Necton Greener Grid Park

on behalf of Statkraft UK

Appendix 4: Great Crested Newt Presence or Absence Survey Report

avian ecology

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V1	28/02/2023	Draft	Z Hinchcliffe *MRes BSc (Hons.)*	C. Wood *MSc ACIEEM*
V2	26/07/2023	Revision 1	Z Hinchcliffe *MRes BSc (Hons.)*	J. Stevens *BSc (Hons)*

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1 INTRODUCTION

1.1 Background

1.1.1 Avian Ecology Ltd. was commissioned to undertake a great crested newt (GCN) *Triturus cristatus* presence or absence survey. The survey was undertaken in relation to a proposed energy storage development and associated infrastructure including proposed grid connection on land near Necton, Norfolk henceforth referred to as 'the Site', as illustrated on **Figure 4.1**.

1.1.2 This report subsequently provides detailed survey methodology and survey results.

1.2 Survey Area

1.2.1 Ponds were identified from aerial images and OS maps on or within 250m of the Site. Due to the low impact of Greener Grid Park developments on GCN habitats, and reflecting guidance published by English Nature[1], ponds beyond 250m from the Site were not considered.

1.2.2 Ponds subject to assessment are identified on **Figure 4.1** and photographs can be found in **Appendix 4.1**.

2 METHODOLOGY

2.1.1 Eight ponds were identified within 250m of the Site from OS and aerial mapping. Seven ponds within 250m of the Site (**Ponds 1, 2a, 2b, 3, 4, 6 & 7** on **Figure 1**) could be accessed for their suitability to support great crested newts using the Habitat Suitability Index (HSI) method as developed by Oldham *et al.* (2000[2]) and as detailed within ARG UK guidance (ARG UK, 2010[3]). Additionally, at the time of surveys, a further two ponds (**Ponds 5 and 9**) were located within 250m of a previous redline boundary (referred to as wider survey area within this report) and both of these were accessed. These ponds are included within this report.

2.1.2 Access to pond 8 was not possible, however the A47 is located between this pond and the Site and considered to provide a significant physical barrier.

2.2 Habitat Suitability Index

2.2.1 The HSI assessment involves the measurement of ten different indices which, when combined, have been found to provide a good indication of the general suitability of ponds for great crested newts. Each of the indices is scored (between 0.01-1) using a series of graphs and figures within the guidance notes (ARG UK, 2010). These scores are then used to calculate an overall Habitat Suitability Score for each pond.

2.2.2 Final scores relate to pond suitability for great crested newt and range from 'poor' to 'excellent'.

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[1] https://mokrady.wbs.cz/literatura_ke_stazeni/great_crested_newt_mitigation_guidelines.pdf

[2] 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, 10(4), pp. 143-155.

[3] ARG UK (2010) ARG UK Advice Note 5: Great Crested Newt Habitat Suitability Index. Amphibian and Reptile Groups of the United Kingdom.

2.2.3 The HSI survey was undertaken alongside the Extended Phase 1 habitat survey conducted on 10th February 2022 by experienced ecologist J. Jones *BSc (Hons.)* and updated on 4th May 2022 by Z. Hinchcliffe *MRes BSc (Hons.)*. Assessments were carried out on all ten ponds within 250m of the previous redline boundary, except for Pond 8 where access was not possible.

2.3 Presence or Likely Absence Surveys

2.3.1 The survey protocol followed that set out in the Great *Crested Newt Mitigation Guidelines* (English Nature, 2001[4]) and in line with discussions with Natural England.

2.3.2 Ponds were visited four times between April and mid-May 2022 (i.e. within the optimum timeframes for undertaking amphibian surveys) to establish presence or likely absence of GCN and other amphibians. Visits were made in suitable weather conditions; avoiding strong winds and heavy rain and with an overnight temperature of >5°C.

2.3.3 Surveys were conducted by the following licenced personnel; J. Stevens *BSc (Hons.)* (License No: 2019-43160-CLS-CLS), Z. Hinchcliffe *MRes BSc (Hons.)* (License Nº: 2019-44230-CLS-CLS), A. Hulme (BSc (Hons.) (License Nº: 2018-33560-CLS-CLS) and authorised individuals/assistants; P. Baker, K Love *BSc (Hons.)* and K. Ward *MSc*.

2.3.4 Three out of the four recognised survey techniques were employed at each pond during each survey visit. Survey methods comprised netting, torch counts and egg searches as described below:

- Netting: A standard professional long handled net was used to sample the water column throughout each pond. The net was swept backwards and forwards through the water and emptied out into a white plastic container, the contents of which were examined and recorded.
- Torch survey: The ponds were searched for great crested newts at night by shining a torch into the water. Surveyors were equipped with 1,000,000 candlepower Clulite lamps to search the accessible margins of the water bodies.
- Egg search: A thorough search was made for newt eggs of any suitable vegetation present along the water's margin. This comprised a search for any folded leaves of emergent or marginal vegetation. To reduce disturbance, once eggs were confirmed in a pond no further egg searches were completed.

2.4 eDNA

2.4.1 Environmental DNA (eDNA) is nuclear or mitochondrial DNA that is released from an organism into the environment. Sources of eDNA include secreted faeces, mucous, gametes, shed skin and carcasses. In aquatic environments, eDNA is diluted and distributed in the water where it persists for 7–21 days, depending on the conditions (Biggs *et al*., 2014a[5]). The technique for determining presence/absence of GCN uses Polymerase Chain Reaction (PCR) laboratory techniques to detect the species eDNA within water samples.

[4] English Nature (2001). Great Crested Newt Mitigation Guidelines. English Nature, Peterborough https://cieem.net/resource/great-crested-newt-mitigation-guidelines/

[5] Biggs J., Ewald N., Valentini A., Gaboriaud C, Griffiths R.A., Foster J., Wilkinson J., Arnett A., Williams P and Dunn F (2014). Analytical and methodological development for improved surveillance of the Great Crested Newt. Defra Project WC1067. Freshwater Habitats Trust: Oxford.

2.4.2 Recent research by the Department for Environment Food and Rural Affairs (Defra) Project WC1067, concludes that the sampling of waterbodies collecting eDNA appears to be a highly effective method for determining whether great crested newts are present or absent during the breeding season, even where eDNA is present in very low concentrations (Biggs *et al*., 2014).

2.4.3 Natural England accepts the use of environmental DNA surveys as evidence of presence or absence of GCN, provided samples are taken when newts are likely to be present (this depends on location and conditions like the weather). Natural England will only accept eDNA survey results undertaken between mid-April and 30th June, in strict accordance with the published technical advice note, by suitably trained, experienced and licensed GCN surveyors.

Field Sampling Technique

2.4.4 Ponds 2a, 2b, 3, 4, 5, 6 and 7 were sampled on 3rd May 2022. Ponds 1 and 9 were dry at the time of survey and no access was possible to Pond 8.

2.4.5 Samples were collected by Z. Hinchcliffe (NE License No. 2019-44230-CLS-CLS) and assisted by P. Baker.

2.4.6 The protocol for sampling followed that outlined within the technical advice note for field and laboratory sampling of GCN (Biggs *et al*., 2014), which required the collection of 20 x 30ml subsamples from each pond, spaced as evenly as possible around the pond margin.

2.4.7 Each sample was then placed within a Whirl-Pak bag and shaken for 10 seconds, before a 15ml sample was pipetted from the bag and placed in a specimen tube for laboratory analysis. Following collection, samples were refrigerated prior to laboratory dispatch.

Laboratory Analysis

2.4.8 Laboratory analysis was undertaken by SureScreen Scientifics:

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Morley Retreat,
Church Lane,
Morley,
Derbyshire,
DE7 6DE
Tel: +44 (0)1332 292003
Email: scientifics@surescreen.com

2.4.9 The laboratory follows the analysis methodology outlined within the Defra Project WC1067 (Biggs *et al*., 2014) using the q-PCR test conducted in two phases.

2.4.10 The sample first goes through an extraction process to acquire as much eDNA as possible to produce a pooled sample. The pooled sample is then tested via 1-PCR.

2.4.11 Each pooled sample is replicated 12 times to ensure results are accurate. If one of the twelve replicates tests positive, the sample is declared positive. The sample is only declared negative if no replicates show amplification. Inhibition and degradation checks are also carried out on each sample using a known DNA marker. Results of these quality control tests are recorded with each sample.

2.4.12 Samples are tested in a clean room and the different phases of testing are kept separate to reduce any risk of cross contamination.

2.5 Biosecurity

2.5.1 A Biosecurity Risk Assessment was completed and followed for the surveys as summarised in Table 2.1:

Table 2.1: Biosecurity Risk Assessment

Hazard	Control to be provided
Spreading of Chytridrobatidis fungus (*Batrachochytrium dendrobatidis*) and / or fish	Remove all debris, plant fragments and mud from Wellington boots, waders, nets etc. Disinfect in a 10% bleach solution for 15 minutes or 1 minute in a 1mg/ml Virkon solution. Fabrics washed on a 40° C cycle (with detergent, ensuring sufficient rinsing). Nets should be boiled for 10 minutes or, if the fabric allows, disinfected with spray bleach and rinsed thoroughly. Rinse with clean water and if possible, allow to dry before next use. Keep field gear (e.g. traps, net frames, buckets) inside plastic bags during transit and storage to reduce the likelihood of transmitting diseases. All used disinfectants should be disposed of appropriately. Sites considered to be 'infected' should be surveyed/visited last during any programme of work

3 RESULTS

3.1 Habitat Suitability Index

3.1.1 Seven ponds were assessed for their suitability for GCN during the survey. Ponds 1 and 9 were dry at the time of survey so a HSI was not possible to determine. Within the Survey Area, Pond 5 was assessed as Average (0.61 Habitat Suitability Index (HSI) score) with the other six ponds within the Survey Area ranging between Poor (<0.5) and Below Average (0.50-0.59).

3.1.2 HSI results are presented in **Table 3.1** and shown separately in **Annex 1**.

Table 3.1: Habitat Suitability Index Assessment

Pond	Zone	Area (m^2)	Drying	Water Quality	Shade	Fowl present?	Fish present?	Pond count	Terrestrial habitat	Macro phytes	HSI	GCN habitat suitability
P2a	A	100	Annual	Poor	100%	Absent	Absent	7	Moderate	0	0.43	**Poor**
P2b	A	130	Annual	Poor	100%	Absent	Absent	7	Moderate	0	0.44	**Poor**
P3	A	120	Annual	Poor	100%	Absent	Absent	6	Poor	0	0.40	**Poor**
P4	A	120	Never	Poor	100%	Absent	Absent	6	Poor	0	0.5	**Below Average**
P5	A	150	Never	Poor	70%	Absent	Absent	6	Poor	20	0.61	**Average**
P6	A	240	Never	Poor	80%	Minor	Absent	6	Poor	10	0.56	**Below Average**
P7	A	450	Annual	Poor	100%	Minor	Absent	7	Poor	0	0.42	**Poor**

3.1.3 Access to Pond 8 was not possible, however due to the location of the pond to the west of the A47, this road was busy and considered likely to present a significant physical barrier from amphibians that may potentially be present within this pond and the Site.

3.2 Presence or Likely Absence Surveys

3.2.1 No evidence of GCN were found during presence or likely absence surveys.

3.2.2 Smooth newts *Lissotriton vulgaris* and their eggs were also observed during the surveys.

3.2.3 The following tables (**3.2-3.8**) present the results of the presence or likely absence surveys.

Table 3.2: Pond 2a - Survey Results

Pond Ref: 2a									
No. of survey visits: 4		**Torch**			**Netting**			**Egg search**	**Larvae**
Date	Air Temp	Male	Female	Imm.	Male	Female	Imm.	Eggs found?	Larvae found?
12/04/2022	15°C	0	0	0	0	0	0	No	No
21/04/2022	12°C	0	0	0	0	0	0	No	No
04/05/2022	12°C	0	0	0	0	0	0	No	No
10/05/2022	17°C	0	0	0	0	0	0	No	No
Peak adult count for this pond in any one visit (torch, egg or net): 0									
Comments & constraints:	None								
Other Amphibians	Survey 1 – None Survey 2 – None Survey 3 – None Survey 4 - None								

Table 3.3: Pond 2b - Survey Results

Pond Ref: 2b									
No. of survey visits: 4		**Torch**			**Netting**			**Egg search**	**Larvae**
Date	Air Temp	Male	Female	Imm.	Male	Female	Imm.	Eggs found?	Larvae found?
12/04/2022	15°C	0	0	0	0	0	0	No	No
21/04/2022	12°C	0	0	0	0	0	0	No	No
04/05/2022	12°C	0	0	0	0	0	0	No	No
10/05/2022	17°C	0	0	0	0	0	0	No	No
Peak adult count for this pond in any one visit (torch, egg or net): 0									
Comments & constraints:	None								
Other Amphibians	Survey 1 – 3 smooth newt torched. Survey 2 – None Survey 3 – None Survey 4 - None								

Table 3.4: Pond 3 - Survey Results

Pond Ref: 3									
No. of survey visits: 4		**Torch**			**Netting**			**Egg search**	**Larvae**
Date	Air Temp	Male	Female	Imm.	Male	Female	Imm.	Eggs found?	Larvae found?
12/04/2022	15ºC	0	0	0	0	0	0	No	No
21/04/2022	12ºC	0	0	0	0	0	0	No	No
04/05/2022	12ºC	0	0	0	0	0	0	No	No
10/05/2022	17ºC	0	0	0	0	0	0	No	No
Peak adult count for this pond in any one visit (torch, egg or net): 0									
Comments & constraints:		50% of bank covered by dense willow scrub and not surveyed. Some turbidity due to sheep entering the pond. Moorhen nesting on third survey so area surround avoided.							
Other Amphibians		Survey 1 –1 Smooth newt torched Survey 2 – None Survey 3 – None Survey 4 - None							

Table 3.5: Pond 4 - Survey Results

Pond Ref: 4									
No. of survey visits: 4		**Torch**			**Netting**			**Egg search**	**Larvae**
Date	Air Temp	Male	Female	Imm.	Male	Female	Imm.	Eggs found?	Larvae found?
12/04/2022	15ºC	0	0	0	0	0	0	No	No
21/04/2022	12ºC	0	0	0	0	0	0	No	No
04/05/2022	12ºC	0	0	0	0	0	0	No	No
10/05/2022	17ºC	0	0	0	0	0	0	No	No
Peak adult count for this pond in any one visit (torch, egg or net): 0									
Comments & constraints:		90% covered by duckweed and 40% of the bank covered by blackthorn/bramble scrub							
Other Amphibians		Survey 1 – None Survey 2 – None Survey 3 – None Survey 4 - None							

Table 3.6: Pond 5 - Survey Results

Pond Ref: 5									
No. of survey visits: 4		**Torch**			**Netting**			**Egg search**	**Larvae**
Date	Air Temp	Male	Female	Imm.	Male	Female	Imm.	Eggs found?	Larvae found?
12/04/2022	15ºC	0	0	0	0	0	0	No	No
21/04/2022	12ºC	0	0	0	0	0	0	No	No
04/05/2022	12ºC	0	0	0	0	0	0	No	No
10/05/2022	17ºC	0	0	0	0	0	0	No	No
Peak adult count for this pond in any one visit (by torch, egg or net): 0									
Comments & constraints:		None							
Other Amphibians		Survey 1 – None Survey 2 – 1 Smooth newt torched Survey 3 – None Survey 4 - None							

Table 3.7: Pond 6 - Survey Results

Pond Ref: 6									
No. of survey visits: 4		**Torch**			**Netting**			**Egg search**	**Larvae**
Date	Air Temp	Male	Female	Imm.	Male	Female	Imm.	Eggs found?	Larvae found?
12/04/2022	15ºC	0	0	0	0	0	0	No	No
21/04/2022	12ºC	0	0	0	0	0	0	No	No
04/05/2022	12ºC	0	0	0	0	0	0	No	No
10/05/2022	17ºC	0	0	0	0	0	0	No	No
Peak adult count for this pond in any one visit (by torch, egg or net): 0									
Comments & constraints:		None							
Other Amphibians		Survey 1 – 3smooth newt torched. 2 newt eggs found folded. Survey 2 – several (presumed) smooth newt eggs folder over in submerged vegetation. Survey 3 – None Survey 4 - None							

Table 3.8: Pond 7 - Survey Results

Pond Ref: 7									
No. of survey visits: 4		**Torch**			**Netting**			**Egg search**	**Larvae**
Date	Air Temp	Male	Female	Imm.	Male	Female	Imm.	Eggs found?	Larvae found?
12/04/2022	15ºC	0	0	0	0	0	0	No	No
21/04/2022	12ºC	0	0	0	0	0	0	No	No
04/05/2022	12ºC	0	0	0	0	0	0	No	No
10/05/2022	17ºC	0	0	0	0	0	0	No	No
Peak adult count for this pond in any one visit (by torch, egg or net): 0									
Comments & constraints:		90% covered with duckweed							
Other Amphibians		Survey 1 – None Survey 2 – None Survey 3 – None Survey 4 - None							

3.3 eDNA

3.3.1 All ponds returned negative results for the presence of GCN as summarised in **Table 3.2**. In addition, the laboratory reports are reproduced in **Annex 3.1**.

Table 3.2: eDNA survey results.

Pond	Sample Ref.	Inhibition Check	Degradation Check	Sample Integrity Score	Result
P2a	2636	Pass	Pass	Pass	Negative 0/12
P2b	2634	Pass	Pass	Pass	Negative 0/12
P3	1278	Pass	Pass	Pass	Negative 0/12
P4	2637	Pass	Pass	Pass	Negative 0/12
P5	2633	Pass	Pass	Pass	Negative 0/12
P6	2632	Pass	Pass	Pass	Negative 0/12
P7	2631	Pass	Pass	Pass	Negative 0/12

4 CONCLUSIONS

4.1.1 The surveys have confirmed the likely absence of GCNs including individuals and eggs within Ponds 1, 2a, 2b, 3 and 4 within 250m of the Site as well as Ponds 5, 6, 7 and 9 within the wider Survey Area.

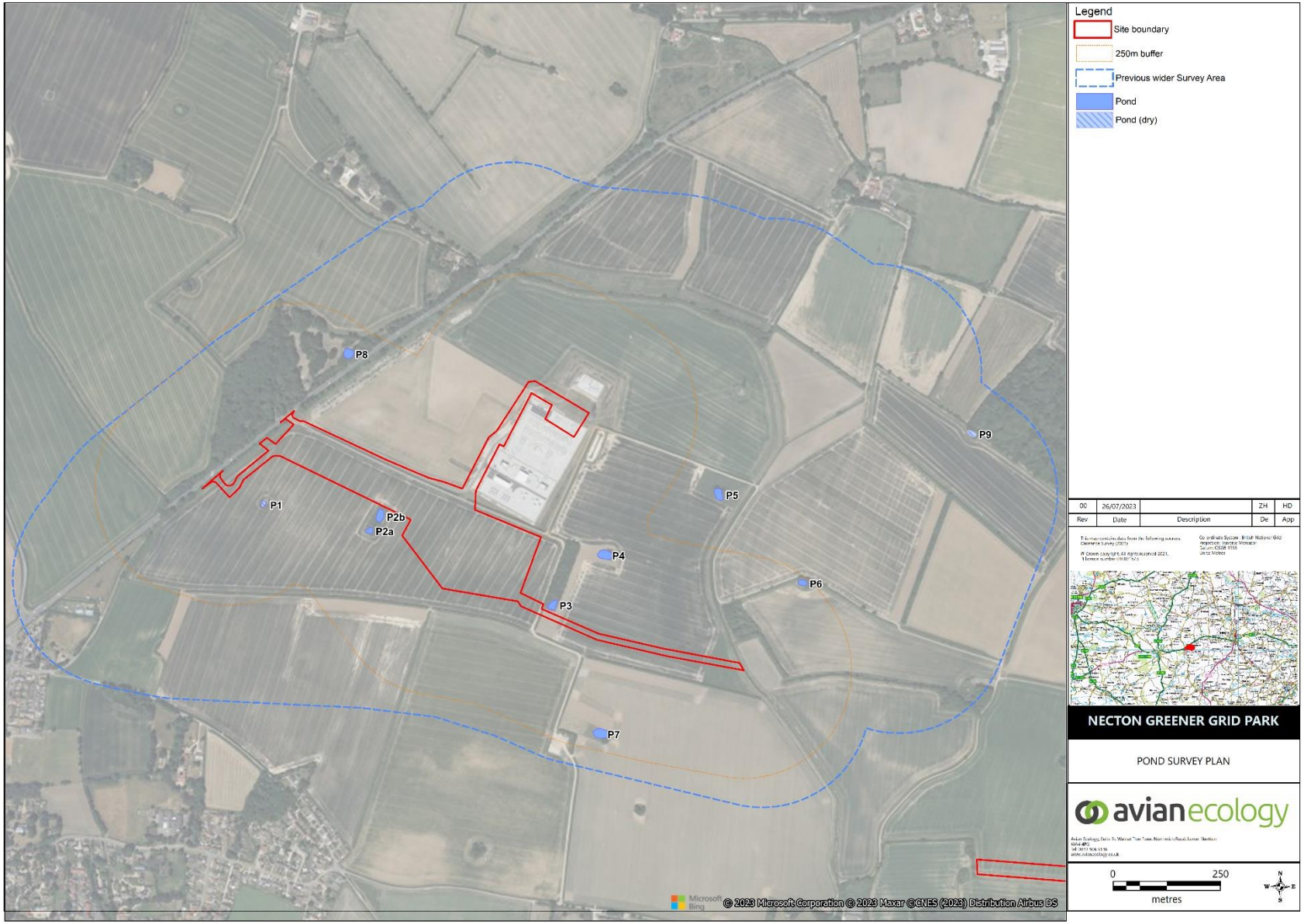
4.1.2 The results of the Presence or Likely Absence surveys were also corroborated by the negative results for all ponds sampled during eDNA surveys.

4.1.3 Aquatic habitats within the Survey Area ranged from Poor to Average and terrestrial habitat, in an arable environment can be considered negligible to low due to habitat isolation, limited feeding opportunities, limited commuting routes and interconnected habitats, as well as the likely negative impacts of agricultural intensification on terrestrial habitats that may be used by amphibians.

4.1.4 Pond 8 was the only pond within the wider Survey Area that was not surveyed due to access constraints, however this pond was located west of the A47, which can be considered significant barrier to commuting amphibians.

4.1.5 GCN are considered likely to be absent from the Site and immediate surrounding area.

FIGURE 4.1: POND LOCATION PLAN



APPENDIX 4.1: PHOTOGRAPHS

Photograph	Description
	Photo 1; Pond 1, dry.
	Photo 2; Pond 2a, poor suitability.

	Photo 3; Pond 2b, poor suitability.
	Photo 4; Pond 3, poor suitability.

	Photo 5; Pond 4, below average suitability.
	Photo 6; Pond 5, average suitability.
	Photo 7; Pond 6, below average suitability.

	Photo 8; Pond 7, poor suitability.

Annex 1: Environmental DNA (eDNA) results

SureScreen Scientifics

Folio No: E13322
Report No: 1
Purchase Order: AE-22-053
Client: AVIAN ECOLOGY LTD
Contact: Rachel Hughes

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: 06/05/2022
Date Reported: 13/05/2022
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
1278	Greener Grid P3		Pass	Pass	Pass	Negative	0
2631	Greener Grid P7		Pass	Pass	Pass	Negative	0
2632	Greener Grid P6		Pass	Pass	Pass	Negative	0
2633	Greener Grid P5		Pass	Pass	Pass	Negative	0
2634	Greener Grid P2B		Pass	Pass	Pass	Negative	0
2636	Greener Grid Pond 2a		Pass	Pass	Pass	Negative	0
2637	Greener Grid P4		Pass	Pass	Pass	Negative	0

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Annex 1: Environmental DNA (eDNA) results

SureScreen Scientifics

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

Reported by: Esther Strafford **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, 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

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Annex 1: Environmental DNA (eDNA) results

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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