Environmental Statement: Volume III

Appendix 9B: Great Crested Newts Surveys

VPI Immingham OCGT Project

Document Ref: 6.4.11
PINS Ref: EN010097

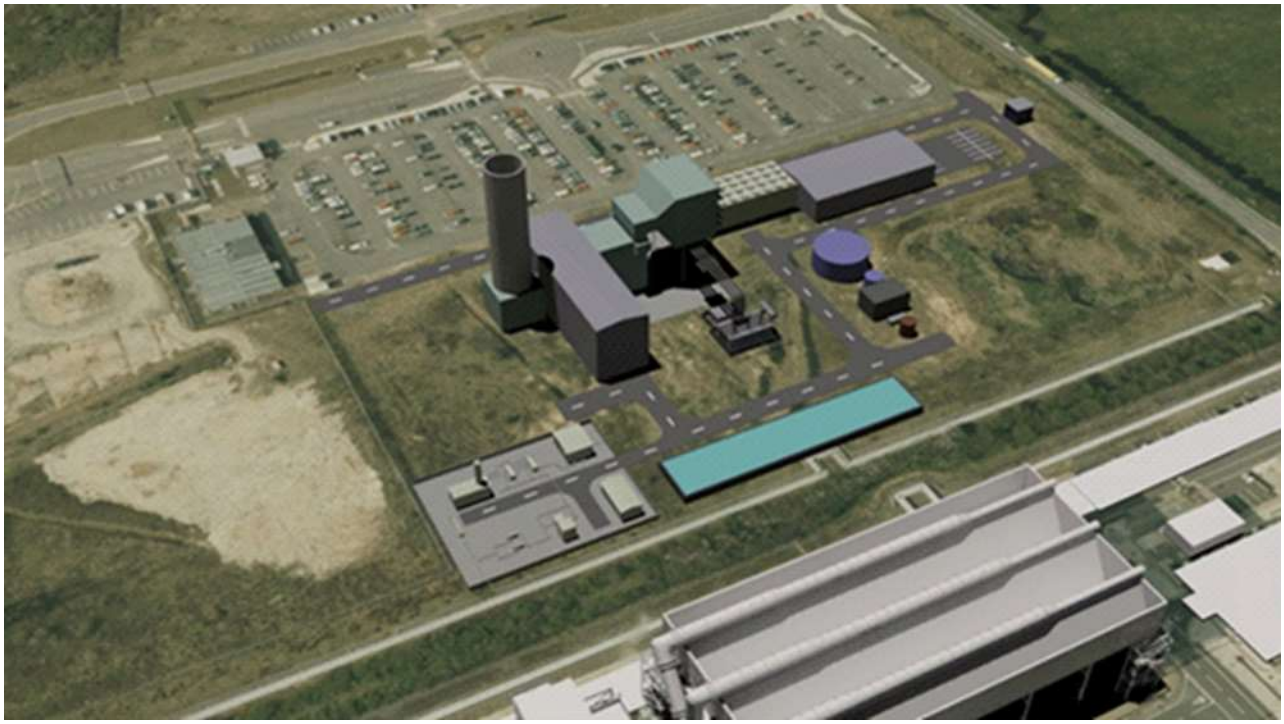
The Immingham Open Cycle Gas Turbine Order

Land to the north of and in the vicinity of the VPI Immingham Power Station, Rosper Road, South Killingholme, Lincolnshire, DN40 3DZ

Environmental Statement Volume III
Appendix 9B: Great Crested Newt Surveys

The Planning Act 2008

The Infrastructure Planning (Applications: Prescribed Forms and Procedure) Regulations 2009 - Regulation 5(2)(q)



Applicant: VPI Immingham B Ltd

Date: April 2019

DOCUMENT HISTORY

Document Ref	**6.4.11**		
Revision	1		
Author	Anna Davies		
Signed	AD	**Date**	April 2019
Approved By	Richard Lowe		
Signed	RL	**Date**	April 2019
Document Owner	AECOM		

GLOSSARY

Abbreviation	Description
EcIA	Ecological Impact Assessment
eDNA	Environmental DNA
ES	Environmental Statement
HSI	Habitat Suitability Index
km	Kilometre
LWS	Local Wildlife Site
m	Metre
m^2	Square metre
NERC	Natural Environment and Rural Communities
OCGT	Open Cycle Gas Turbine
PEA	Preliminary Ecological Appraisal
PINS	The Planning Inspectorate
TLOR	Total Lindsey Oil Refinery

CONTENTS

TABLES

1.0 INTRODUCTION

1.1.1 This Appendix to the Environmental Statement (ES) presents the results of great crested newt (*Triturus cristatus*) surveys undertaken for the Proposed Development. The terms of reference used in this report are consistent with those defined within the main chapters of the ES (Volume I, Application Document Ref.6.2). This Appendix is intended to be read in conjunction with Chapter 9: Ecology of ES Volume I and the Preliminary Ecological Assessment (PEA, Appendix 9A, ES Volume III).

1.2 Survey Scope

1.2.1 The 'Study Area' for great crested newts incorporated the Site boundary and all land within 250 m of the boundary as this is the typical terrestrial range of great crested newts from their breeding ponds (English Nature, 2001). A 250 m survey radius is widely accepted as an appropriate search area for breeding ponds within the potential zone of influence of a particular development. Natural England (Natural England, 2016) guidance states that requirements for great crested newt survey should be proportionate and risk based, and that surveys of ponds of greater than 250 m distance (up to a maximum survey radius of 500 m) are only likely to required where a specific combination of circumstances are met. Following review of this guidance, it was concluded that the Proposed Development was of a type whereby surveys of more distant waterbodies were not necessary or proportionate.

1.2.2 The scope of works for the great crested newt surveys were as follows:

- Identify all waterbodies within the Study Area through a combination of review of aerial photographs and 1:25,000 Ordnance Survey maps, and field survey;
- Complete Habitat Suitability Index (HSI) assessment of all potentially suitable waterbodies within the Study Area to indicate their likely suitability for great crested newts; and
- Undertake eDNA survey of all waterbodies with potential to support great crested newts in the Study Area to determine likely presence or absence.

1.3 Relevant Legislation

1.3.1 The great crested newt is listed 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 the intentional, deliberate or reckless:

- Killing, injuring, taking or disturbance of great crested newts;
- Damaging, destroying or obstructing any place used by great crested newts for the purposes of breeding, sheltering or protection; and
- Selling and/or advertising for sale a great crested newt or any part thereof.

1.3.2 The 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. 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.

2.0 METHODS

2.1 Desk Study

2.1.1 A desk study was undertaken as part of the scope of works for the Phase 1 Habitat survey and is reported in detail in the PEA Report (Appendix 9A). Great crested newt records were obtained from the local ecological records centre (Greater Lincolnshire Nature Partnership) for the entirety of the Proposed Development and a radius of 1 km around it.

2.2 Habitat Suitability Index (HSI) Assessment

2.2.1 Six waterbodies were identified as present within the Study Area (Ponds 1-6). These six waterbodies were assessed for their potential to support great crested newt using the HSI assessment in accordance with standard methodology (Oldham et al. 2000). The assessments were undertaken during April (Ponds 1, 2, 4, 5 and 6) and November 2018 (Pond 3). The locations of these waterbodies are shown on Figure 9B.1.

2.2.2 The HSI assessment considers the following ten habitat attributes that are considered to influence the suitability of a waterbody for breeding great crested newts:

- Location – within a UK-wide context reflecting the differences in national distribution of this species;
- Area – waterbodies between 100 and 300 m² in size are considered to represent the most suitable habitat for great crested newt;
- Drying – the number of years in which a pond dries over a ten year period. Occasional drying kills fish which is beneficial for great crested newt, but the species predominantly favours ponds that do not dry out every year;
- 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;
- Shade – percentage of pond perimeter shaded to at least 1 m from the shore. Great crested newt favours lightly shaded waterbodies;
- 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;
- 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;
- Number of waterbodies within 1 km – great crested newt populations are typically best developed where they have access to a network of ponds, and therefore the species is more likely to be found where there are several ponds within 1 km that are linked by suitable terrestrial habitat; and
- Macrophyte cover – percentage of pond surface area occupied by macrophyte cover. Female great crested newts require aquatic vegetation for egg-laying.

2.3 Environmental DNA (eDNA) Survey

2.3.1 Water samples were collected by an AECOM ecologist who is registered to use the Natural England great crested newt survey licence from Ponds 1, 2, 4, 5 and 6. These samples were collected on 16th April 2018 and sent to ADAS for analysis for eDNA in accordance with approved field and laboratory protocols. Waterbodies were not entered by surveyors during sample collection, and new sterile equipment supplied by ADAS was used to collect each water sample, to prevent contamination between samples.

2.3.2 The eDNA survey was carried out in accordance with Biggs et al. (2014) which is an alternative to the traditional methods by Natural England, for assessing the presence or likely absence of great crested newts in waterbodies. Environmental DNA analysis can detect traces of great crested newt for up to two weeks after the species has been present in a waterbody. Water samples must be collected between mid-April and end-June to capture the peak breeding season.

2.3.3 For each waterbody, a total of 20 water samples were taken from different areas which were considered suitable to support for great crested newts, using sterile kits provided by ADAS, and taking care to not collect sediment from the bottom. All sampling was carried out from the banks and the water was not entered, as this may risk DNA from elsewhere being transferred between waterbodies (e.g. from the ecologist's boots). All water samples were transferred into a whirl pack and mixed thoroughly. Once mixed a pipette was used to transfer 15ml of the water sample into a tube of ethanol to preserve the eDNA, filling the tube up to 50ml. The tube was fastened, labelled and shaken. This process was completed until six tubes had been prepared for each waterbody. Following the survey all the tubes were safely packed and sent to the laboratory for testing.

2.3.4 The presence or absence of great crested newt from each of the surveyed waterbodies 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.

2.3.5 It was not possible to collect water samples from Pond 3 due to health and safety concerns, this is further explained below.

2.4 Refugia Surveys

2.4.1 A total of 70 amphibian refugia (carpet tiles) were installed around Pond 3 in suitable great crested newt terrestrial habitat on 19th March 2019 by two AECOM ecologists registered to use the Natural England great crested newt class survey licence. The amphibian refugia were checked twice a week for a period of 5 weeks between 26th March and 26th April 2019 (surveys are currently ongoing). The locations of the amphibian refugia are shown on Figure 9B.1. The purpose of the surveys was to record any great crested newts which may have emerged from their hibernation sites and are moving towards ponds for breeding purposes. This generally occurs in March and April.

2.5 Limitations

2.5.1 It was not possible to undertake presence/absence surveys for great crested newt within Pond 3 due to health and safety concerns (steep sided banks and deep water). There is

one safe accessible point of entry (via a small step way), which means that egg searches, bottle trapping, netting and eDNA surveys are not feasible and would not meet the survey method requirements when undertaking presence/absence surveys for great crested newts (English Nature, 2001 and Biggs et. al 2014).

2.5.2 Due to this restriction, the HSI assessment undertaken of Pond 3, in November 2018 was updated during additional surveys in March/April 2019, and amphibian refugia installed around the perimeter of Pond 3 as an additional survey method. This is in accordance with advice from Natural England as reported in Chapter 9: Ecology (ES Volume I, Application Document Ref. 6.2).

3.0 SURVEY RESULTS

3.1 Desk Study

3.1.1 The closest record to the Survey Area is approximately 160 m to the west of the OCGT Power Station Site. There are also records from Station Road Field Local Wildlife Site, which is located 0.3 km to the north of the Site at its closest point.

3.2 HSI Assessment

3.2.1 Table 9B.1 below provides descriptions and HSI scores for each waterbody within 250 m of the Proposed Development. Further details on the HSI scores are presented within Annex 9B.1. Photographs of each waterbody are presented within Annex 9B.2.

Table 9B.1: Waterbody Descriptions and HSI Scores

Pond Reference	Grid Reference/ Location	Description	HSI Score
Pond 1	TA 16780 17471 Within the Site	This was a medium-size pond approximately 30 x 40 m that is dominated by bulrush (*Typha latifolia*). The south-eastern pond margin is bordered by an earthen cliff on two levels, the first being 2 m high and climbing steeply to 5 m high at its tallest point. The pond margins were not clearly defined at the time of eDNA sampling, but a newly-dug access track borders the pond to the south-west and marshy grassland/tall ruderal vegetation immediately surrounds the pond, with neutral semi-improved grassland further afield. Approximately 40 % of the pond was accessible for eDNA sampling along the south-west margin as the steep cliff to the south-east and marshy ground from the north-west to the east prevented safe access.	Excellent
Pond 2	TA 16774 17499 Within the Site	This pond is an irregular shaped, medium-sized natural pond approximately 15 x 10 m, typified by marginal and emergent bulrush and rushes (*Juncus* spp.). The water level was relatively low at the time of eDNA sampling. Beyond the extent of the pond the habitat was surrounding by semi-improved neutral grassland and tall ruderal vegetation, with patches of scrub and saplings to the east of the pond.	Good
Pond 3	TA 1646 1730 160 m west of the Site	Man-made concrete lined lagoon with limited marginal and aquatic vegetation. The lagoon is steep sided with fluctuating water depth.	Good

Pond Reference	Grid Reference/ Location	Description	HSI Score
Pond 4	TA 16669 17451 Within the Site	This was a medium-sized irregularly shaped pond with a good cover of aquatic plants. The pond had earth banks to the south and west, marshy grassland with scattered rushes to the east and north, and neutral semi-improved grassland further to the south and east. The northern bank of the pond was not accessible for taking eDNA samples as it was covered by dense bramble (*Rubus fruticosus* agg.) scrub. The pond margins contained plentiful egg-laying plants for newts, including water forget-me-not (*Myosotis scorpioides*). A female smooth newt (*Lissotriton vulgaris)* was seen in this pond during eDNA sampling.	Average
Pond 5	TA 16649 17405 Within the Site	This pond has developed in a former archaeological trial trench (50 m x 2 m), which is likely to dry annually. The northern section of the pond was marshy and had too low a water level to sample for eDNA. The bank was largely accessible but had dense hawthorn (*Crataegus monogyna*) scrub on the eastern bank in places. The primary habitat surrounding the pond was neutral semi-improved grassland with areas of tall ruderal e.g. teasel (*Dipsacus fullonum*). Areas of standing water in the trench supported a low cover of aquatic plants.	Below Average
Pond 6	TA 16572 17340 30 m west of the Site	This is a rectangular former archaeological trench approximately 50 m x 2 m in area that holds standing water along most of its length. Small stands of emergent vegetation were present within the pond. The northern bank was largely inaccessible during eDNA due to dense hawthorn scrub, while patches of this species were also present on the southern bank as well as bramble to a lesser degree. The primary habitat surrounding the pond was neutral semi-improved grassland with stands of scrub and tall ruderal vegetation also present.	Poor

3.3 Environmental DNA (eDNA) Survey

3.3.1 Ponds 1, 2, 4, 5 and 6 were sampled for eDNA. None returned a positive result for great crested newt eDNA. The full eDNA results from the laboratory are provided as Annex 9B.3.

3.4 Refugia Surveys

3.4.1 The results of the refugia surveys are presented within Table 9B.2 below. These surveys are currently on-going and as such data between 12th and 26th April has not yet been captured. The results of the surveys will be reported via a supplementary report.

Table 9B.2: Refugia Survey Results

Date	Results
26/03/09	One common toad (*Bufo bufo*).
28/03/19	One common toad.
02/03/19	Nothing recorded.
05/04/19	One smooth newt (*Lissotriton vulgaris*).
09/04/19	Two smooth newts.
12/04/19	Survey not yet undertaken.
16/04/19	Survey not yet undertaken.
18/04/19	Survey not yet undertaken.
23/04/19	Survey not yet undertaken.
26/04/19	Survey not yet undertaken.

4.0 CONCLUSIONS AND EVALUATION

4.1.1 None of the ponds within the Survey Area or within 250 m that were subject to eDNA survey in 2018 returned positive results for great crested newt. If great crested newt was present in Pond 3, given the good habitat connectivity between this pond and Ponds, 1, 2, 4, 5 and 6, it would be reasonable to expect that great crested newt would be also present in those waterbodies.

4.1.2 To date no great crested newts have been recorded during the amphibian refugia checks.

4.1.3 It is therefore thought highly unlikely that great crested newts are present within the Study Area, however if the presence of this species is identified a mitigation strategy will be agreed with the Local Planning Authority.

5.0 REFERENCES

Biggs, J., Ewald, N., Valentini, A., Gaboriaund, C., Griffiths, R.A., Foster, J., Wilkinson, J., Arnett, A., Williams, P. & Dunn, F. (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

English Nature (2001) Great Crested Newt Mitigation Guidelines. English Nature (now Natural England), Peterborough

Natural England (2016) Great Crested Newt Method Statement for EPS Licence Application. https://www.gov.uk/government/publications/great-crested-newts-apply-for-a-mitigation-licence

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

Annex 9B.1 Habitat Suitability Index

Table A9B.2: HSI Survey Pond 1

Suitability Index	Habitat Attribute	Field Score	SI Score
SI1	Location	A	1
SI2	Pond Area	350 m²	0.7
SI3	Pond Drying	Sometimes	0.5
SI4	Water Quality	Moderate	0.67
SI5	Shade	0%	1
SI6	Fowl	Absent	1
SI7	Fish	Absent	1
SI8	Ponds per km	3.82	0.98
SI9	Terrestrial Habitat	Moderate	0.67
SI10	Macrophyte Cover	60%	0.9
HSI SCORE			**0.82 = Excellent Suitability**

Table A9B.3: HSI Survey Pond 2

Suitability Index	Habitat Attribute	Field Score	SI Score
SI1	Location	A	1
SI2	Pond Area	150 m²	0.3
SI3	Pond Drying	Sometimes	0.5
SI4	Water Quality	Moderate	0.67
SI5	Shade	0%	1
SI6	Fowl	Absent	1
SI7	Fish	Absent	1
SI8	Ponds per km	3.82	0.98
SI9	Terrestrial Habitat	Moderate	0.67
SI10	Macrophyte Cover	50%	0.8
HSI SCORE			**0.75 = Good Suitability**

Table A9B.3: HSI Survey Pond 3

Suitability Index	Habitat Attribute	Field Score	SI Score
SI1	Location	A	1
SI2	Pond Area	576 m²	1
SI3	Pond Drying	Sometimes	0.5
SI4	Water Quality	Poor	0.33
SI5	Shade	0%	1
SI6	Fowl	Absent	1
SI7	Fish	Absent	1
SI8	Ponds per km	11	0.97
SI9	Terrestrial Habitat	Moderate	0.67
SI10	Macrophyte Cover	0%	0.3
HSI SCORE			**0.71 = Good**

Table A9B.4: HSI Survey Pond 4

Suitability Index	Habitat Attribute	Field Score	SI Score
SI1	Location	A	1
SI2	Pond Area	350 m²	0.7
SI3	Pond Drying	Annually	0.1
SI4	Water Quality	Moderate	0.67
SI5	Shade	0%	1
SI6	Fowl	Absent	1
SI7	Fish	Absent	1
SI8	Ponds per km	3.5	0.97
SI9	Terrestrial Habitat	Moderate	0.67
SI10	Macrophyte Cover	10%	0.4
HSI SCORE			**0.64 = Average Suitability**

Table A9B.5: HSI Survey Pond 5

Suitability Index	Habitat Attribute	Field Score	SI Score
SI1	Location	A	1
SI2	Pond Area	40 m²	0.08
SI3	Pond Drying	Annually	0.1
SI4	Water Quality	Moderate	0.67
SI5	Shade	0%	1
SI6	Fowl	Absent	1
SI7	Fish	Absent	1
SI8	Ponds per km	3.5	0.97
SI9	Terrestrial Habitat	Moderate	0.67
SI10	Macrophyte Cover	5%	0.35
HSI SCORE			**0.51 = Below Average Suitability**

Table A9B.6 HSI Survey Pond 6

Suitability Index	Habitat Attribute	Field Score	SI Score
SI1	Location	A	1
SI2	Pond Area	25 m²	0.05
SI3	Pond Drying	Annually	0.1
SI4	Water Quality	Moderate	0.67
SI5	Shade	0%	1
SI6	Fowl	Absent	1
SI7	Fish	Absent	1
SI8	Ponds per km	3.5	0.97
SI9	Terrestrial Habitat	Moderate	0.67
SI10	Macrophyte Cover	0%	0.3
HSI SCORE			**0.47 = Poor Suitability**

Annex 9B.2 Photographs

Pond Reference	Photograph
Pond 1	
Pond 2	

Pond Reference	Photograph
Pond 3	
Pond 4	

Pond Reference	Photograph
Pond 5	
Pond 6	Photograph not available.

Annex 9B.3 Environmental DNA Results

Client: [redacted],
AECOM Infrastructure and Environment Ltd,
2 City Walk,
Leeds,
LS11 9AR

ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

Tel: 01159 516747
Email: Helen.Rees@adas.co.uk

www.adas.uk

Sample ID: 2018-0143	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: D	Description: pond water samples in preservative	
Date of Receipt: 18/04/2018	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	19/04/2018
Degradation Control[§]	Within Limits	Real Time PCR	19/04/2018
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	19/04/2018
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:	[redacted]	Signed:	[redacted]
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	19/04/2018	Date of issue:	19/04/2018

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.

Client: [redacted],
AECOM Infrastructure and Environment Ltd,
2 City Walk,
Leeds,
LS11 9AR

ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

Tel: 01159 516747
Email: Helen.Rees@adas.co.uk

www.adas.uk

Sample ID: 2018-0144	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: B	Description: pond water samples in preservative	
Date of Receipt: 18/04/2018	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control†	2 of 2	Real Time PCR	19/04/2018
Degradation Control§	Within Limits	Real Time PCR	19/04/2018
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	19/04/2018
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:	[redacted]	Signed:	[redacted]
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	19/04/2018	Date of issue:	19/04/2018

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.

Client: [redacted],
AECOM Infrastructure and Environment Ltd,
2 City Walk,
Leeds,
LS11 9AR

ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

Tel: 01159 516747
Email: Helen.Rees@adas.co.uk

www.adas.uk

Sample ID: 2018-0145	Condition on Receipt: Good	Volume: Passed
Client Identifier: E	Description: pond water samples in preservative	
Date of Receipt: 18/04/2018	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control†	2 of 2	Real Time PCR	19/04/2018
Degradation Control§	Within Limits	Real Time PCR	19/04/2018
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	19/04/2018
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:	[redacted]	Signed:	[redacted]
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	19/04/2018	Date of issue:	19/04/2018

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.

Client: [redacted],
AECOM Infrastructure and Environment Ltd,
2 City Walk,
Leeds,
LS11 9AR

ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

Tel: 01159 516747
Email: Helen.Rees@adas.co.uk

www.adas.uk

Sample ID: 2018-0146	Condition on Receipt: White Precipitate	Volume: Passed
Client Identifier: A	Description: pond water samples in preservative	
Date of Receipt: 18/04/2018	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control†	0 of 2	Real Time PCR	19/04/2018
Degradation Control§	Within Limits	Real Time PCR	19/04/2018
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	19/04/2018
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:	[redacted]	Signed:	[redacted]
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	19/04/2018	Date of issue:	19/04/2018

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.

Client: [redacted],
AECOM Infrastructure and Environment Ltd,
2 City Walk,
Leeds,
LS11 9AR

ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

Tel: 01159 516747
Email: Helen.Rees@adas.co.uk

www.adas.uk

Sample ID: 2018-0147	Condition on Receipt: Good	Volume: Passed
Client Identifier: C	Description: pond water samples in preservative	
Date of Receipt: 18/04/2018	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	1 of 2	Real Time PCR	19/04/2018
Degradation Control[§]	Within Limits	Real Time PCR	19/04/2018
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	19/04/2018
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:	[redacted]	Signed:	[redacted]
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	19/04/2018	Date of issue:	19/04/2018

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).

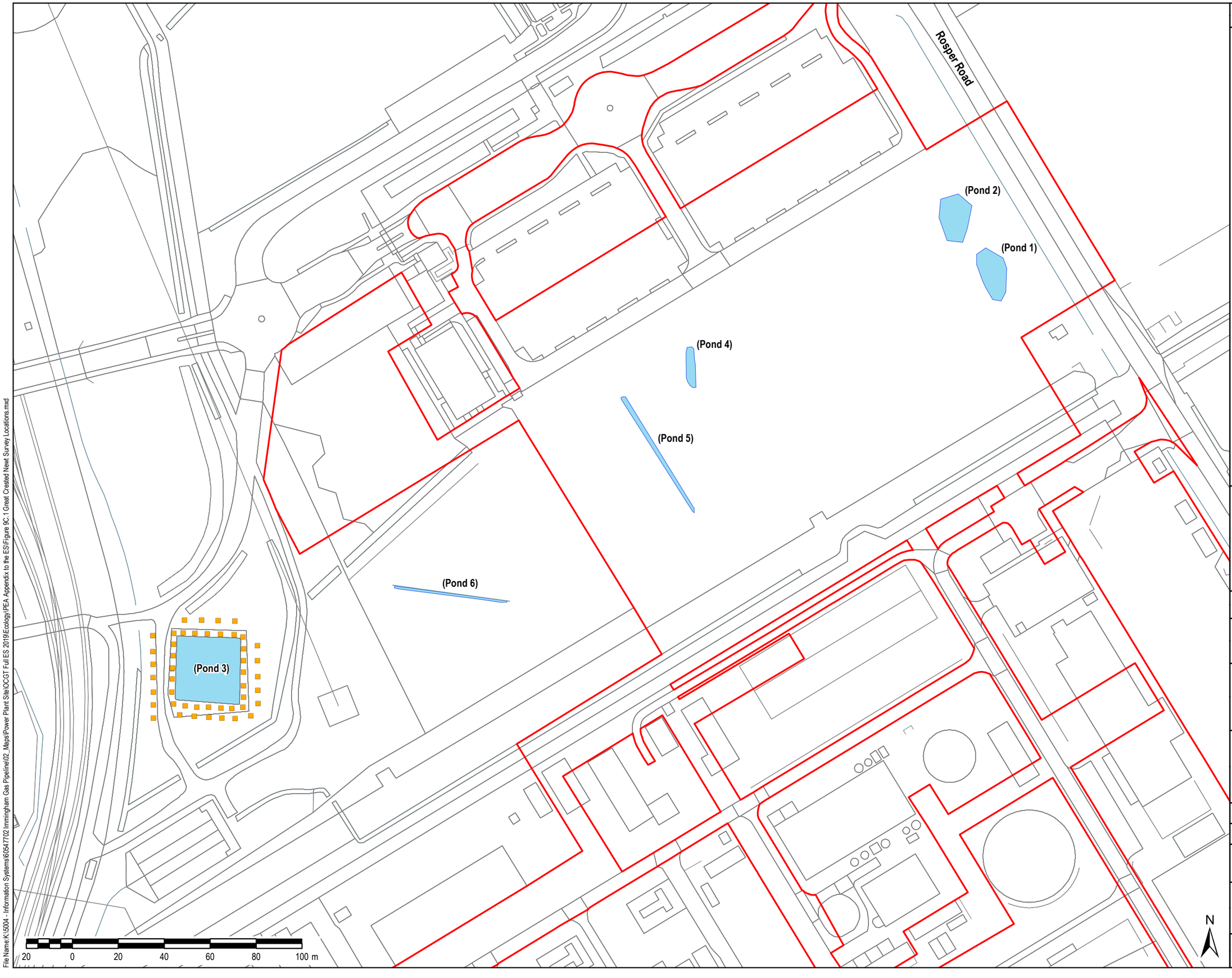
A negative result means that DNA from the great crested newt has not been detected in your sample.

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)

Figure 9B.1 Great Crested Newt Survey Locations



File Name:K:\5004 - Information Systems\60547702 Immingham Gas Pipeline\02_Maps\Power Plant Site\OCGT Full ES 2019\Ecology\PEA Appendix to the ES\Figure 9C.1 Great Crested Newt Survey Locations.mxd

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

LEGEND

- Proposed Development
- Location of Ponds
- Standing Water
- Location of Amphibian Refugia

Purpose of Issue
ENVIRONMENTAL STATEMENT

Client
VPI IMMINGHAM B LTD

Project Title
PROPOSED 299MW OCGT POWER STATION

Drawing Title
GREAT CRESTED NEWT SURVEY LOCATIONS FIGURE 9C.1

Drawn	Checked	Approved	Date
JW	BB	MS	28/03/2019

AECOM Internal Project No.	Scale @ A3
60547702	1:1,500

AECOM
Midpoint
Alençon Link, Basingstoke
Hampshire, RG21 7PP
Telephone (01256) 310200
Fax (01256) 310201
www.aecom.com

DCO Document Ref:	Rev
6.4.12	