Cotmoor Solar Farm
on behalf of JBM Solar Projects 6 Ltd

Appendix 2: GCN Presence or Absence (eDNA) Survey Report

avian ecology

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V2	29/05/2020	Draft with updated Figure	B Walker *MSc GradCIEEM*	U Maginn *MSc MCIEEM*
V3	08/07/2020	Final	B Walker *MSc GradCIEEM*	U Maginn *MSc MCIEEM*

CONTENTS

1 INTRODUCTION

1.1 Background

1.1.1 Avian Ecology Ltd. was commissioned by JBM Solar Projects 6 Ltd to undertake a great crested newt (GCN) *Triturus cristatus* environmental DNA (eDNA) survey. The survey was undertaken in relation to a proposed solar energy development on land at Cotmoor Solar Farm, Halloughton, Nottinghamshire, henceforth referred to as 'the Site', as illustrated on **Figure 1.**

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

1.2 Survey Area

1.2.1 Ponds were identified form aerial images and OS maps on or within 250m of the Site. Due to the low impact of solar energy, battery stations and associated infrastructure 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 Eighteen ponds were identified on and within 250m of the Site from OS and aerial mapping. Of these, only three lay in close proximity to the Site Boundary, and no ponds were present within the Site itself. Four ponds (Ponds 12, 13, 14 and 15 on **Figure 1**) could be accessed and assessed for their suitability to support GCN using the HSI (Habitat Suitability Index) Assessment methodology as developed by Oldham *et al.* (2000[1]) and as detailed within ARG UK guidance (ARG UK, 2010[2]). These ponds were also subject to eDNA survey sampling to determine the presence or likely absence of GCN.

2.2 HSI

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 7–21 days, depending on the conditions (Biggs *et al*., 2014a[3]). 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 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).

2.3.3 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.

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[1] 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.

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

[3] 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.

Field Sampling Technique

2.3.4 Ponds were sampled on **7th May 2020**.

2.3.5 Samples were collected by Miss B. Walker (NE Licence No. 2016-20749-CLS-CLS) and Mr. M. Walker as a health and safety second.

2.3.6 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.

2.3.7 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.

2.3.8 This process was repeated for each sampled pond.

Laboratory Analysis

2.3.9 Laboratory analysis was undertaken by SureScreen Scientifics:

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2.3.10 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.

2.3.11 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.

2.3.12 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.

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

3 RESULTS

3.1.1 The summary of the HSI and eDNA survey results are summarised in **Table 3.1** and **Table 3.2**.

3.2 HSI

3.2.1 The habitat suitability of the ponds ranged between poor and good suitability for GCN. Pond 12 was a kidney shaped pond which had inlet and outlet pipes and forms part of the ditch network when surface water flows are in full spate. At the time of the survey the water levels were low and the waterbody formed a self-contained feature surrounded by scattered trees and tall ruderal vegetation. Pond 13 lay within a grazed field, with signs of stock poaching (trampling and erosion) at the banks. The open water contained water lilies and supported nesting moorhen. Pond 14 is a large lake surrounded by grazed pasture which also had signs of poaching along the banks and a number of waterfowl were present at the time of the survey. Pond 15 was an oval shaped pond on a field boundary which has been largely overtaken by bramble scrub and was shaded by willow trees.

Table 3.1: eDNA survey results.

Suitability Indices	Pond Reference			
	P12	P13	P14	P15
SI1 – Location	1.00	1.00	1.00	1.00
SI2 – Pond area	0.30	0.30	N/A >2,000m²	0.15
SI3 – Pond drying	0.50	1.00	0.90	1.00
SI4 – Water quality	0.33	0.67	0.01	0.33
SI5 –Shade	1.00	1.00	1.00	0.50
SI6 – Fowl	0.67	0.67	0.01	0.67
SI7 – Fish	1.00	1.00	0.67	1.00
SI8 – Ponds	1.00	1.00	1.00	1.00
SI9 – Terrestrial habitat	0.33	0.33	0.33	0.67
SI10 – Macrophytes	0.30	0.90	0.90	0.60
HSI	0.56	0.72	0.00	0.61
Suitability	Below average	Good	Poor	Average

3.3 eDNA

3.3.1 Ponds 12 and 13 returned a **Positive** result for the presence of GCN. Ponds 14 and 15 returned a **Negative** result for the presence of GCN as summarised in **Table 3.2**. The laboratory report is reproduced in **Annex 1**.

Table 3.2: eDNA survey results.

Pond	Sample Ref.	Inhibition Check	Degradation Check	Sample Integrity Score	Result
P12	2023	Pass	Pass	Pass	**Positive** 3/12
P13	2022	Pass	Pass	Pass	**Positive** 7/12
P14	2020	Pass	Pass	Pass	Negative 0/12
P15	2021	Pass	Pass	Pass	Negative 0/12

4 CONCLUSIONS

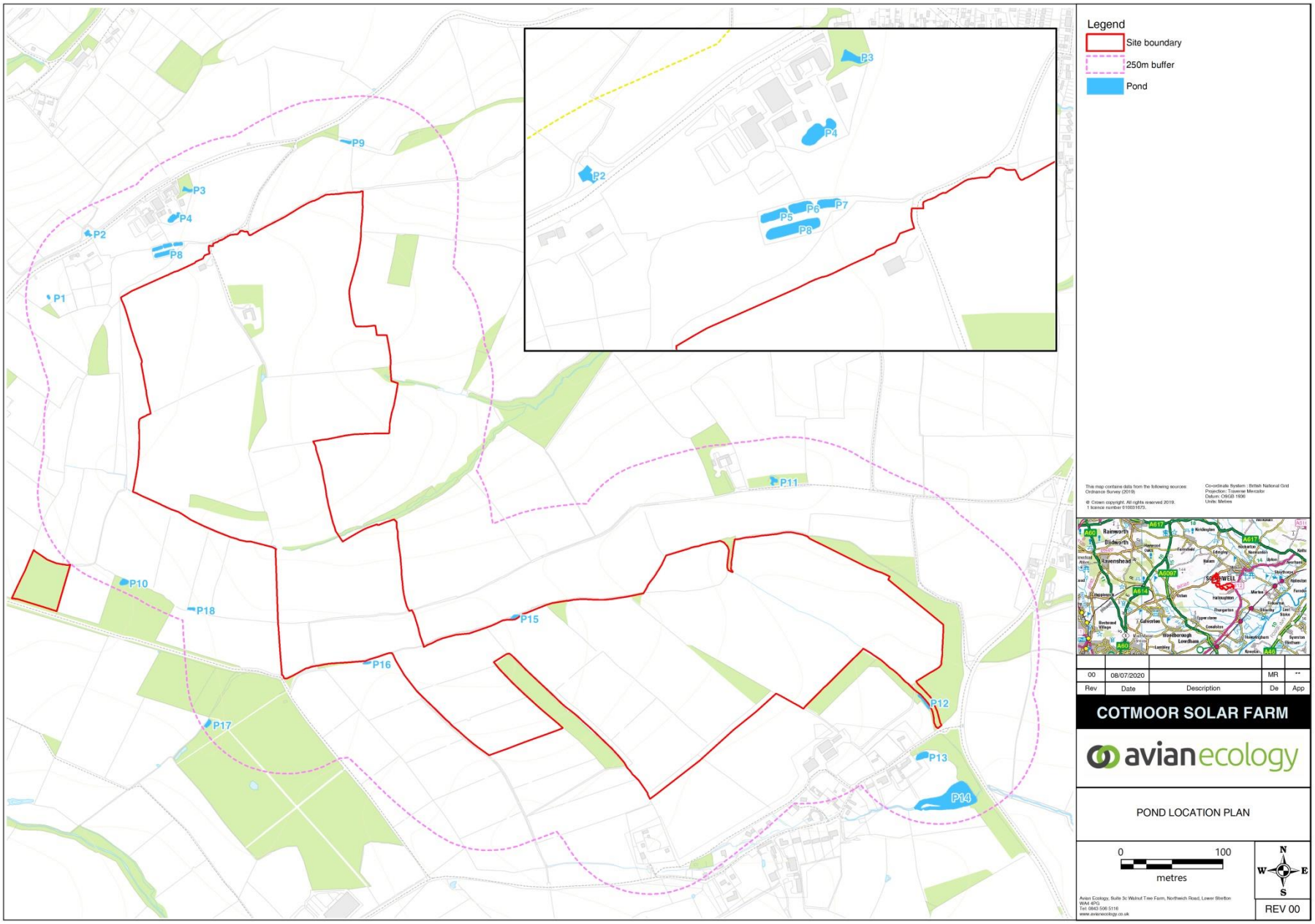
4.1.1 The eDNA sampling and analysis identified the presence of GCN at two ponds (Pond 12 & 13). Pond 12 lies immediately adjacent to the access route into the main body of the Site while Pond 13 lies outside the Site to the south and separated from it by a road as shown on Figure 1.

4.1.2 Ponds 14 and 15 returned negative results for GCN. To generate the e-DNA results, all of the samples from each pond are combined to produce one eDNA extract, following from this, twelve separate analyses are undertaken. If one or more of these analyses are positive, the pond is declared positive for the presence of GCN. It may be assumed that small fractions of positive analyses suggest low level presence, but this cannot currently be reliably used for population studies. In accordance with Natural England protocol, even a score of 1/12 is declared positive.

4.1.3 The presence of GCN in the locality will need to be considered as part of any future planning application, with appropriate mitigation implemented during construction to ensure compliance with the Wildlife and Countryside Act 1981 (as amended) and The Conservation of Habitats and Species Regulation 2017 (as amended).

Figure 1:

Pond Survey Plan


Legend
Site boundary
250m buffer
Pond
P1
P2
P3
P4
P5
P6
P7
P8
P9
P10
P11
P12
P13
P14
P15
P16
P17
P18
This map contains data from the following sources:
Ordnance Survey (2019)
© Crown copyright. All rights reserved 2019.
Licence number 01003 1673.
Co-ordinate System : British National Grid
Projection: Transverse Mercator
Datum: OSGB 1936
Units: Metres
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08/07/2020
MR
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Rev
Date
Description
De
App
COTMOOR SOLAR FARM
avianecology
POND LOCATION PLAN
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Avian Ecology, Suite 3c Walnut Tree Farm, Northwich Road, Lower Stretton WA4 4PG
Tel: 0843 508 5116
www.avianecology.co.uk
REV 00

Annex 1 – e-DNA Laboratory Results

SureScreen Scientifics

Folio No: E7271
Report No: 1
Purchase Order: AE-20-041
Client: AVIAN ECOLOGY
Contact: Beth Walker

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: 11/05/2020
Date Reported: 14/05/2020
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
2020	P14, Halloughton	SK 69198 51663	Pass	Pass	Pass	Negative	0
2021	P15, Halloughton	SK 68022 52117	Pass	Pass	Pass	Negative	0
2022	P13, Halloughton	SK 69097 51769	Pass	Pass	Pass	Positive	7
2023	P12, Halloughton	SK 69100 51909	Pass	Pass	Pass	Positive	3

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

Reported by: Sarah Evans **Approved by:** Chris Troth

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SureScreen Scientifics

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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