Belvoir Solar Farm

on behalf of JBM Solar Projects 10 Ltd

Great Crested Newt Presence or Absence (eDNA) Survey Report

avian ecology

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V2	11/11/2021	Pond plan updated with new red line	S. Turner *MCIEEM*	B. Walker *MSc MCIEEM*
V3	09/09/2022	Updated– any amendments from previous version marked in red	B. Walker *MSc MCIEEM*	N. Robinson *MSc BSc (Hons) ACIEEM*

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1 INTRODUCTION

1.1 Background

1.1.1 Avian Ecology Ltd. was commissioned by JBM Solar to undertake a great crested newt (GCN) *Triturus cristatus* environmental DNA (eDNA) survey in relation to a the pproposed solar energy development on land to the west of the village Muston, Leicestershire, henceforth referred to as 'the Site', as illustrated on **Figure 5.6.1.**

1.1.2 This report subsequently provides detailed survey methodology and survey results updated to reflect a change in Site boundary and Proposed Development layout.

1.2 Survey Area

1.2.1 Ponds were identified form aerial images and OS maps on or within 250m of the preliminary Site boundary and which encompasses the updated Site boundary and areas within 250m.

1.2.2 Due to the low impact of solar energy developments on GCN habitats, and reflecting guidance published by Natural England, ponds beyond 250m from updated Site boundary were not considered.

1.2.3 Ponds subject to assessment are identified on **Figure 5.6.1**.

2 METHODOLOGY

2.1.1 In 2020, 11 ponds were identified for survey on the basis of the preliminary Site boundary, through a review of OS and aerial mapping.

2.1.2 One pond, P1 located within approximately 250m of the preliminary Site boundary, is now no longer located within 250m of the updated Site boundary. Pond P1 is therefore no longer considered in relation to the Proposed Development.

2.1.3 Of the other ten ponds, only three are present within the updated Site boundary(Ponds P8, P9 and P10), and which were found to be dry during the Extended Phase 1 Habitat Survey undertaken in May 2020 and eDNA survey in June 2020.

2.1.4 One pond within the study area was accessed and subject to survey, pond P2 as shown on **Figure 5.6.1.**

2.1.5 The pond was assessed for its suitability to support GCN using the Habitat Suitability Index (HSI) Assessment methodology as developed by Oldham *et al.* (2000[1]) and as detailed within ARG UK guidance (ARG UK, 2010[2]). This pond was 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.

[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.

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.

Field Sampling Technique

2.3.4 The pond was sampled on 22nd June 2020. Samples were collected by Mr A. Hulme (NE Licence No. 2018-33563-CLS-CLS) and Mr. A. Morley (NE Licence No. 2020-44980-CLS-CLS).

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:

SureScreen Scientifics Division Ltd,
Morley Retreat,
Church Lane,
Morley,
Derbyshire,
DE7 6DE

[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.

Tel: +44 (0)1332 292003
Email: scientifics@surescreen.com

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.

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.

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 pond P2 was determined as good. Its a small oval shaped pond at the corner of a large arable field. At the time of the survey, there were areas of open water with reedmace *Typha latifolia* present within the water body. Marginal vegetation consisted of rushes *Juncus sp*, sedges, flag iris *Iris pseudacorus* and water mint *Mentha aquatica*. Water at the time of the survey was deep at the centre but evidence of drying was seen on the banks of the pond. The water was turbid and algal bloom was also present.

Table 3.1: eDNA survey results.

Suitability Indices	**P2**
SI1 – Location	1.00
SI2 – Pond area	0.2
SI3 – Pond drying	1.00
SI4 – Water quality	0.33
SI5 –Shade	1.00
SI6 – Fowl	1.00
SI7 – Fish	1.00
SI8 – Ponds	1.00
SI9 – Terrestrial habitat	0.67
SI10 – Macrophytes	1.00

HSI	0.73
Suitability	**Good**

3.3 eDNA

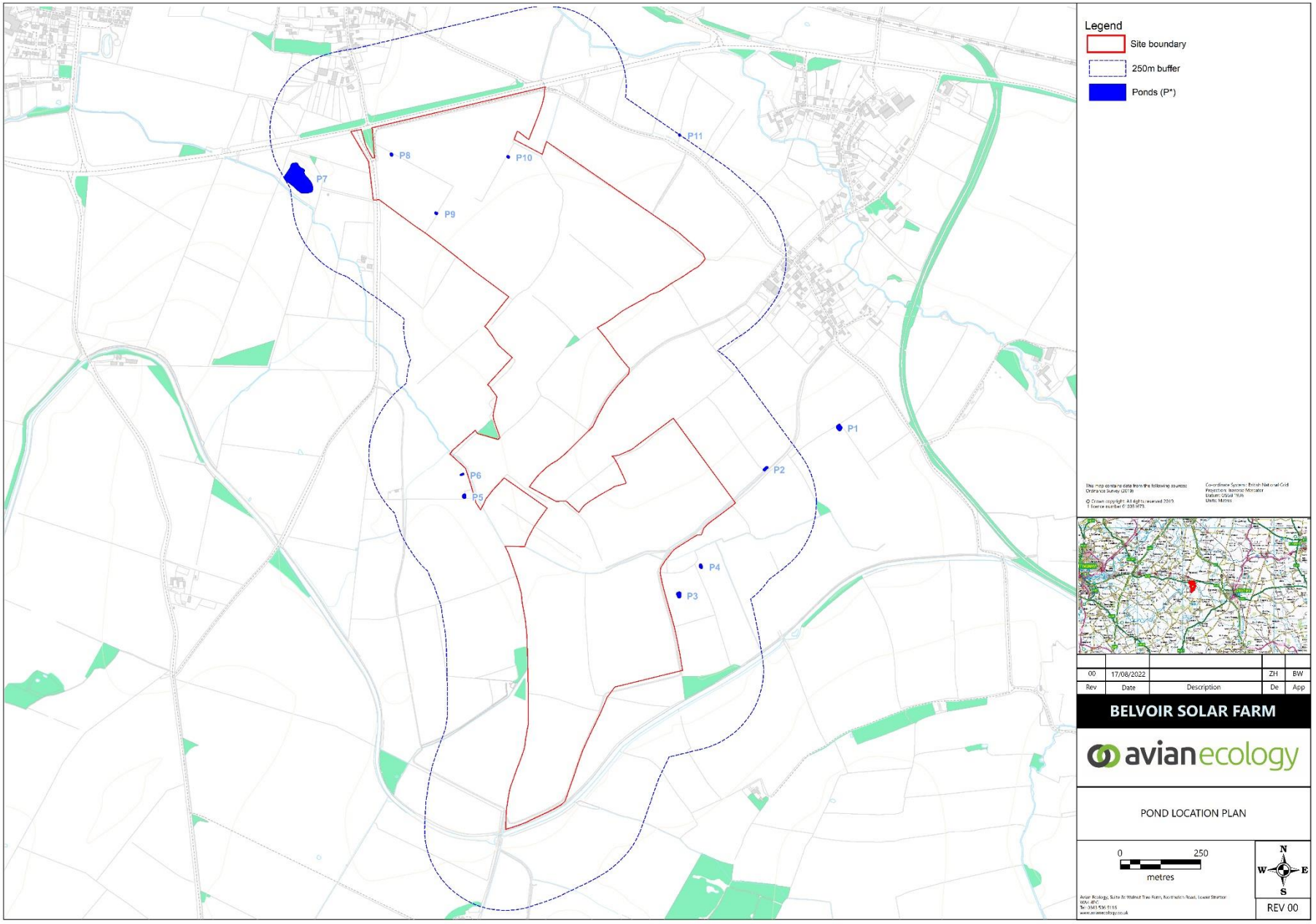
3.3.1 Pond 2 returned a **Negative** result for the presence of GCN as summarised in **Table 3.2**. The laboratory report is reproduced in **Annex 1**.

Table 3.2: eDNA survey results.

Pond	Sample Ref.	Inhibition Check	Degradation Check	Sample Integrity Score	Result
P2	1750	Pass	Pass	Pass	Negative 0/12

Figure 5.6.1:

Pond Location Plan


Legend
Site boundary
250m buffer
Ponds (P*)
P1
P2
P3
P4
P5
P6
P7
P8
P9
P10
P11
00
17/08/2022
ZH
BW
Rev
Date
Description
De
App
BELVOIR SOLAR FARM
avianecology
POND LOCATION PLAN
0
250
metres
N
W
E
S
REV 00

Annex 1 – e-DNA Laboratory Results

SureScreen Scientifics

Folio No: E7943
Report No: 1
Purchase Order: AE-20-107
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: 23/06/2020
Date Reported: 29/06/2020
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
1750	Pond 2 Belvior		Pass	Pass	Pass	Negative	0

[redacted]

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

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

Forensic Scientists and Consultant Engineers
SureScreen Scientifics Ltd, Morley Retreat, Church Lane, Morley, Derbyshire, DE7 6DE
UK Tel: +44 (0)1332 292003 Email: scientifics@surescreen.com
Company Registration No. 08950940

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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