Great Crested Newt Survey Report

Margam, National Grid Electricity Transmission

July 2025

Prepared for:
National Grid Electricity Transmission

Prepared by:
Stantec, UK Limited

Project Number:
331201497

Revision	Description	Date	Author	Quality Check	Independent Review
V1	First Issue	June 2024	TL	HM	HE
V2	Second Issue with 2025 survey data	July 2025	DS	HM	HE
V3	Update following client and legal comments	July 2025	HM	HE	HE

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Executive Summary

Stantec UK Limited was commissioned by National Grid Electricity Transmission (NGET) to complete environmental DNA (eDNA) surveys and Habitat Suitability Index (HSI) assessments for great crested newt (GCN) *Triturus cristatus* to determine their presence/ likely absence within waterbodies on land owned by NGET at Margam, Neath Port Talbot; hereafter referred to as 'the Site'. NGET subsequently commissioned Stantec to also undertake a GCN survey of the land to the south of the Site, owned by BOC Ltd, following NGET securing access permission to this area from September 2024; this area is hereafter referred to as 'the BOC land'.

Samples were collected from three ponds and four ditches within the Site for eDNA analysis on 23rd May 2024 and 24th May 2024, during the peak GCN eDNA survey season. In addition, a further two ponds within the Tata Steel golf course to the north of the Site were assessed using the GCN HSI assessment method, based on observations from adjacent land. A further seven ditches within the BOC land were surveyed on 17th April 2025 for eDNA analysis.

The two ponds located within the Tata Steel golf course to the north of the Site concluded Good and Average suitability for GCN. The eDNA results from the fourteen waterbodies sampled within the Site and the BOC land determined likely absence of GCN based on negative eDNA returns.

Taking into account the negative eDNA survey results, along with historical survey data for the Site (submitted with the 2009 planning application) also recording "likely absence" for GCN, and recent survey work undertaken on behalf of Tata Steel on land adjacent to the Site also recording "likely absence" for GCN, it is considered that GCN are likely absent from the Site and BOC land.

1 Introduction

1.1 Overview

1.1.1 Stantec UK Limited (Stantec) was commissioned by National Grid Electricity Transmission (NGET) to complete great crested newt surveys (GCN) *Triturus cristatus* of the area of land owned by NGET at Margam, Neath Port Talbot; hereafter referred to as 'the Site'. NGET subsequently commissioned Stantec to also undertake a GCN survey of the land to the south of the Site, owned by BOC Ltd, following NGET securing access permission to this area from September 2024; this area is hereafter referred to as 'the BOC land'.

1.1.2 The GCN surveys included environmental DNA (eDNA) surveys to determine the presence/ likely absence of GCN and Habitat Suitability Index (HSI) assessments within waterbodies to determine their suitability for GCN.

1.2 Project Context

Site Location and Description

1.2.1 The Site is located in Margam, Port Talbot, at approximate central grid reference SS 78000 85000. The Site comprises an existing substation to the east of the Tata Steel Works and Network Rail railway line; the Site also lies to the south of the Tata Steel Sports and Social Club (golf course), to the west of woodland and to the north of the BOC Ltd works area and fields owned by BOC Ltd. Beyond the immediate Site surroundings, the M4 corridor lies to the east, Swansea Bay lies to the west, Eglwys Nunydd Reservoir to the south and Margam town to the north. The BOC land considered in this GCN report lies immediately to the south of the Site, between the Site and the road to the north of the Eglwys Nunydd Reservoir, Heolcae'r Bont. The location of the Site and the BOC land is shown in Figure 1.

1.2.2 The Site was subject to habitat surveys completed in 2024 which identified that the Site comprises a mosaic of reedbed, scrub, grassland and small areas of open water associated with a number of ditches within the Site.

Description of Works

1.2.3 The Site and the BOC land are proposed for an extension to the existing substation and associated cabling works, with the substation extension proposed to the east of the existing substation within the Site and cabling works passing through the Site and the BOC land. The proposed substation extension and associated works within the Site will be progressed under a planning application. The cabling and associated temporary works to link the proposed new substation extension at Margam to the Port Talbot Steelworks will be progressing under NGET’s permitted development rights..

Historic Project Understanding and Ecological Context

1.2.4 The Site was subject to a previous successful planning application for a new substation within NGET land which received planning consent in 2009. However, the development was not progressed by NGET. A suite of ecological surveys was completed to inform the planning application. The results of the survey work were presented within the Margam 275kV Substation Environmental Report (National Grid 2009).

1.3 Purpose of Report

1.3.1 The purpose of this report is to:

- set out the methods used for the desk study and field survey, including any limitations;
- describe the results of the desk study and field survey, including both the eDNA and Habitat Suitability Index assessment results, and determine likely presence/absence of GCN within the Site and BOC land;
- use the results to inform an evaluation of the Site and BOC land.

2 Legislation

2.1.1 Great crested newts are protected under the Conservation of Habitats and Species Regulations, 2017 (as amended) and the Wildlife and Countryside Act 1981 (as amended), which combined makes it an offence to:

- deliberately capture, injure or kill a great crested newt;
- deliberately disturb a great crested newt;
- damage or destroy a great crested newt breeding site or resting place; or obstruct access to a place of shelter or protection.

2.1.2 Disturbance includes, but is not limited to, any disturbance which is likely:

- to impair their ability –
  - to survive, to breed or reproduce, or to rear or nurture their young; or
  - in the case of animals of a hibernating or migratory species, to hibernate or migrate; or
  - to affect significantly the local distribution or abundance of the species to which they belong.

2.1.3 Works affecting great crested newts can only take place under licence from Natural Resources Wales. The Licence can only be issued for specific purposes, if the following three tests are met:

- the purpose of the work meets one of those listed in the Habitats Regulations;
- that there is no satisfactory alternative; and
- that the action authorised will not be detrimental to the maintenance of the population of the species concerned at a favourable conservation status in their natural range.

2.1.4 The Habitats Regulations permits licences to be issued for a specific set of purposes including preserving public health or public safety or other imperative reasons of over-riding public interest including those of a social or economic nature and beneficial consequences of primary importance for the environment.

2.1.5 Great crested newts are also listed under Section 7 of the Environment (Wales) Act 2016 and as such are considered species of principal importance for the purpose of maintaining and enhancing biodiversity in relation to Wales. Section 7 replaces the duty described under Section 42 of the NERC (Natural Environment and Rural Communities) Act, 2006.

3 Methods

3.1 Overview

3.1.1 The section below sets out the methods undertaken as part of the assessment for GCN at the Site and BOC land.

3.2 Desk Study

3.2.1 A 2 km data search was requested from South East Wales Biodiversity Records Centre (SEWBReC), the local biological records centre in October 2024. To ensure that the information is as current as possible, records within the last ten years were considered most relevant. However, records older than this were analysed for their potential relevance to the Site.

3.2.2 Waterbodies for eDNA and HSI survey were identified using OS maps at 1:25,000 scale and MAGIC website colour mapping within 250m of the Site and the BOC land. 250m is considered the average migratory distance of GCN from breeding ponds during their terrestrial phase (Griffiths et al., 2010).

3.2.3 In addition, previous ecological reports from the Site and nearby land were reviewed. This included a report relating to the previous planning application for the Site (National Grid, 2009) and surveys undertaken on Tata Steel land in 2022, located to the west of the BOC land on the other side of the railway line (RSK Biocensus, 2024).

3.3 Survey Approach and Strategy

3.3.1 The survey methods employed comprised of environmental DNA (eDNA) sampling and/or Habitat Suitability Index (HSI) assessments to determine either the presence/likely absence of GCN within a waterbody and the suitability of the aquatic habitat for supporting GCN.

3.3.2 Together, the results of the eDNA and HSI survey methods provide a stratified sampling strategy to determine the presence/ likely absence of GCN at the Site, and whether it is likely that the waterbodies on the Site and the BOC land would support breeding GCN.

3.4 Environmental DNA (eDNA) Survey

3.4.1 Water samples were collected from waterbodies on site and then analysed to determine the presence or absence of GCN DNA within each water sample via a specific laboratory protocol. Natural Resources Wales (NRW) has approved this method for the determination of GCN presence/likely absence. Samples were taken on 23rd May 2024 and 24th May 2024 within the Site and on 17th April 2025 within the BOC land, during the peak GCN eDNA survey season which runs from 15th April to 30th June (inclusive) in line with guidance (Biggs *et al.*, 2014).

3.4.2 A single visit to each waterbody was undertaken during the GCN breeding season (mid-April to June) in accord with guidance. Samples were collected during the daytime and in reasonable weather conditions; periods of heavy rain were avoided to decrease the risk of cross contamination. Samples of water were taken from 20 locations around the perimeter of each waterbody, ensuring that the sediment was not disturbed. Samples were stored in a cool location and sent by courier to the ADAS or Surescreen laboratory for analysis approximately 48 hours after samples were collected.

3.5 Great Crested Newt Habitat Suitability Index Assessment

3.5.1 The waterbodies were visited by suitably experienced ecologists on 24th September 2024 and assessed using the GCN HSI assessment method developed by Oldham *et al.* (2000) and modified by Amphibian and Reptile Groups of the UK (ARG) (2010). This involves visiting

each waterbody and recording scores against certain parameters such as pond area and habitat variables.

3.5.2 The Habitat Suitability Index (HSI) is a simple model that provides an informed view of the suitability of ponds to support breeding populations of GCN. The HSI assessment involves assessing ponds based on the following 10 habitat parameters that influence breeding populations of GCN:

1. Geographic location
2. Pond area
3. Pond permanence
4. Water quality
5. Pond shading
6. Number of waterfowl
7. Occurrence of fish
8. Pond density
9. Terrestrial Habitat
10. Macrophyte cover of pond

3.5.3 A score between 0 and 1 is assigned to each of these 10 habitat variables, based on observations made in the field and interpreting the guidance provided by ARG (2010). The HSI score is the geometric mean of the ten habitat variables listed above, obtained by multiplying each of the variable scores together, and then taking the 10th root of the product. The resulting HSI scores were cross-referenced with the ARG (2010) guidance (as shown below in Table 3.1) to categorise the suitability of each waterbody to support a breeding population of GCN.

Table 3 1 HSI for Great Crested Newt (ARG, 2010).

HSI Score	Habitat Suitability Result
0.00 – 0.49	Poor
0.50 – 0.59	Below Average
0.60 – 0.69	Average
0.70 – 0.79	Good
0.80 – 1.00	Excellent

3.6 Limitations

3.6.1 Species records provided by local records centres are not often collected as a result of systematic surveys and therefore geographic, temporal, and species coverage are not often representative. As such, a lack of records of a species in an area does not necessarily mean an absence of this species.

3.6.2 It was not possible to undertake eDNA analysis and a HSI assessment of all waterbodies within the Site and its 250 m buffer due to lack of land access permission and dense vegetation. A summary of the limitations for the survey at each waterbody is provided in Table 3 2.

Table 3 2. Summary of Surveys Undertaken and Associated Limitations

Waterbody	HSI survey undertaken	eDNA survey undertaken	Summary of Surveys Undertaken and Limitations
Ponds 4 & 5	Yes	No	No access, located within Tata Steel land. HSI undertaken from adjacent land holding.
Ponds 6 & 7	No	No	No access, located within Tata Steel land.
Upper Mother Ditch	No	No	No access from the Site due to dense vegetation. Access not available to undertake survey of water body from Tata Steel land.
Ditch 2, 3, 6, 8, 9, & 10	No	No	No access due to dense vegetation
Ditches 1, 4, 5 & 7	No	Yes	HSI not required as eDNA survey undertaken. Due to dense vegetation, access to the full length of ditches was not always possible. However, best effort was made to collect samples from different areas of each waterbody. Therefore, this is not considered to be a significant limitation to the survey results.
Ditch 13	Yes	No	No access for eDNA in 2024 and ditch was dry at time of 2025 visit
Other waterbodies within 250m	No	No	No access

3.6.3 As outlined in the Section 3.3, the results of the eDNA and HSI surveys provide a stratified sampling strategy of waterbodies within the Site and BOC land, sufficient to determine the presence/ likely absence of GCN at the Site and the BOC land, given the connected nature of the waterbodies within the Site and BOC land.

3.6.4 The HSI survey was preceded by a period of heavy rainfall and light rain and cloud was present on the day of the survey. This is not considered to be a significant limitation to the survey as the ecological parameters set out in Section 3.5 were not significantly impacted by higher water levels to make a reasonable judgement on the suitability of the water bodies to support GCN.

3.7 Report Qualification

3.7.1 The survey described here was undertaken in accordance with the current best practice methodologies at the time of commissioning. Site circumstances, scientific knowledge or methodological requirements can change during the course of a project, and these external factors may impact on the scope of subsequent work requirements.

3.7.2 All ecological surveys have an expected validity period owing to the tendency of the natural environment to change over time. This validity period varies from receptor to receptor and is also dependent on the degree of change in a site's management and overall landscape ecology. Where the potential for change is considered to be relevant to the Site, this is highlighted in the appropriate section.

3.7.3 This report does not purport to provide detailed, specialist legal advice. Where legislation is referenced, the reader should consult the original legal text, and/or the advice of a qualified environmental lawyer.

4 Results

4.1 Overview

4.1.1 This section presents the results from the desk study and field surveys.

4.2 Desk Study

4.2.1 Using OS mapping, seven ponds were identified within and up to 250 m from the Site and the BOC land, along with 18 ditches within the Site and BOC land, and further ditches within the 250 m buffer (see Figure 2).

4.2.2 The data trawl from the local biological records centre returned eight records for GCN within 2 km of the Site. The closest record pertained to four individual adult GCN, recorded in 2005 approximately 450 m west of the Site, within the Tata Steel land holding.

4.2.3 The surveys reported in the 2009 planning application for the Site found no evidence of GCN and recorded fish from a number of ditches within the Site, including the Mother Ditch. GCN surveys undertaken within the Tata Steel landholding included surveys of three water bodies within 250 m of the BOC land, as well as a number of waterbodies across the Tata Steel landholding. All of the waterbodies surveyed, aside from one, returned a negative result for GCN eDNA. This included the waterbody likely associated with the record returned from the local records centre which was found to no longer support GCN. The waterbody that returned a positive result is located within a dune system to the south of the Tata Steel works, located approximately 1.5 km south-west of the BOC land.

4.3 eDNA Results and HSI Scores

4.3.1 Table 4.1 provides a summary of the eDNA survey and HSI assessment results from the Site, BOC land and waterbodies visible from the Site. eDNA surveys confirmed likely absence of GCN within all waterbodies surveyed and HSI surveys identified waterbodies with Poor to Good suitability for GCN. As outlined in the limitations (Section 3.6) it was not possible to survey all waterbodies within a 250 m buffer. However, given the access constraints due to the dense vegetation around the waterbodies within the Site and BOC land, the sampling strategy of waterbodies employed is considered sufficient to determine the presence/ likely absence of GCN at the Site and the BOC land, given the connected nature of the waterbodies within the Site and BOC land.

4.3.2 The results are shown on Figure 3.

4.3.3 The raw scores for the HSI assessments are shown in Appendix A and a copy of the full eDNA results can be found in Appendix B.

Table 4.1: Great Crested Newt Presence/Likely Absence eDNA results and HSI Scores

Waterbody Reference	eDNA Result	HSI Numerical Score	HSI Score
Pond 1	Negative	-	-
Pond 2	Negative	-	-
Pond 3	Negative	-	-
Pond 4	-	0.72	Good
Pond 5	-	0.66	Average
Ditch 1	Negative	-	-
Ditch 4	Negative	-	-

Waterbody Reference	eDNA Result	HSI Numerical Score	HSI Score
Ditch 5	Negative	-	-
Ditch 7	Negative	-	-
Ditch 10	-	0.50	Below Average
Ditch 11	Negative	0.38	Poor
Ditch 12	Negative	0.57	Below Average
Ditch 13	-	0.40	Poor
Ditch 14	Negative	0.60	Average
Ditch 15	Negative	0.57	Below Average
Ditch 16	Negative	0.52	Below Average
Ditch 17	Negative	0.51	Below Average

5 Evaluation and Conclusion

5.1.1 The desk study identified GCN records within the 2 km study area. The closest record was 450 m west of the Site within the Tata Steel land, from 2005. The ponds within this area were surveyed during the Tata Steel surveys in 2022 which confirmed likely absence of GCN (RSK Biocensus, 2024). The next closest GCN record is located approximately 1.5 km south-west of the BOC land (at Margam Burrows, in land owned by Tata Steel UK Ltd), which is considered beyond the maximum 1 km dispersal distance that GCN are likely to naturally disperse (Griffiths, 2004).

5.1.2 GCN were confirmed likely absent in all waterbodies that were sampled for GCN eDNA within the Site and the BOC land. Given the similar habitat compositions, habitat connectivity and lack of close-by desk study records, it is also considered that the waterbodies which did not undergo eDNA sampling would be likely absent of GCN.

5.1.3 HSI assessments indicated that most waterbodies had Poor or Below Average suitability for GCN. One pond within the Tata Steel golf course to the north of the Site was assessed as Good, and another as Average. Additionally, one ditch within the BOC land returned an Average suitability score. Waterbodies with Poor or Below Average suitability are considered unlikely to support breeding GCN, and the overall likelihood of GCN presence across the Site and BOC land remains low, supported by negative eDNA results and limited habitat suitability.

5.1.4 If a GCN were to be encountered during works, works must cease where safe to do so and a consideration would be undertaken by a suitably qualified ecologist to determine if the Works can proceed avoiding contravention of legislation. If effects on GCN are subsequently confirmed, works should not proceed until a licence from Natural Resources Wales has been obtained to legally facilitate the works.

5.1.5 This report is valid for a period of 24 months from the eDNA and HSI surveys were undertaken. If works have not commenced within this timeframe, update surveys should be undertaken on waterbodies surveyed in 2024 to determine the latest presence/likely absence status of GCN.

6 References

Biggs J, Ewald N, Valentini A, Gaboriaud C, Griffiths RA, Foster J, Wilkinson J, Arnett A, Williams P and 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.

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National Grid (2009). Margam 275kV Substation – Environmental Report Technical Appendices. National Grid

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RSK Biocensus (2024) Tata Steel UK Limited - Electric Arc Furnace. Great Crested Newt Survey Report. Report for Tata Steel UK Limited.

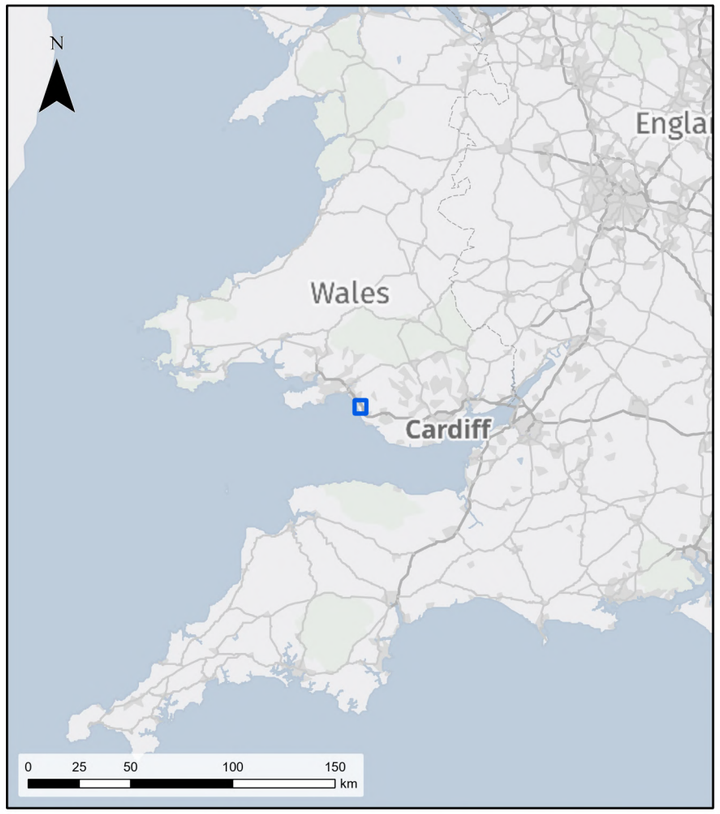
Stantec (2024). Margam, National Grid – Preliminary Ecological Appraisal

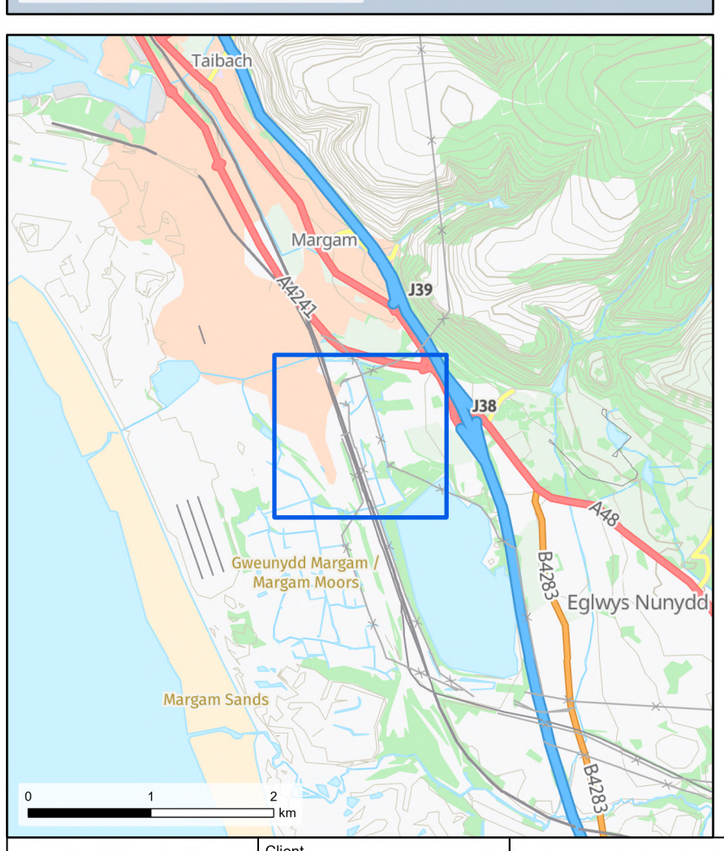
7 Figures

Figure 1: Site Location Plan

Figure 2: Great Crested Newt Survey: Waterbodies

Figure 3: Great Crested Newt Survey Results





Client

nationalgrid

MARGAM PORT TALBOT

Site Location

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Stantec

Client: nationalgrid

MARGAM PORT TALBOT

Great Crested Newt Waterbodies

0 300 600 m

Esri Community Maps Contributors, Esri UK, Esri, TomTom, Garmin, Foursquare, GeoTechnologies, Inc, METI/NASA, USGS
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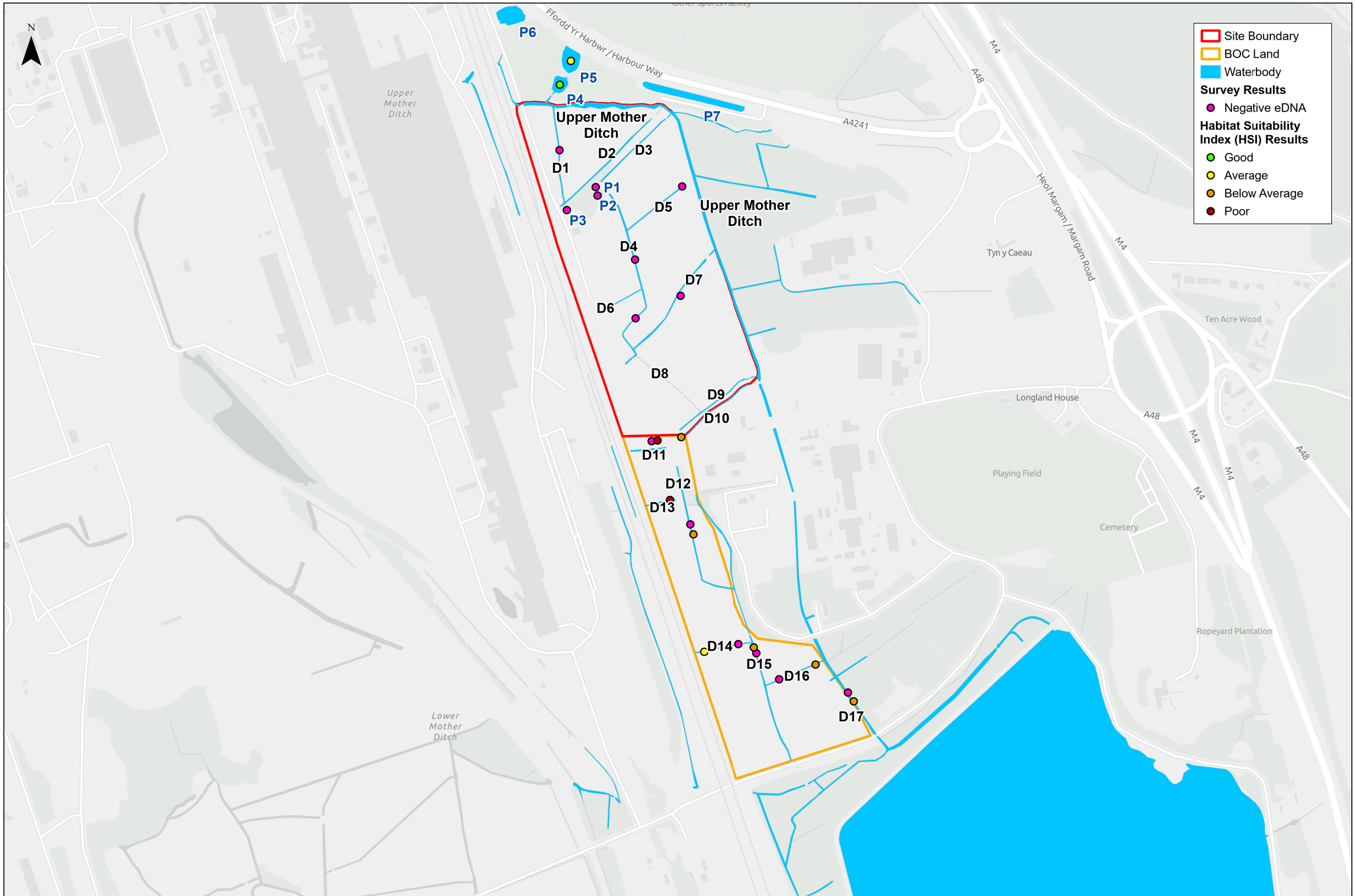

Stantec

Client: nationalgrid

MARGAM PORT TALBOT

Great Crested Newt Survey Results

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Appendix A Habitat Suitability Index Assessment Raw Scores

Waterbody	Factor										Score
	Geographic Location	Pond Area	Permanence	Water Quality	Shade	Waterfowl	Fish	Pond Count	Terrestrial	Macrophytes	
Pond 4	0.5	1	0.9	0.67	1	1	0.67	0.6	0.33	0.9	0.72
Pond 5	0.5	0.9	0.9	0.33	1	1	0.67	0.6	0.33	0.9	0.66
Ditch 10	0.5	0.2	0.5	0.33	0.4	1	1	0.8	0.67	0.3	0.50
Ditch 11	0.5	0.2	0.9	0.01	0.4	1	1	0.8	0.67	0.3	0.38
Ditch 12	0.5	0.2	0.5	0.33	1	1	1	0.8	0.67	0.4	0.57
Ditch 13	0.5	0.2	0.1	0.33	0.2	1	1	0.8	1	0.3	0.42
Ditch 14	0.5	0.2	0.9	0.33	0.85	1	1	0.8	1	0.85	0.60
Ditch 15	0.5	0.2	0.5	0.33	1	1	1	0.8	0.67	0.4	0.57
Ditch 16	0.5	0.2	0.9	0.33	0.2	1	1	0.8	1	0.3	0.52
Ditch 17	0.5	0.1		0.33	0.2	1	1	0.8	1	0.3	0.51

Appendix B eDNA Results

Client: Zak Mitchell,
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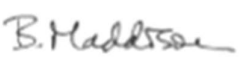
ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

Tel: 01159 229249
Email: Helen.Rees@adas.co.uk

www.adas.uk

Sample ID: ADAS-5429	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: Ditch 2, Margam	Description: pond water samples in preservative	
Date of Receipt: 30/05/2024	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control†	2 of 2	Real Time PCR	03/06/2024
Degradation Control§	Within Limits	Real Time PCR	03/06/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	03/06/2024
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:	04/06/2024	Date of issue:	04/06/2024

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: Zak Mitchell,
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ADAS
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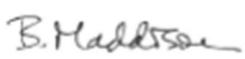
Tel: 01159 229249
Email: Helen.Rees@adas.co.uk

www.adas.uk

Sample ID: ADAS-5430	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: Ditch 1, Margam	Description: pond water samples in preservative	
Date of Receipt: 30/05/2024	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control†	2 of 2	Real Time PCR	03/06/2024
Degradation Control§	Within Limits	Real Time PCR	03/06/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	03/06/2024
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:	04/06/2024	Date of issue:	04/06/2024

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† 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.

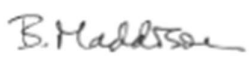
Client: Zak Mitchell,
Stantec

ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

Tel: 01159 229249
Email: Helen.Rees@adas.co.uk

www.adas.uk

Sample ID: ADAS-5432	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: Pond 1, Margam	Description: pond water samples in preservative	
Date of Receipt: 30/05/2024	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control†	2 of 2	Real Time PCR	03/06/2024
Degradation Control§	Within Limits	Real Time PCR	03/06/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	03/06/2024
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
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Stantec

ADAS
Spring Lodge
172 Chester Road
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WA6 0AR

Tel: 01159 229249
Email: Helen.Rees@adas.co.uk

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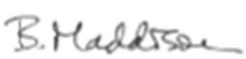
Sample ID: ADAS-5433	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: Ditch 4, Margam	Description: pond water samples in preservative	
Date of Receipt: 30/05/2024	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control†	2 of 2	Real Time PCR	03/06/2024
Degradation Control§	Within Limits	Real Time PCR	03/06/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	03/06/2024
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:	04/06/2024	Date of issue:	04/06/2024

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ADAS
Spring Lodge
172 Chester Road
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Tel: 01159 229249
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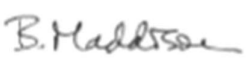
Sample ID: ADAS-5434	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: Pond 2, Margam	Description: pond water samples in preservative	
Date of Receipt: 30/05/2024	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control†	2 of 2	Real Time PCR	03/06/2024
Degradation Control§	Within Limits	Real Time PCR	03/06/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	03/06/2024
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:	04/06/2024	Date of issue:	04/06/2024

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† 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: Zak Mitchell,
Stantec

ADAS
Spring Lodge
172 Chester Road
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Email: Helen.Rees@adas.co.uk

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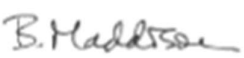
Sample ID: ADAS-5437	Condition on Receipt: Good	Volume: Passed
Client Identifier: Pond 3, Margam	Description: pond water samples in preservative	
Date of Receipt: 30/05/2024	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control†	2 of 2	Real Time PCR	03/06/2024
Degradation Control§	Within Limits	Real Time PCR	03/06/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	03/06/2024
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:	04/06/2024	Date of issue:	04/06/2024

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Folio No:	259-2025
Purchase Order:	ECOLOGYPT01
Contact:	Levan Ecology
Issue Date:	23.04.2025
Received Date:	17.04.2025

SureScreen Scientifics

GCN eDNA Analysis

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 analyzing water samples, we can detect these small traces of environmental DNA (eDNA) to confirm GCN habitation or establish GCN absence.

Results

Lab ID	Site Name	OS Reference	Degradation Check	Inhibition Check	Result	Positive Replicates
GCN25 1883	Port Talbot - D17	SS 79023 85500	Pass	Pass	Negative	0/12
GCN25 1886	Port Talbot - D12	SS 79023 85500	Pass	Pass	Negative	0/12
GCN25 1888	Port Talbot - D14	SS 79023 85500	Pass	Pass	Negative	0/12
GCN25 1889	Port Talbot - D16	SS 79023 85500	Pass	Pass	Negative	0/12
GCN25 1890	Port Talbot - D11	SS 79023 85500	Pass	Pass	Negative	0/12
GCN25 1891	Port Talbot - D15	SS 79023 85500	Pass	Pass	Negative	0/12

Matters affecting result: none

Reported by: Lauryn Jewkes

Approved by: Chelsea Warner