
Hob Lane Solar Farm

on behalf of Hob Lane Solar Ltd

Appendix 2: Great Crested Newt Presence or Absence (eDNA) Survey Report



Document Control				
Project Name:		Hob Lane Solar farm		
Project / Report Number:		Bellt-648-6694		
Report Title		Great Crested Newt Presence or Absence (eDNA) Survey Report		
Issue	Date	Notes	Prepared	Reviewed
V1	09/04/2025	First issue	A Crone BSc (Hons.)	J. Stevens BSc (Hons) Principal Ecologist
V2	16/04/2025	For planning	A Crone BSc (Hons.)	J. Stevens BSc (Hons) Principal Ecologist

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1 INTRODUCTION

1.1 Background

1.1.1 Avian Ecology Ltd. was commissioned by Belltown Power UK Limited on behalf of Hob Lane Solar Farm Ltd to undertake great crested newt (GCN) *Triturus cristatus* presence/absence surveys adopting the environmental DNA (eDNA) sampling methodology. The surveys were in relation to the proposed installation of a Solar Farm with associated infrastructure on land located north and south of Rake Lane, Dunham-on-the-Hill, Cheshire (the 'Site'), as illustrated on the Site Location Plan (**Figure 1**) within the *Ecological Assessment Report*¹.

1.1.2 This report subsequently provides detailed survey methodology and results.

1.2 Survey Area

1.2.1 Ponds were identified from aerial images and Ordnance Survey (OS) maps on site or within the 250m buffer around the site boundary. Due to the low impact of solar energy developments on GCN habitats, and reflecting guidance published by Natural England, ponds beyond 250m from the Site were not considered.

1.2.2 Ponds subject to assessment are identified on **Figure 1**.

2 METHODOLOGY

2.1.1 Five ponds are located within the Site with a further 26 ponds located within the 250 buffer.

2.1.2 Ponds were assessed for their suitability to support great crested newt using the Habitat Suitability Index (HSI) Assessment methodology as developed by Oldham *et al.* (2000²) and as detailed within ARG UK guidance (ARG UK, 2010³). These ponds were also subject to eDNA survey sampling to determine the presence or likely absence of GCN.

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'.

2.3 eDNA

2.3.1 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

¹ Avian Ecology. (2025). *Hob Lane Solar Farm, Ecological Assessment Report*.

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.

7–21 days, depending on the conditions (Biggs *et al.*, 2014a⁴). The technique for determining presence/absence of GCN uses Polymerase Chain Reaction (PCR) laboratory techniques to detect the species eDNA within water samples.

- 2.3.2 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).
- 2.3.3 Natural England accepts the use of eDNA 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

- 2.3.4 The onsite ponds were sampled on 14th June 2023 by suitably experienced and licensed GCN surveyor, Z. Hinchcliffe (NE Licence number; 2020-44328-CLS-CLS) and F. Wilde. This is within the period accepted by Natural England.
- 2.3.5 The protocol for sampling followed that outlined within the technical advice note for field and laboratory sampling of great crested newts (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.
- 2.3.6 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

- 2.3.7 Laboratory analysis was undertaken by SureScreen Scientifics, an approved laboratory for eDNA testing:

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- 2.3.8 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.
- 2.3.9 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.

⁴ 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.

- 2.3.10 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.
- 2.3.11 Samples are tested in a clean room and the different phases of testing are kept separate to reduce any risk of cross contamination.


2.4 Limitations



- 2.4.1 No substantive limitations were encountered during the course of surveys.
- 2.4.2 It should be acknowledged that suitability of ponds and occupation by GCN is likely to change between years dependent on factors such as rainfall and management of surrounding habitat. As such, results present a snapshot in time and ponds found not be occupied by GCN may be in other years as part of a wider meta-population.



3 RESULTS



- 3.1.1 Photographs and brief descriptions of the ponds surveyed are provided in **Table 1** below. Pond locations are shown in **Figure 1**.



Table 1: Pond Information



Pond Reference	Approximate Location	Photograph
P1	250m buffer	 <p>This pond is situated within an area of trees bordering an arable field containing stubble at the time of the survey. The pond is approximately 30m from the Site boundary.</p> <p>The ponds banks are inhabited by species such as oak, willow, bramble, hawthorn, willow, crab-apple and bullrush. The heavy vegetation and trees creating a barrier for ecologist to access the water of the pond, resulting in c. 10% of the bank being able to be accessed by ecologists.</p>



P2	250m buffer	 <p>The pond is located within an enclosed area of woodland. The pond is located approximately 15m from the Site boundary.</p> <p>The ponds banks are inhabited by species such as oak, willow, bramble, sedge, willow and elder and willow. The pond was fully accessible.</p>
P3	250m buffer	 <p>Located in a crop field approximately 30m from the Site boundary.</p> <p>Bullrushes encircle the entire pond with two willow trees, gorse, hawthorn and bramble present within the vicinity of the pond. The pond was fully accessible.</p>

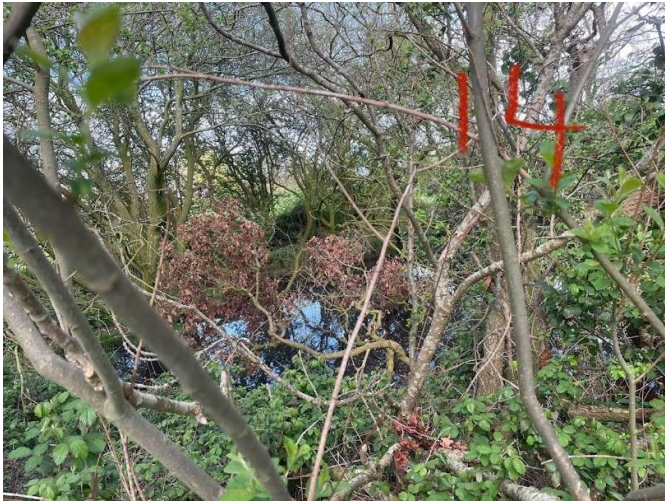

P4	250m buffer	 <p>Located along the edge of a crop field this pond is located approximately 25m from the Site boundary.</p> <p>Soft rush and bullrush can be found along the pond edge with patches of hawthorn, willow and reed also present. The pond was fully accessible.</p>
P5	250m buffer	 <p>Located within a crop field this pond is approximately 100m from the site boundary.</p> <p>Bullrush, rush and reed are present throughout the pond with a hawthorn hedge along the south side. 90% of the bank was accessible.</p>



<p>P6</p>	<p>250m buffer</p>	 <p>The pond is situated within a crop field approximately 105m from the Site boundary .</p> <p>Soft rush is present along the whole edge of the pond with a small area of the pond edge taken up by a willow with some bulrushes. The pond was fully accessible.</p>
<p>P7</p>	<p>250m buffer</p>	 <p>The pond is situated within a patch of grassland approximately 160m from the Site boundary.</p> <p>Bullrush was present throughout the pond with willowherb and sallow along the pond edge. 90% of the pond was accessible.</p>



P8	250m buffer	 <p>This large pond is found along the boundary of the arable field it is situated in and is located approximately 170m from the Site boundary.</p> <p>The pond is bordered by grassland with the pond bank being occupied by thick areas of oak, gorse and willow. Soft rush is also present on the ponds bank and within the pond itself. Due to dense vegetation on the bank only 40% of the pond was accessible.</p>
P9	Within the Site	 <p>This pond is located in a crop field within the Site.</p> <p>The pond has bullrush throughout with a small patch of willow along its banks. Willow herb and soft rush can be found in areas near the bank where the bullrush is less prevalent. The pond was fully accessible.</p>

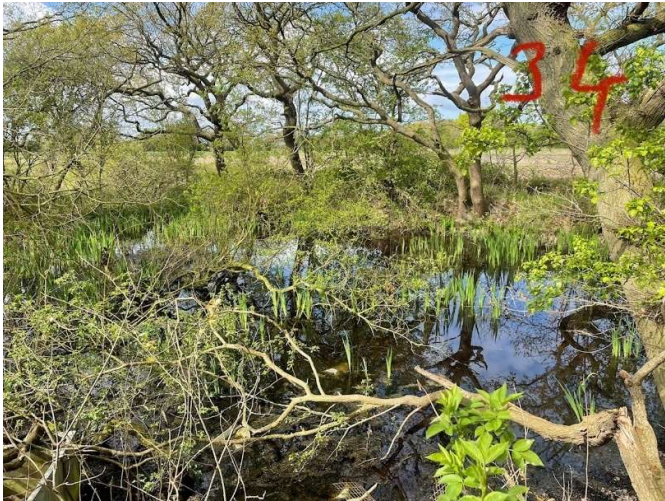

<p>P10</p>	<p>250m buffer</p>	 <p>This pond is located within the middle of an arable field approximately 105m from the Site boundary.</p> <p>The pond itself is dominated by sallow with the banks containing rush and iris. The pond was fully accessible.</p>
<p>P11</p>	<p>250m buffer</p>	 <p>This pond is located along an arable field boundary immediately adjacent to the Site boundary.</p> <p>The pond has bullrush throughout with bramble, willowherb and blackthorn surrounding the pond. Due to vegetation obstructing access to 80% of the ponds bank was accessible.</p>


<p>P12</p>	<p>250m buffer</p>	 <p>The pond is located along the border of arable field immediately outside the Site boundary. Willow is growing through the pond with rush around the banks extending into the pond itself. 50% of the bank was able to be accessed.</p>
<p>P13</p>	<p>250m buffer</p>	 <p>This pond is situated between two arable field boundaries immediately adjacent to the Site boundary.</p> <p>Species present were willow, oak, and hawthorn. Due to the dense vegetation only 20% of the pond was able to be accessed.</p>

P14	250m buffer	 <p>This pond is situated between two arable field boundaries immediately adjacent to the Site boundary.</p> <p>Species present were willow, oak, and hawthorn. Due to the dense vegetation only 20% of the pond was able to be accessed.</p>
P15	250m buffer	 <p>P15 was found not to be a pond but part of a wet ditch, and as such no water samples have been taken.</p>

P16	250m buffer	 <p>This pond is situated centrally in an arable field approximately 85m south of the Site.</p> <p>Willow is growing throughout most of the waterbody with the bank of the pond being populated by soft rush. No samples were taken from this pond, as the water was too shallow for viable samples to be taken.</p>
P28	250m buffer	 <p>This pond is located within a field boundary approximately 90m from the Site boundary.</p> <p>The pond had little emergent vegetation, but the banks had numerous willow, hawthorn, oak, gorse, bramble and soft rush surrounding the pond. Samples could not be taken from this pond as the water levels were too low at the time of the survey.</p>

<p>P29</p>	<p>Within the Site</p>	 <p>Situated within a pasture field within the Site.</p> <p>This pond is surrounded by plant species such as soft rush, sallow, willowherb and reeds, with the entire surface of the pond covered by horsetail. At the time of the survey this pond was dry so no samples could be taken.</p>
<p>P30</p>	<p>Within the Site</p>	 <p>Located in a pasture field within the Site</p> <p>Bullrushes are present along the ponds edge and centre with some soft rush also present. A single hawthorn is present growing near the bank. The pond was fully accessible.</p>

P34	250m buffer	 <p>This pond is enclosed within a small area of sparse woodland located on the boundary of two arable fields immediately adjacent to the Site.</p> <p>The pond itself has reeds growing throughout the water body with a mixture of oak, elder and hawthorn growing from its banks. It was noted during the site visit that, parts of this water seemed to be polluted. This coupled with low water levels resulted in no samples being taken for eDNA analysis.</p>
P43	Within the Site	 <p>This pond is located within the Site situated to the west of a farm track that links the arable fields to Common Lane.</p> <p>This pond has an estimated 20 percent of its surface area taken up by bullrushes with willowherb and soft rush comprising of most of the vegetation that is found on the banks of the pond. Dense bullrush partially restricted access with approximately 80% of the pond able to be accessed.</p>

<p>P44</p>	<p>Within the Site</p>	 <p>A large pond located towards the boundary of a field within the Site. Willowherb and soft rush surround the pond with willow, hawthorn and bramble on the banks and edges of the pond. The pond was fully accessible.</p>
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3.1.1 The summary of the HSI and eDNA survey results are provided in **Table 2** and **Table 3**, respectively.

3.2 HSI

3.2.1 Ponds P3, P4, P8, P30 and P43 received an HSI score of >0.8 indicating 'excellent' habitat suitability for GCN. Ponds P1, P9, P10, P12 P13 and P28 received an HSI score of 0.7– 0.79 indicating 'good' suitability for GCN. Ponds P5, P6, P7, P14, P29 and P34 received an HSI score of 0.6-0.69 indicating 'average' habitat suitability for GCN. Ponds P11, P15 and P16 received a HSI score of <0.5 indicating 'poor' habitat suitability for GCN

Table 2: HSI survey results

Suitability Indices	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12
SI1 – Location	1	1	1	1	1	1	1	1	1	1	1	1
SI2 – Pond area	0.4	0.7	0.3	0.7	0.6	0.5	0.4	1	0.8	0.45	0.2	0.3
SI3 – Pond drying	0.9	0.9	1	1	0.1	0.1	0.1	0.9	0.1	0.5	0.1	0.5
SI4 – Water quality	0.67	0.67	1	0.67	0.33	0.67	0.33	1	0.67	0.67	0.01	0.33
SI5 – Shade	0.6	1	1	1	1	1	1	1	1	0.8	1	1
SI6 – Fowl	1	0.67	1	1	1	1	1	1	0.67	1	1	1
SI7 – Fish	1	1	1	1	1	1	1	0.67	1	1	1	1
SI8 – Ponds	1	1	1	1	1	1	1	1	1	1	1	1
SI9 – Terrestrial habitat	1	1	1	0.67	0.67	0.67	1	1	1	0.67	1	1
SI10 – Macrophytes	0.5	0.4	0.7	0.35	0.8	0.6	0.8	0.35	0.8	1	0.8	0.8
HSI	0.77	0.80	0.86	0.80	0.63	0.65	0.63	0.86	0.73	0.78	0.42	0.72
Suitability	Good	Excellent	Excellent	Excellent	Average	Average	Average	Excellent	Good	Good	Poor	Good

Suitability Indices	P13	P14	P15	P16	P28	P29	P30	P34	P43	P44
SI1– Location	1	1	1	1	1	1	1	1	1	1
SI2 – Pond area	0.3	0.2	No data/Ditch	0.3	0.5	0.8	1	0.2	0.4	1
SI3 – Pond drying	0.5	0.5	1	0.5	1	0.1	0.9	0.5	0.9	0.9
SI4 – Water quality	0.67	0.67	0.67	0.67	0.67	0.33	1	0.33	1	0.67
SI5 – Shade	0.6	0.6	1	0.6	0.4	1	1	0.6	1	1
SI6 – Fowl	1	1	1	1	1	1	1	1	0.67	0.67
SI7 – Fish	1	1	1	1	1	1	1	1	1	1
SI8 – Ponds	1	1	1	1	1	1	1	1	1	1
SI9– Terrestrial habitat	1	1	1	0.67	1	1	1	0.67	1	1
SI10– Macrophytes	0.6	0.6	No data	No data	0.4	0.8	0.6	0.8	0.5	0.35
HSI	0.72	0.69	0.00	0.00	0.75	0.68	0.94	0.63	0.81	0.82
Suitability	Good	Average	Poor	Poor	Good	Average	Excellent	Average	Excellent	Excellent

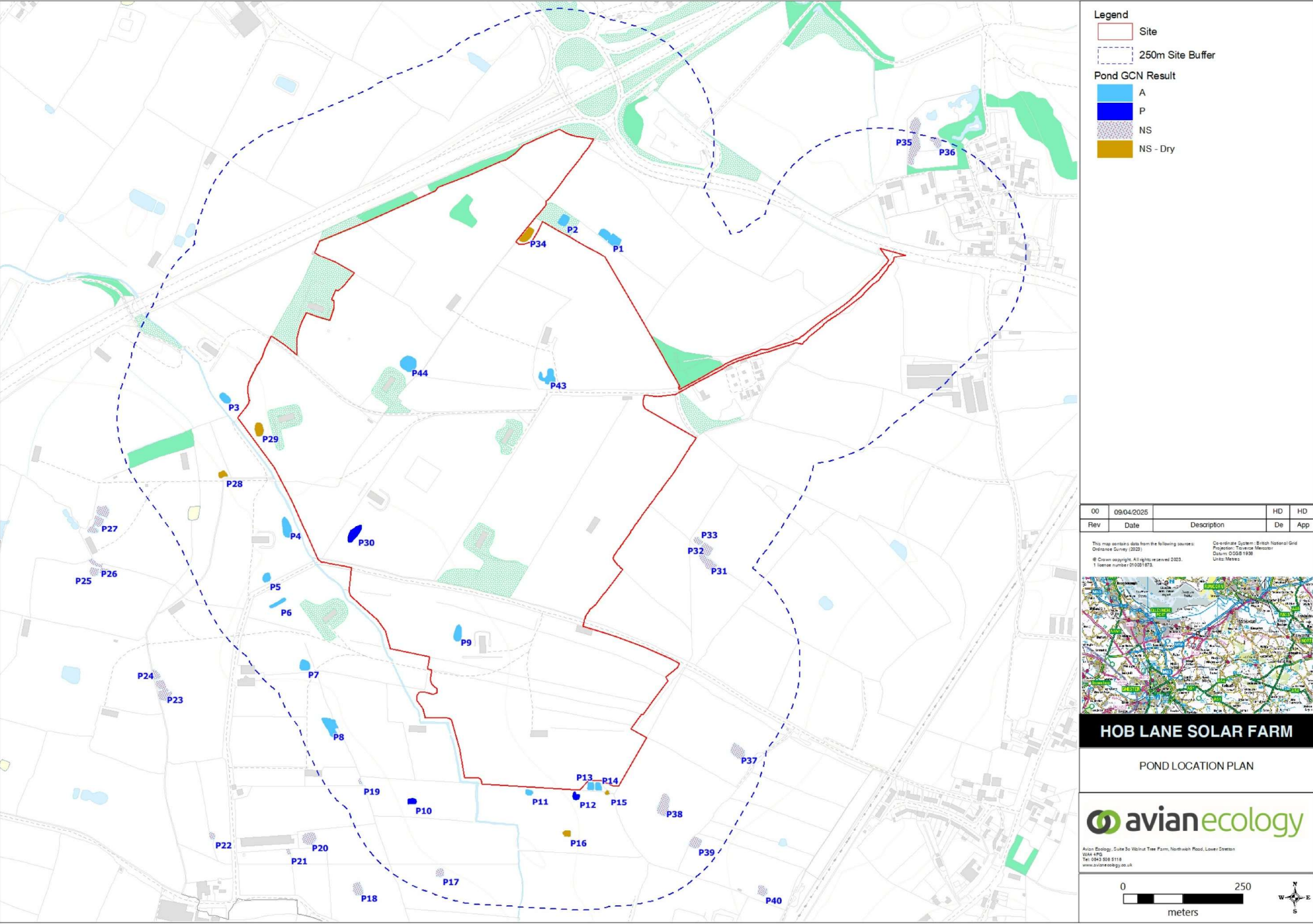
3.3 eDNA

3.3.1 Ponds 10, 12 and 30 returned a positive result for the presence of GCN while ponds P1 -9, P11, P13, P14 P43 and P44 returned negative results, as summarised in **Table 3**. In addition, the laboratory reports are reproduced in **Annex 1**.

3.3.2 No samples were able to be taken from Ponds P28, P29 and P34 as outlined above.

Table 3: eDNA survey results

Pond	Sample Ref.	Degradation Check	Inhibition Check	Result
P1	4859	Pass	Pass	Negative 0/12
P2	4913	Pass	Pass	Negative 0/12
P3	4857	Pass	Pass	Negative 0/12
P4	4858	Pass	Pass	Negative 0/12
P5	4865	Pass	Pass	Negative 0/12
P6	R448	Pass	Pass	Negative 0/12
P7	4863	Pass	Pass	Negative 0/12
P8	4910	Pass	Pass	Negative 0/12
P9	R449	Pass	Pass	Negative 0/12
P10	4861	Pass	Pass	Positive 8/12
P11	4866	Pass	Pass	Negative 0/12
P12	4912	Pass	Pass	Positive 7/12
P13	4914	Pass	Pass	Negative 0/12
P14	3817	Pass	Pass	Negative 0/12
P30	4864	Pass	Pass	Positive 1/12
P43	4862	Pass	Pass	Negative 0/12
P44	4860	Pass	Pass	Negative 0/12



Annex 1 – e-DNA Laboratory Results



Folio No: E18147
Report No: 1
Purchase Order: AA-SS-23-044
Client: AVIAN ECOLOGY LTD
Contact: Stacey Whiteley

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: 20/06/2023
Date Reported: 27/06/2023
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
3817	Hob Lane - P14	-	Pass	Pass	Pass	Negative	0
4857	Hob Lane - P3	-	Pass	Pass	Pass	Negative	0
4858	Hob Lane - P4	-	Pass	Pass	Pass	Negative	0
4859	Hob Lane - P1	-	Pass	Pass	Pass	Negative	0
4860	Hob Lane - P44	-	Pass	Pass	Pass	Negative	0
4862	Hob Lane - P43	-	Pass	Pass	Pass	Negative	0
4864	Hob Lane - P30	-	Pass	Pass	Pass	Positive	1
4865	Hob Lane - P5	-	Pass	Pass	Pass	Negative	0
4866	Hob Lane -	-	Pass	Pass	Pass	Negative	0



Forensic Scientists and Consultant Engineers
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Company Registration No. 08950940

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P11								
4912	Hob Lane - P12	-	Pass	Pass	Pass	Positive	7	
4913	Hob Lane - P2	-	Pass	Pass	Pass	Negative	0	
4914	Hob Lane - P13	-	Pass	Pass	Pass	Negative	0	
R448	Hob Lane - P6	-	Pass	Pass	Pass	Negative	0	

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

Reported by: Chris Troth

Approved by: Jennifer Higginbottom

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.



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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 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

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Folio No: E18382
 Report No: 1
 Purchase Order: AESS-23-051
 Client: AVIAN ECOLOGY LTD
 Contact: Stacey Whiteley

TECHNICAL REPORT

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RESULTS

Date sample received at Laboratory: 27/06/2023
Date Reported: 04/07/2023
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
4861	Hobs Lane - P10	SJ 46121 73185	Pass	Pass	Pass	Positive	8
4863	Hobs Lane - P7	SJ 45915 73482	Pass	Pass	Pass	Negative	0
4910	Hobs Lane - P8	-	Pass	Pass	Pass	Negative	0
R449	Hobs Lane - P9	SJ 46232 73535	Pass	Pass	Pass	Negative	0

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

Reported by: Chris Troth

Approved by: Jackson Young



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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 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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