as expected.

This test procedure was developed using research funded by the Department of Environment, Food and Rural Affairs.

Issuing officer: Steven Bryce
Tel: 01904 462 070
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18186-AE-AJ-1 Order number: AEC-17-AJ

Great Crested Newt eDNA Results

Company: AECOM
Address: 2 City Walk, Leeds LS11 9AR
Contact: Jo Atkinson
Project code|Task code: TBC
Date of Report: 29 May 2018
Number of samples: 1

Thank you for sending your sample for analysis by NatureMetrics. Your sample has been processed in accordance with the protocol set out in Appendix 5 of Biggs et al. (2014).

DNA was precipitated via centrifugation at 14,000 x g and then extracted using Qiagen Blood and Tissue extraction kits.

qPCR amplification was carried out in 12 replicates per sample, using the primers and probe described by Biggs et al. (2014), in the presence of both positive and negative controls.

Results indicate GCN absence in your sample. No degradation or inhibition was detected, and all controls performed as expected. Conclusive results are therefore presented.

Results are based on the samples as supplied by the client to the laboratory. Incorrect sampling methodology may affect the results. Note that a negative result does not preclude the presence of Great Crested Newts at a level below the limits of detection.

Sample	Pond ID	Date arrived	Inhibition	Degradation	eDNA score	GCN status
GCN18-1058	'Pond 2'	22-May-18	No	No	0	Negative

End of report

Report issued by: Dr. Cuong Tang

Contact: ct@naturemetrics.co.uk | 01491 829042

Understanding your results

Positive: GCN DNA has been detected in this sample, meaning that at least one of the 12 replicates has amplified. Remember that this is not a quantitative test, so you should not interpret a high eDNA score (e.g. 12/12) as necessarily indicating a larger population of GCN than a low eDNA score (e.g. 1/12).

Negative: No GCN DNA has been detected in this sample, and the internal and external controls worked as expected. This tells us that if there had been GCN DNA in the sample, we would have detected it, so we can be confident in its absence from the sample provided.

Inconclusive: No GCN DNA was detected in the sample, but the internal controls failed to amplify as expected. This means that any GCN DNA in the sample might also have failed to amplify properly, so we cannot have confidence in this negative result. Inconclusive results can be caused by degradation of the DNA (when the DNA marker contained in the ethanol in the kits fails to amplify) or by inhibition of the reaction (when the marker added in the lab fails to amplify) caused by certain chemicals or organic compounds that may be present in the water sample.

18186-AE-AJ-2 Order number: AEC-17-AJ

Great Crested Newt eDNA Results

Company: AECOM
Address: 2 City Walk, Leeds LS11 9AR
Contact: Jo Atkinson
Project code|Task code: TBC
Date of Report: 30 May 2018
Number of samples: 1

Thank you for sending your sample for analysis by NatureMetrics. Your sample has been processed in accordance with the protocol set out in Appendix 5 of Biggs et al. (2014).

DNA was precipitated via centrifugation at 14,000 x g and then extracted using Qiagen Blood and Tissue extraction kits.

qPCR amplification was carried out in 12 replicates per sample, using the primers and probe described by Biggs et al. (2014), in the presence of both positive and negative controls.

Results indicate GCN absence in your sample. Inhibition was detected, which could not be overcome with DNA dilution. An inconclusive result is therefore presented.

Results are based on the samples as supplied by the client to the laboratory. Incorrect sampling methodology may affect the results. Note that a negative result does not preclude the presence of Great Crested Newts at a level below the limits of detection.

Sample	Pond ID	Date arrived	Inhibition	Degradation	eDNA score	GCN status
GCN18-1057	'pond 1'	22-May-18	Yes	No	0	Inconclusive

End of report

Report issued by: Dr. Cuong Tang

Contact: ct@naturemetrics.co.uk | 01491 829042

Understanding your results

Positive: GCN DNA has been detected in this sample, meaning that at least one of the 12 replicates has amplified. Remember that this is not a quantitative test, so you should not interpret a high eDNA score (e.g. 12/12) as necessarily indicating a larger population of GCN than a low eDNA score (e.g. 1/12).

Negative: No GCN DNA has been detected in this sample, and the internal and external controls worked as expected. This tells us that if there had been GCN DNA in the sample, we would have detected it, so we can be confident in its absence from the sample provided.

Inconclusive: No GCN DNA was detected in the sample, but the internal controls failed to amplify as expected. This means that any GCN DNA in the sample might also have failed to amplify properly, so we cannot have confidence in this negative result. Inconclusive results can be caused by degradation of the DNA (when the DNA marker contained in the ethanol in the kits fails to amplify) or by inhibition of the reaction (when the marker added in the lab fails to amplify) caused by certain chemicals or organic compounds that may be present in the water sample.

Figure 10C.1 Site Location Plan

THIS DRAWING IS TO BE USED ONLY FOR THE PURPOSE OF ISSUE THAT IT WAS ISSUED FOR AND IS SUBJECT TO AMENDMENT

LEGEND

Application Boundary

Purpose of Issue
ENVIRONMENTAL STATEMENT

Client
EP SHB

Project Title
SOUTH HUMBER BANK ENERGY CENTRE

Application Document Ref
SITE LOCATION PLAN

Drawn	Checked	Approved	Date
LC	SD	JA	06/11/2018

AECOM Internal Project No.
60580855

Scale @ A3
1:50,000

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Drawing Ref
FIGURE 10C.1

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Figure 10C.2 Statutory and Non-statutory Designations

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LEGEND

- Application Boundary
- Application Boundary 2km buffer
- Application Boundary 10km buffer

National designated sites within 2km

- Priority Habitat

Non-statutory designations within 2km

- Local wildlife site
- Site of nature conservation interest

Statutory designations within 10km

- RAMSAR
- Special area of conservation
- Special protection area
- Site of special scientific interest

Purpose of Issue
ENVIRONMENTAL STATEMENT

Client
EP SHB

Project Title
SOUTH HUMBER BANK ENERGY CENTRE

Application Document Ref
STATUTORY AND NON-STATUTORY DESIGNATIONS

Drawn	Checked	Approved	Date
LC	SD	JA	06/11/2018

AECOM Internal Project No.	Scale @ A3
60580855	1:80,000

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Drawing Ref
FIGURE 10C.2

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0 2 4 6 8 10 km

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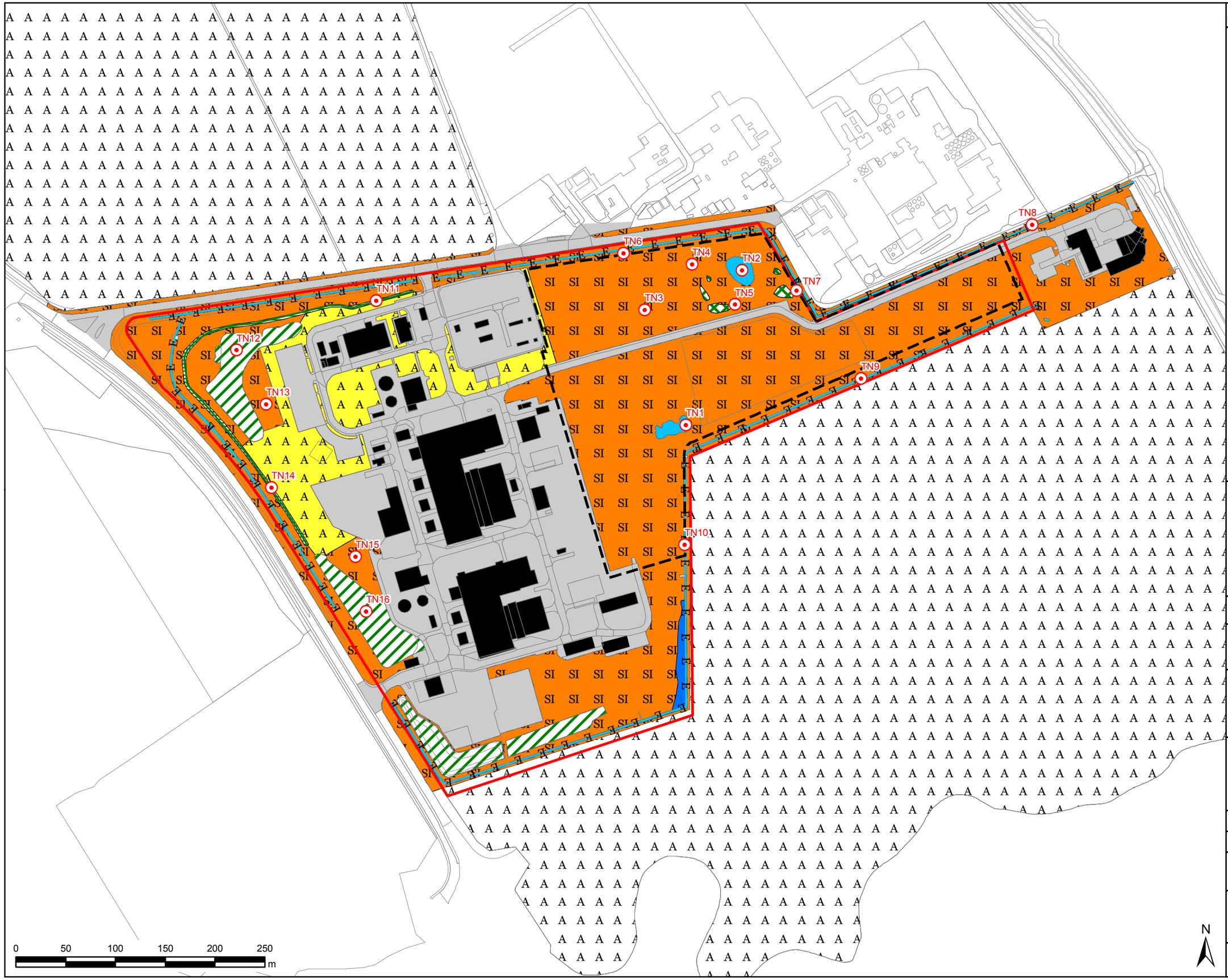
Figure 10C.3 Phase 1 Habitat Map



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LEGEND

- Application Boundary
- Main development area (Indicative)
- Target note
- G2.1 - Running water - eutrophic
- J2.1.2 - Intact hedge - species-poor
- A1.1.2 - Broadleaved woodland - plantation
- A2.1 - Scrub - dense/continuous
- B2.2 - Neutral grassland - semi-improved
- F1 - Swamp
- G1 - Standing water
- G1.1 - Standing water - eutrophic
- J1.1 - Cultivated/disturbed land - arable
- J1.2 - Cultivated/disturbed land - amenity grassland
- J2.1.2 - Intact hedge - species-poor
- J3.6 - Buildings & Structures
- J5 - Other Habitat - Hard Standing

Purpose of Issue
ENVIRONMENTAL STATEMENT

Client
EP SHB

Project Title
SOUTH HUMBER BANK ENERGY CENTRE

Application Document Ref
PHASE 1 HABITAT SURVEY

Drawn	Checked	Approved	Date
LC	SD	JA	06/11/2018

AECOM Internal Project No.	Scale @ A3
60580855	1:3,500

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Drawing Ref
FIGURE 10C.3

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