One Earth Solar Farm

Volume 6.0 Environmental Statement [EN010159]

Volume 3: Technical Appendices Supporting ES Volume 2

Appendix 6.6: Great Crested Newt Baseline

July 2025

Document Reference: EN010159/APP/6.21.1

Revision 02

Infrastructure Planning (Applications: Prescribed Forms and Procedure) Regulations 2009 - Reg 5 (2) (a)

Contents

Appendix 6.6: Great Crested Newt Baseline

A.6.1 Introduction

Background

A.6.1.1. This Appendix should be read in conjunction with Chapter 6 of the Environmental Statement (ES) which is provided in support of the delivery of an Environmental Impact Assessment (EIA) associated with the One Earth Solar Farm, hereafter referred to as the 'Proposed Development'.

Purpose of this Appendix

A.6.1.2. The purpose of the Appendix is to present the methods and findings of the baseline great crested newt surveys which were undertaken to identify the presence, or likely absence of great crested newt (GCN). This baseline is required to inform the (EIA) in assessing potential impacts of the Proposed Development.

A.6.1.3. Surveys were completed during the spring and summer period (April to June) in 2023 with updated surveys undertaken in May and June 2025 following best practice guidance.

A.6.1.4. This report does not allude to any requirements for mitigation and/or compensation in respect of GCN, nor does it assess the potential impacts that proposals might have upon GCN, as both issues are covered in detail in Chapter 6 of the ES.

A.6.1.5. The document references have not been updated from the original submission. Please refer to the Guide to the Application [EN010159/APP/1.3.2] for the list of current versions of documents.

Structure of this Appendix

A.6.1.6. The remainder of this Appendix is structured as follows:

- **Section 2**Error! Reference source not found.**:** Methods
- **Section 3:** Results
- **Section 4:** Summary
- **Section 5:** References

The Order Limits

A.6.1.7. The Order Limits are located on the boundary between Lincolnshire and Nottinghamshire, along the River Trent, with a central grid reference of

SK816718 (see **Figure 1.1**). The Order Limits cover 1,409 hectares (ha) and are bisected north to south by the River Trent, with 799 ha lying to the west and the remaining 604 ha to the east. The River Trent itself encompasses approximately 6 ha.

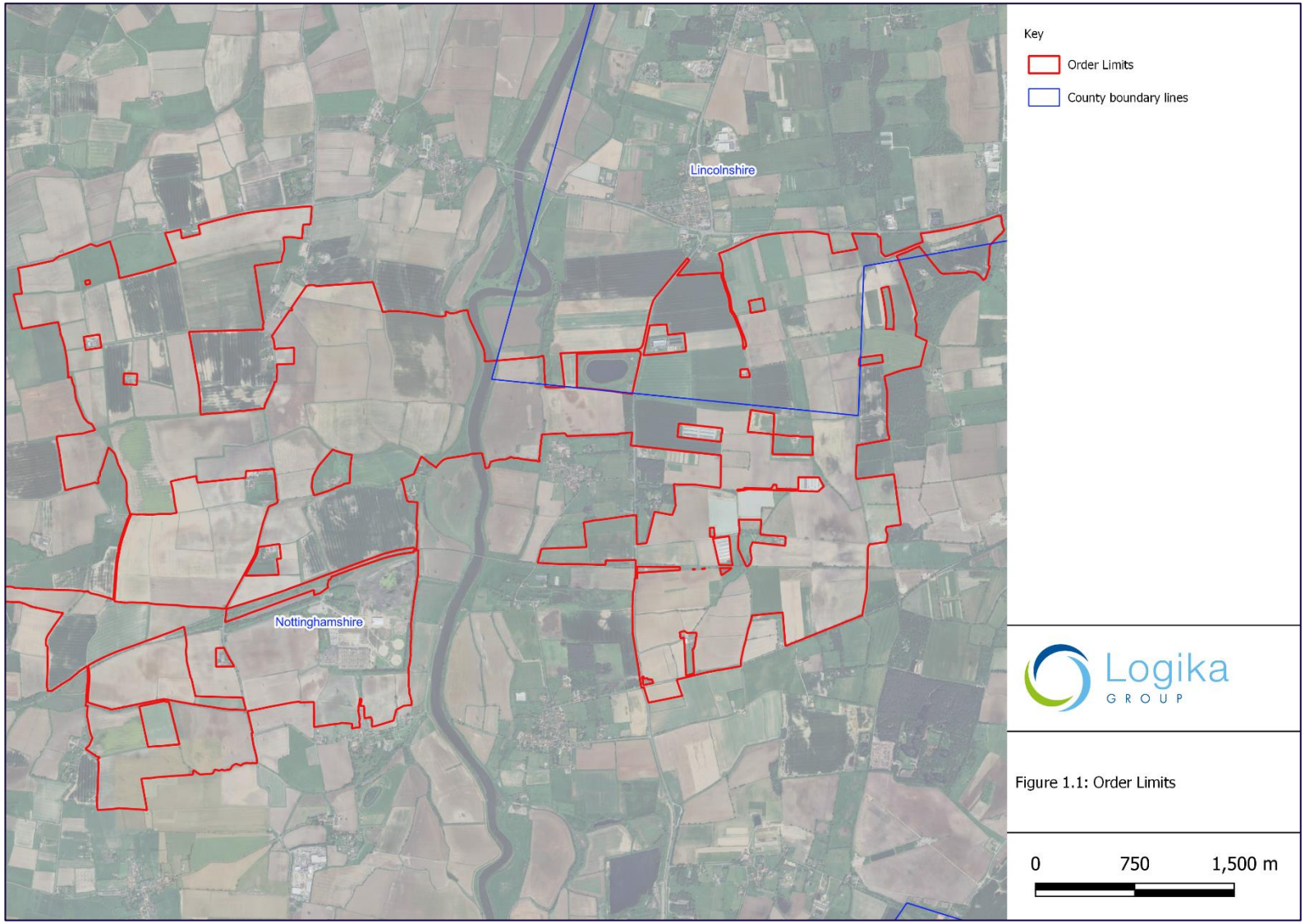

Figure 2.1 Order Limits

A.6.2 Methodology

Survey Guidance

A.6.2.1. The following survey guidance has been taken into account in the methodology design. Any deviation from standard industry practice is noted in the appropriate section.

- Analytical and methodological development for improved surveillance of the Great Crested Newt. Technical advice note for field and laboratory sampling of great crested newt (*Triturus cristatus*) environmental DNA. Freshwater Habitats Trust, Oxford. (Biggs *et al*, 2014)
- Herpetofauna Workers Manual. JNCC, Peterborough. (Gent & Gibson, 2003).
- Great Crested Newt Habitat Suitability Index. ARG UK Advice Note 5 (Amphibian and Reptile Groups of the United Kingdom, 2010).

Desk Study

A.6.2.2. An environmental desk study was undertaken in September 2023 to identify records of GCN within 2 kilometres (km) of the Order Limits. Data was collected from:

- Greater Lincolnshire Nature Partnership (GLNP)
- Nottinghamshire Biological and Geological Records Centre (NBGRC)
- Multi-Agency Geographic Information for the Countryside (MAGIC)[1] for records of European Protected Species Licences (EPSL) granted.

Survey Scope and Design

A.6.2.3. In line with CIEEM guidance (CIEEM, 2018), surveys were focussed on those areas in which works associated with the Proposed Development could contribute to significant negative effects on GCN populations or could result in contravention of the legislation protecting GCN.

A.6.2.4. Initial scoping was undertaken to assess habitats within the Order Limits for their potential suitability to support GCN. This exercise was conducted using a combination of aerial imagery and walkover surveys across all habitat types.

A.6.2.5. For ease, the scoping survey was divided into habitats with the potential to support GCN in their terrestrial phase (rough grassland and

[1] An internet-based Geographic Information Systems database developed and maintained by the Department for Environment, Foods and Rural Affairs (DEFRA) (Defra, 2024). Located here: MAGIC

scattered scrub habitats), their breeding phase (waterbodies and watercourses), and habitat features with potential to support GCN in the hibernation period (stone walls, log piles, etc).

A.6.2.6. Surveys were designed to identify the presence of GCN specifically within breeding ponds. Typically all ponds within a 500 metre (m) buffer are incorporated into the assessment where there are no barriers to movement of GCN, however, due to the large extent of the Order Limits and the limited anticipated impacts on vegetated habitat features, 250 m was considered a suitable buffer.

Habitat Suitability Index (HSI)

A.6.2.7. Waterbodies within the Order Limits were assessed for their potential to support breeding GCN following the suitability index created by Oldham *et al* (2000). Indices are allocated to ten separate features of the waterbody, these are then used to calculate a probability score indicating the likelihood of it being used by breeding GCN. Features include:

- Geographic location;
- Area of waterbody or watercourse;
- Permanence (frequency of drying);
- Water quality (indicated by invertebrate diversity);
- Levels of overshading by adjacent vegetation;
- Presence of waterfowl;
- Presence of fish;
- Total count of waterbodies/watercourses within 1 km;
- Surrounding terrestrial habitats; and
- Presence of macrophytes (emergent vegetation and floating plants.

A.6.2.8. The result provides a number between 0 and 1, for which:

- < 0.5 = poor
- 0.5 - 0.59 = below average
- 0.6 - 0.69 = average
- 0.7 - 0.79 = good
- > 0.8 = excellent

A.6.2.9. In addition to HIS, a habitat suitability assessment was undertaken of the ditch systems. Ditches were assessed using the following criteria:

- Geographic location
- Permanence
- Level of shading
- Presence of fish
- Presence of macrophytes/emergent vegetation
- Presence of waterfowl
- Water quality
- Pond count/connectivity
- Flow rate

Environmental DNA Sampling

A.6.2.10. Waterbodies and suitable watercourses which contained water at the time of the initial survey visit (in April 2023) were sampled during a follow up visit in June 2023 to confirm the presence or likely absence of GCN using environmental DNA (eDNA) sampling techniques. Additional surveys were undertaken in May and June 2025. This method of collection follows Biggs *et al.,* (2014) and involves a single visit between 15th April and 30th June.

A.6.2.11. A sterile collection cup was used to collect water at 20 locations around the margin of the waterbody, where access was available, each of which was added to a sterile bag. The bag was then thoroughly mixed before adding 30 millilitres (ml) to six sample pots with a fixing solution. These were immediately sent for analysis at SureScreen Laboritories (who are a Natural England (NE) accredited laboratory).

A.6.2.12. The samples were collected on 19th June 2023 and 8th May and 27th June 2025 by suitably qualified ecologists (holding a NE Class licences for GCN survey).

A.6.2.13. Following two inconclusive results for ditches 3 and 5 during the May 2025 surveys, repeat sampling was undertaken in June 2025. The results from this are shown in section A.6.3.11.

Limitations

A.6.2.14. Of the 40 waterbodies initially identified within the Order Limits and the 250 m buffer, access was not gained (either due to land owner permissions or dense vegetation presenting a physical barrier) to 29. As such, only ten were subject to eDNA sampling, with one found to be dry during initial survey. A description and full access details for each water body is provided in **Annex A**.

A.6.2.15. Comments following the Preliminary Environmental Impact Review (PEIR) in 2024 indicated the requirement for further eDNA sampling. Access was therefore requested for a further eight waterbodies outside of the DCO Limits, however, landowner access was not granted. Further surveys were instead undertaken within the DCO Limits, focusing on the ditch systems near to the eight waterbodies where access was unavailable. The results from these are provided in **Annex C**.

A.6.3 Results

Scoping

A.6.3.1. Initial satellite imagery and Ordnance Survey (OS) mapping identified 40 waterbodies within the Order Limits and the 250 m buffer, see **Figure 3.1**. Watercourses, including streams and agricultural drainage ditches, were considered unsuitable to support breeding GCN due to the flow of water and presence of wildfowl and fish.

A.6.3.2. The Order Limits are largely comprised of habitats that are of low suitability for GCN in their terrestrial phase. Agricultural habitat (arable fields), with narrow, intensively managed field margins and species-poor hedgerows cover over three quarters of the Order Limits. Features considered suitable to support hibernating GCN were also limited, with a single brash pile located along Sewer Dyke to the east of the River Trent, and a piles of large rocks in a arable field margin in the northwest of the Order Limits. Additional features may have been present within vegetation either side of the Fledborough Viaduct, but these habitats could not be accessed due to their dense neture.

Desk Study

A.6.3.3. Following the finalisation of Order Limits, 15 of the waterbodies initially identified no longer fell within the Order Limits or its 250 m buffer. Of the remaining 25, five are within the Order Limits and 20 fall within the 250 m buffer. Roughly half are to the west of the River Trent and half to the east.

A.6.3.4. The data search returned six records of GCN within 2 km of the Order Limits in the past 10 years. The closest GCN record was located within the Order Limits at High Marnham, west of the River Trent, with the most recent record being in 2019.

A.6.3.5. Historic records were returned from 1977 to 2010 and included a record at waterbody 11 (within the 250 m buffer) in 2006.

A.6.3.6. The MAGIC search identified no GCN EPSL within 2 km of the Order Limits.

Habitat Suitability Index

A.6.3.7. Fourteen waterbodies were either accessible or visible from Public Rights of Way (PRoW) to conduct an HSI. Full results are presented in **Annex B** with a summary in **Table 3.1**.

A.6.3.8. Five ditches were accessible to conduct a habitat suitability assessment. The results are presented below:

- Ditch 1 was considered poor due to the influence of adjacent arable fields on water quality, high presence of fish and waterfowl, low macrophyte cover, minimal shading and a lack of suitable egg laying habitat.
- Ditch 2 was considered poor, primarily due to poor water quality possibly influenced by surrounding arable fields, high presence of fish and waterfowl, absence of shading, poor terrestrial habitat, minimal macrophyte coverage and unsuitable conditions for egg laying.
- Ditch 3 was considered poor due to poor water quality from adjacent arable land, poor terrestrial habitat and high levels of shading.
- Ditch 4 was considered poor.It comprised a running stream, which unsuitable for GCN, poor water quality, high shade levels, low macrophyte cover, poor surrounding terrestrial habitat and a possible presence of fish.
- Ditch 5 was considered poor, comprising of a running stream unsuitable for GCN. Additional concerns included poor water quality, minimal shading, major waterfowl presence, possible fish presence, low macrophyte cover, and poor surrounding terrestrial habitat.

Table 3.1 Habitat Suitability Index Results for Waterbodies within the Order Limits and a 250 m Buffer

Ref	HSI Score	Pond suitability	Location
1	0.684	Average	Within Order Limits
2	0.39	Poor	250 m buffer
3	0.70	Good	No longer falls within the Order Limits or the 250 m buffer
4	0.37	Poor	250 m buffer
5	0.66	Average	No longer falls within the Order Limits or the 250 m buffer
6	0.80	Excellent	250 m buffer
7	0.83	Excellent	250 m buffer
8	0.25	Poor	No longer falls within the Order Limits or the 250 m buffer

Ref	HSI Score	Pond suitability	Location
9	0.66	Average	Within Order Limits
10	0.81	Excellent	250 m buffer
14	0.13	Poor	250 m buffer
15	0.73	Good	250 m buffer
17	N/A No water present		
27	0.30	Poor	250 m buffer

Environmental DNA Sampling

A.6.3.9. Access was gained to collect water samples from ten water bodies in 2023 (referenced 1 to 10 in **Figure 3.1**), all of which tested negative for GCN eDNA. Additional surveys conducted in May and June 2025 focused on the ditch network surrounding Bubble Dyke, Fledborough Beck, Old Trent, and a Sewer Drain that feeds into ditches 2 and 3. Ditch 2 extends from Little London to Wigsley Woods, while ditch 3 runs from Little London to South Clifton. The locations of the ditch sampling sites are shown in **Figure 2.2**.

A.6.3.10. The results from SureScreen Scientifics concluded negative results for ditches 1, 2 and 4. Results for ditches 3 and 5 initially came back inconclusive, which could be due to some underlying degradation or inhibition which could be affecting the interpretation of results. This could be due to sediment, algae or plant matter within the sample, or an unusually high concentration of inhibitory molecules within the water sample. For inconclusive results, it is recommended that analysis is repeated with a fresh sample from the site in question.

A.6.3.11. Following guidlines from SureScreen repeated eDNA surveys were undertaken for ditches 3 and 5 in June 2025. The results of the repeated surveys were negative (see Annex C).

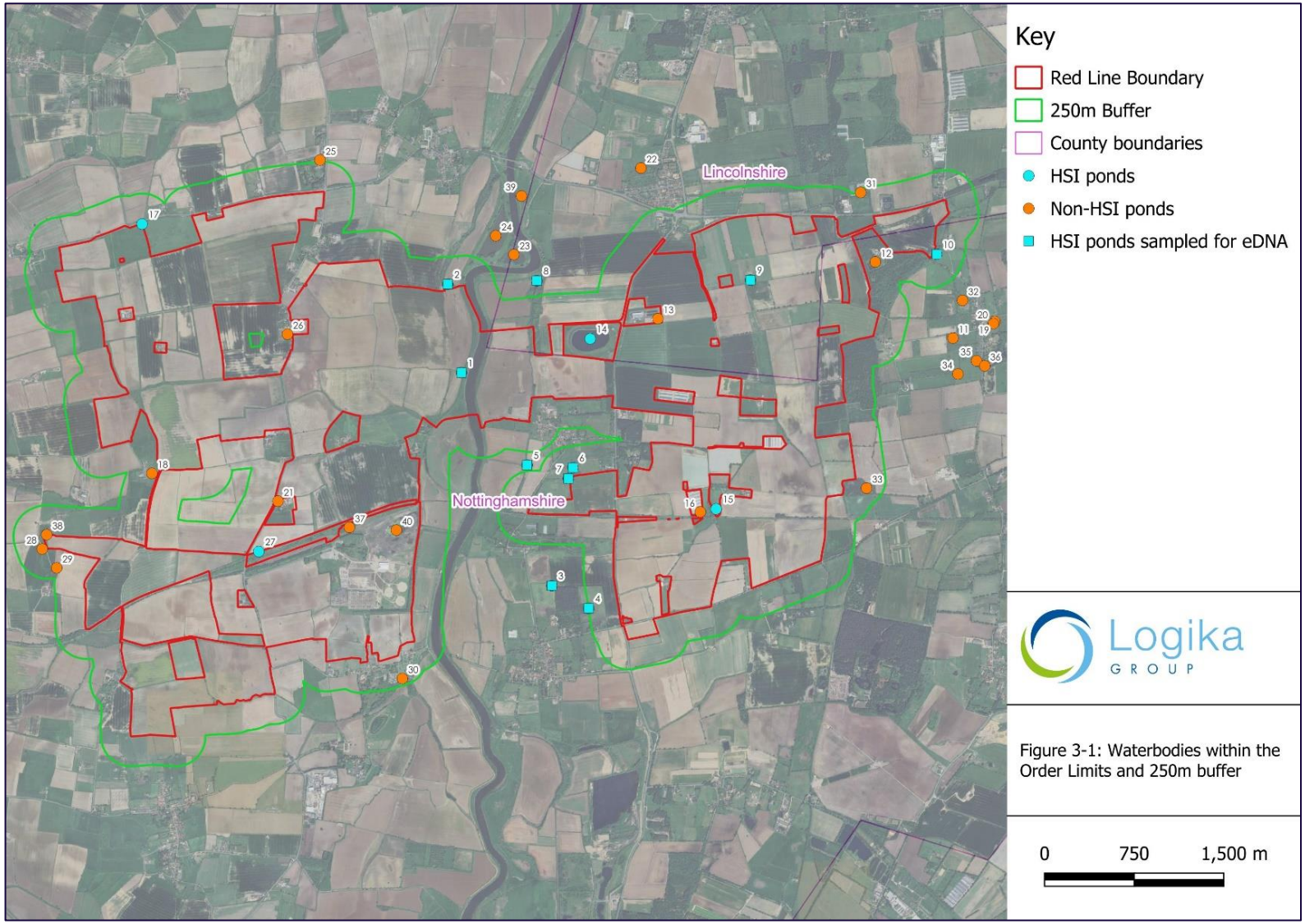

Figure 2.1 Waterbodies within the Order Limits and the 250 m buffer

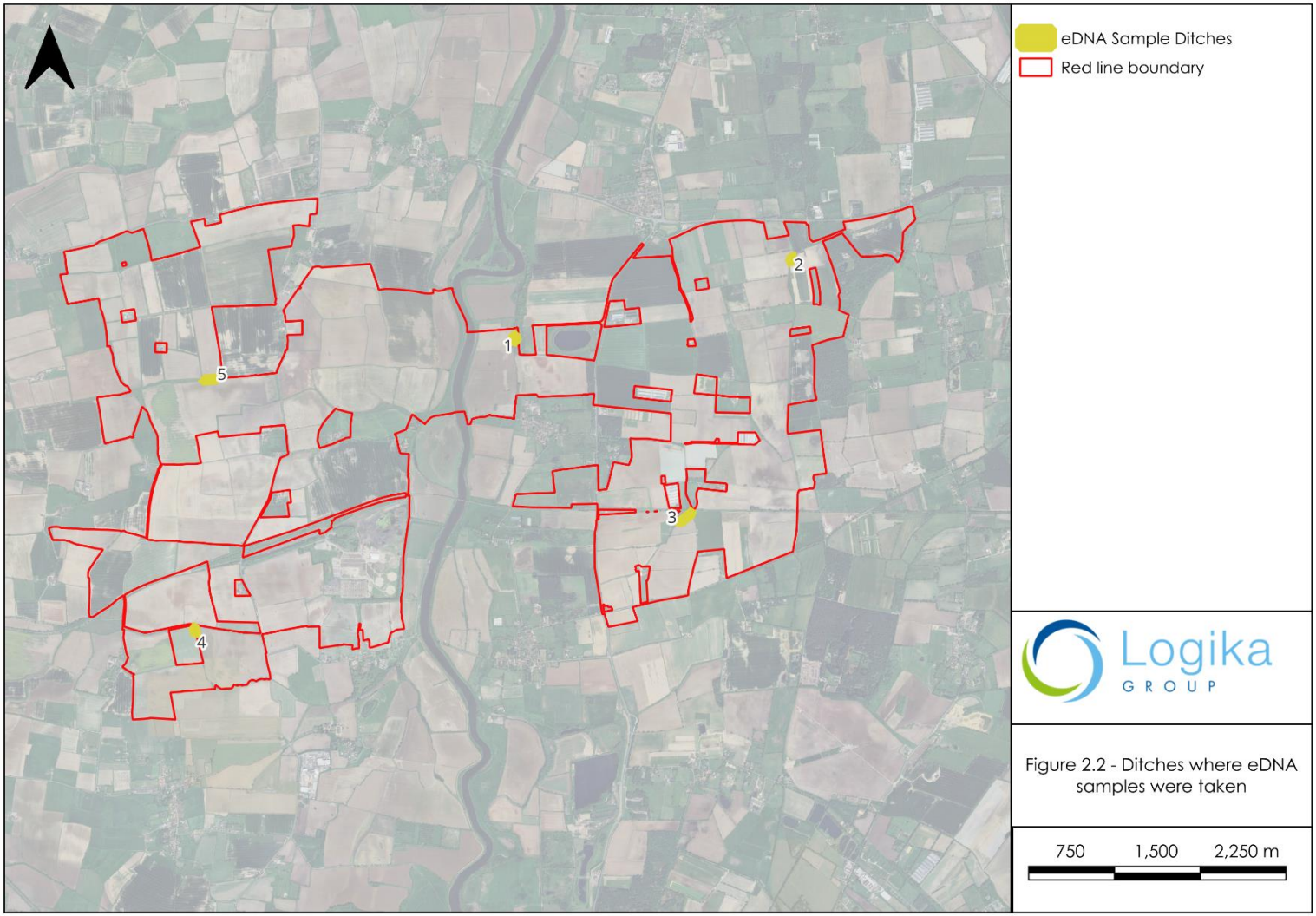

Figure 2.2 Ditches where eDNA samples were taken

A.6.4 Summary

A.6.4.1. The scoping process identified a limited area of suitable terrestrial habitats within the Order Limits and two hibernation features.

A.6.4.2. Twenty-five waterbodies are located within the Order Limits and the 250 m buffer, five of which are located within the Order Limits (1, 9, 17, 37 and 40), however, waterbody 17 was dry and has remained so.

A.6.4.3. Fifteen waterbodies were sampled for eDNA, including waterbodies 1 and 9, and ditches 1 to 5 within the Order Limits, all of which proved negative for GCN.

A.6.4.4. Further water sampling was due to be conducted within waterbodies 37 and 40 within the Order Limits and as many as possible within the 250m Order Limits, however access was not granted. Further water samples were instead taken from five separate ditches, as detailed in Section A.6.3.8, to establish the presence or likely absence of GCN. .

A.6.5 References

- Amphibian and Reptile Groups of the United Kingdom (2010) ARG UK Advice Note 5: *Great Crested Newt Habitat Suitability Index*. ARGUK.
- 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.
- English Nature (2001) *Great Crested Newt Mitigation Guidelines*. English Nature, Peterborough.
- Gent T and Gibson S (2003). *Herpetofauna Workers Manual*. JNCC, Peterborough.
- Oldham, R.S., Keeble, J., Swan, M.J.S and Jeffcote, M. (2000) *Evaluating the Suitability of Habitat for the Great Crested Newt (Triturus cristatus)*. Herpetological Journal Vol. 10, pp. 143-155

Annex A - Waterbody Description and Access

Table A.1 Description and Access to Waterbodies

Ref	Description	Location	HSI	eDNA
1	Pond immediately surrounded by scrub and trees with arable in the wider landscape and the River Trent to the east	Order Limits	Yes	Yes
2	Reed bed feeding agricultural drainage to the River Trent	250 m buffer	Yes	Yes
3	Woodland pond	Outside buffer	Yes	Yes
4	Woodland pond	250 m buffer	Yes	Yes
5	Pond immediately surrounded by scrub and trees with arable in the wider landscape	Outside buffer	Yes	Yes
6	Woodland pond	250 m buffer	Yes	Yes
7	Woodland pond	250 m buffer	Yes	Yes
8	Reed bed feeding agricultural drainage to the River Trent	Outside buffer	Yes	Yes
9	Pond immediately surrounded by scrub and trees with arable in the wider landscape	Order Limits	Yes	Yes
10	Woodland pond	250 m buffer	Yes	Yes
11	Woodland pond – dense vegetation prevents access	Outside buffer	No	No
12	Woodland pond – no access permissions in place	250 m buffer	No	No
13	Farmland pond – no access permissions in place	250 m buffer	No	No
14	Large reservoir - no access permissions in place	250 m buffer	Yes	No
15	Farmland pond, steep banks – unsafe to access	250 m buffer	Yes	No
16	Farmland pond – no access permissions in place	250 m buffer	No	No
17	Depression in grassland field - dry	Order Limits	Yes	No
18	Farmland pond – no access permissions in place	250 m buffer	No	No
19	Garden pond, private residence – no access	Outside buffer	No	No
20	Garden pond, private residence – no access	Outside buffer	No	No

Ref	Description	Location	HSI	eDNA
21	Garden pond, private residence – no access	250 m buffer	No	No
22	Garden pond, private residence – no access	Outside buffer	No	No
23	Large recreational lake	Outside buffer	No	No
24	Large recreational lake	Outside buffer	No	No
25	Garden pond, private residence – no access	Outside buffer	No	No
26	Garden pond, private residence – no access	250 m buffer	No	No
27	Garden pond, private residence – no access	250 m buffer	Yes	No
28	Garden pond, private residence – no access	250 m buffer	No	No
29	Garden pond, private residence – no access	250 m buffer	No	No
30	Garden pond, private residence – no access	250 m buffer	No	No
31	Garden pond, private residence – no access	250 m buffer	No	No
32	Garden pond, private residence – no access	Outside buffer	No	No
33	Woodland pond - no access permissions in place	250 m buffer	No	No
34	Garden pond, private residence – no access	Outside buffer	No	No
35	Garden pond, private residence – no access	Outside buffer	No	No
36	Garden pond, private residence – no access	Outside buffer	No	No
37	Woodland pond – dense vegetation prevents access	Order Limits	No	No
38	Garden pond, private residence – no access	250 m buffer	No	No
39	Pond in grassland field - no access permissions in place	Outside buffer	No	No
40	Appears to be waterlogged ground but only viewed from satellite images. Land access restrictions in place.	Order Limits	No	No

Annex B - Habitat Suitability Index Results

Table B.1 Habitat Suitability Index Results for Waterbodies within the Order Limits and a 250 m Buffer

Ref.	Habitat Suitability Index Factor											
	1	2	3	4	5	6	7	8	9	10	Index	Result
1	1	0.6	1	0.67	1	0.67	0.01	1.91	0.67	0.35	0.684	Average
2	1	0.9	0.1	0.67	1	0.67	0.01	1.59	0.67	0.35	0.39	Poor
3	1	0.92	1	0.67	0.9	1	0.33	0.64	0.67	0.35	0.70	Good
4	1	0.01	1	1	0.2	1	0.33	0.32	0.67	0.35	0.37	Poor
5	1	0.95	1	0.33	0.2	1	0.33	4	0.67	0.3	0.66	Average
6	1	1	1	0.67	0.2	1	0.67	4	1	0.3	0.80	Excellent
7	1	0.3	1	1	0.9	1	0.67	3	1	0.3	0.83	Excellent
8	1	0.01	0.1	1	1	0.67	0.01	0.63	0.67	0.35	0.25	Poor
9	1	0.3	1	0.67	0.6	1	0.67	2	0.33	0.3	0.66	Average
10	1	0.98	0.5	0.67	0.5	0.67	0.67	5	1	0.35	0.81	Excellent
14	1	0.01	0.9	0.67	1	0.01	0.01	0.63	0.01	0.3	0.13	Poor
15	1	0.8	0.9	0.67	1	0.67	0.67	1	0.67	0.3	0.73	Good
17	N/A – no water present											
27	1	0.1	0.1	0.33	1	1	1	0.63	0.01	0.3	0.30	Poor

Annex C - SureScreen Scientific Outputs

SureScreen Scientifics

Folio No: E18306
Report No: 1
Purchase Order: LCL2021
Client: Logika
Contact: Alex Jackson

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: 26/06/2023
Date Reported: 04/07/2023
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
5806	Pond 9, One Earth Solar	SK 83954 73461	Pass	Pass	Pass	Negative	0
5807	Pond 8, One Earth Solar	SK 82146 73432	Pass	Pass	Pass	Negative	0
5810	Pond 10, One Earth Solar	SK 85304 73683	Pass	Pass	Pass	Negative	0
5812	Pond 6, One Earth Solar	SK 82459 71891	Pass	Pass	Pass	Negative	0
5813	Pond 7, One Earth Solar	SK 82425 71805	Pass	Pass	Pass	Negative	0
5815	Pond 5, One Earth Solar	SK 82072 71911	Pass	Pass	Pass	Negative	0
5814	Pond 3, One Earth Solar	SK 82283 70907	Pass	Pass	Pass	Negative	0

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5816	Pond 2, One Earth Solar	SK 81406 73403	Pass	Pass	Pass	Negative	0
5817	Pond 4, One Earth Solar	SK 82594 70727	Pass	Pass	Pass	Negative	0
5818	Pond 1, One Earth Solar	SK 81525 72697	Pass	Pass	Pass	Negative	0

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

Reported by: Chris Troth **Approved by:** Chris Troth

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,

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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.

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Company Registration No. 08950940

Folio No:	1353-2025
Purchase Order:	LCL2381
Contact:	Logika Consultants
Issue Date:	27.05.2025
Received Date:	12.05.2025

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 0939	One Earth - D4	SU 79301 70444	Pass	Pass	Negative	0/12
GCN25 0940	One Earth - D5	SU 79380 72611	Pass	Inhibited Sample	Inconclusive	-/12
GCN25 0945	One Earth - D2	SU 84542 73695	Pass	Pass	Negative	0/12
GCN25 0946	One Earth - D1	SU 82106 72984	Pass	Pass	Negative	0/12
GCN25 0947	One Earth - D3	SU 83637 71436	Pass	Inhibited Sample	Inconclusive	-/12

Matters affecting result: none

Reported by: Amy Bermudez

Approved by: Vanessa Hind

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Methodology

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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 attention to detail to prevent the 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 analytical security.

SureScreen Scientifics Ltd is ISO9001 accredited and participates in Natural England's proficiency testing scheme for GCN eDNA testing.

Interpretation of Results

Sample Integrity Check:	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. Any samples which fail this test are rejected and eliminated before analysis.
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.
Inhibition Check:	**Pass/Fail.** The presence of inhibitors within a sample is assessed using a DNA marker. If inhibition is detected, samples are purified and re-analyzed. 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 the WC1067 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. **Inconclusive:** Controls indicate inhibition or degradation of the sample, resulting in the inability to provide conclusive evidence for GCN presence or absence.

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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 8693	One Earth - 14529AD5	SU 79380 72611	Pass	Pass	Negative	0/12
GCN25 8694	One Earth - 14529AD3	SU 83640 71433	Pass	Pass	Negative	0/12

Matters affecting result: none

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one earth

solar farm