Brogan Solar Farm

on behalf of Fuse Renewables Ltd.

Appendix 4: Great Crested Newt Survey Report

avian ecology

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CONTENTS

FIGURES

ANNEX

1 INTRODUCTION

1.1 Background

1.1.1 Avian Ecology Limited (AEL) was commissioned by Pegasus Planning Group, on behalf of their client Fuse Renewables Limited, to undertake great crested newt (GCN) presence/absence surveys adopting the environmental DNA (eDNA) sampling methodology. The surveys were carried out in relation to the proposed construction of a solar farm and associated infrastructure (the 'Proposed Development') on land located to the east of the B4393 near Llanfyllin, North Wales, SY22 5LQ; central Ordnance Survey (OS) grid reference: SJ 17662 18915 (hereafter referred to as the 'Site') as illustrated on **Figure 4.1: Site Location Plan.**

1.1.2 This report provides detailed survey methodology and results of the GCN eDNA surveys undertaken to establish the baseline conditions with regards to GCN at the Site. This appendix report supports the Brogan Solar Farm Ecological Assessment Report (EAR).

1.1.3 The objectives of this report are to:

- Identify the suitability, location and extent of suitable GCN habitat at the Site; and
- Establish the presence or likely absence of GCN at ponds located within 250 m of the Site.

1.1.4 Common species names are used throughout the text of this report, with scientific names only presented where there is the potential for confusion.

1.2 Site overview

1.2.1 The Proposed Development is predominantly located within a rural landscape, on agricultural land located to the east of the B493 and located *c.* 2.5 km east of the town of Llanfyllin in North Wales. The Site is *c.* 12.1 hectares (ha) in extent.

1.3 Quality assurance and environmental management

1.3.1 This report has been subject to AELs internal quality assurance checks in line with ISO9001:2015.

1.3.2 All surveys and assessments were undertaken by suitably experienced ecologists as per the Chartered Institute of Ecology and Environmental Management (CIEEM) competency framework (CIEEM, 2024[1]) and have been undertaken with reference to the recommendations given in BS 42020:2013 Biodiversity: Code of practice for planning and development (British Standards Institute, 2013[2]).

1.3.3 As per the advice note from CIEEM (2019[3]) On the Lifespan of Ecological Reports & Surveys; the findings presented in this report are considered valid for up to 18 months from the date of survey, providing there is no significant change to the baseline conditions at the Site.

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[1] Chartered Institute of Ecology and Environmental Management (2024). *Competency Framework.* Competency-Framework-2024-V7-Web.pdf (Accessed: 8th January 2026).

[2] British Standards Institute (2013). *BS 42020:2013 Biodiversity: Code of Practice for Planning and Development.*

[3] Chartered Institute of Ecology and Environmental Management (2019). *Advice Note on the Lifespan of Ecological Reports and Surveys* - Advice-Note.pdf (Accessed: 8th January 2026).

1.3.4 Following this time period, the survey data should be reviewed and, if appropriate, updated to ensure baseline conditions remain valid.

2 METHODOLOGY

2.1 Survey area

2.1.1 Ponds located within the Site and a surrounding buffer of 250 m were identified from aerial images and OS maps. Following guidance published by Natural England (2022[4]), ponds beyond 250 m from the Site were not considered.

2.1.2 Ponds subject to assessment are illustrated on **Figure 4.2: Pond Location Plan.**

2.1.3 No ponds were identified within the Site; however, two ponds were located within 250 m of the Site.

2.1.4 Ponds were assessed for their suitability to support GCN using the Habitat Suitability Index (HSI) Assessment methodology as developed by Oldham *et al.* (2000[5]) and as detailed within ARG UK guidance (ARG UK, 2010[6]). 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 GCN. 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 GCN and range from 'poor' to 'excellent'.

2.2.3 The ranges and corresponding HSI scores are:

- Poor - < 0.5;
- Below average – 0.50 – 0.59;
- Average – 0.60 – 0.69;
- Good – 0.7 – 0.79; and,
- Excellent – > 0.8.

2.3 eDNA

2.3.1 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*., 2014[7]). The technique for determining presence/absence of GCN uses

[4] Natural England (2022) Great crested newts: advice for making planning decisions.

[5] 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.

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

[7] 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.

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 (Biggs *et al.,* 2014), concludes that the sampling of waterbodies collecting eDNA appears to be a highly effective method for determining whether GCN are present or absent during the breeding season, even where eDNA is present in very low concentrations.

2.3.3 Natural Resource Wales (NRW) 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). NRW will only accept eDNA survey results undertaken between mid-April and 30th June, in accordance with the published technical advice note (Rees *et al.,* 2023)[8], by suitably trained, experienced and licensed GCN surveyors.

Field sampling technique

2.3.4 The ponds were sampled on 20th June 2025 by a suitably experienced and licenced GCN surveyor, along with a health and safety second. This is within the period accepted by NRW.

2.3.5 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 30 ml 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 15 ml 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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Church Lane,
Morley,
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DE7 6DE
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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 are

[8] Rees. H. C., Baker, C.B., Maddison, B.C., (2023). An evidence review for great crested newt eDNA monitoring protocols. Natural England Commissioned Reports, Number NECR476.

declared positive. 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 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, the results present a snapshot in time and ponds which are found not to be occupied by GCN may be occupied in other years as part of their wider meta-population and habitat use dynamics.

3 RESULTS

3.1.1 Brief descriptions of the ponds surveyed are provided in **Table 3:1** below, photographs are provided in **Annex 1** and pond locations are illustrated in **Figure 4.2**.

Table 3:1. Pond information

Pond reference	Grid reference	Distance from Site	Description
P1	SJ 17422 18547	150 m south	The pond surface was completely covered by duckweed. An island of soft rush, willow herb, nettles and willow scrub was present in the centre of the pond. The pond was located within a field, grazed by horses, with signs of poaching present along the banks. A hedgerow was located within proximity of the pond.
P2	SJ 17852 18628	55 m southeast	A large pond with some poaching present along the banks, however a fence is now present surrounding the pond. Some marginal vegetation was present including scattered soft rush and a stand of bullrush. Aquatic vegetation comprised of pondweed and waterweeds.

3.1.2 The HSI assessment results are provided in **Table 3:2** and the eDNA survey results in **Table 3:3.**

3.2 HSI

3.2.1 Ponds P1 and P2 both achieved HSI scores between 0.6 – 0.69 indicating 'average' habitat suitability for GCN.

Table 3:2. HSI survey results

Suitability indices	Pond number	
	P1	P2
SI1 – Location	0.5	0.5
SI2 – Pond area	0.6	0.95
SI3 – Pond drying	0.9	0.9
SI4 – Water quality	0.33	0.67
SI5 – Shade	1	1
SI6 – Fowl	0.33	0.33
SI7 – Fish	1	0.67
SI8 – Ponds	1	0.55
SI9 – Terrestrial habitat	0.67	0.67
SI10 – Macrophytes	0.35	0.95
HSI	**0.61**	**0.68**
Habitat Suitability	**Average**	**Average**

3.3 eDNA

3.3.1 Water samples were taken at ponds P1 and P2 to test for presence of GCN eDNA. Both ponds returned positive eDNA results, indicating GCN presence, as summarised in **Table 3.3**.

3.3.2 P1 returned 12 of 12 replicates as positive for GCN eDNA, whilst P2 returned only two of 12 replicates as positive for GCN eDNA. Whilst it may be assumed that small fractions of positive analyses suggest low level presence, eDNA testing cannot be used for population estimates and even a score of one out of 12 is deemed positive eDNA results which indicate GCN presence. The SureScreen laboratory reports are presented in **Annex 2.**

Table 3:3. eDNA survey results

Pond reference	Sample reference	Degradation check	Inhibition check	Result
P1	GCN25 5698	Pass	Pass	Positive 12/12
P2	GCN25 5699	Pass	Pass	Positive 2/12

4 SUMMARY

4.1.1 Two ponds (P1 and P2) were identified within 250 m of the Site boundary; both ponds were subjected to a HSI assessment and eDNA survey.

4.1.2 The HSI assessment resulted in both ponds being classified as having 'average' habitat suitability to support GCN.

4.1.3 The eDNA survey found both ponds to be positive for the presence of GCN, concluding the presence of GCN within proximity of the Site.

Figure 4.1: Site Location Plan

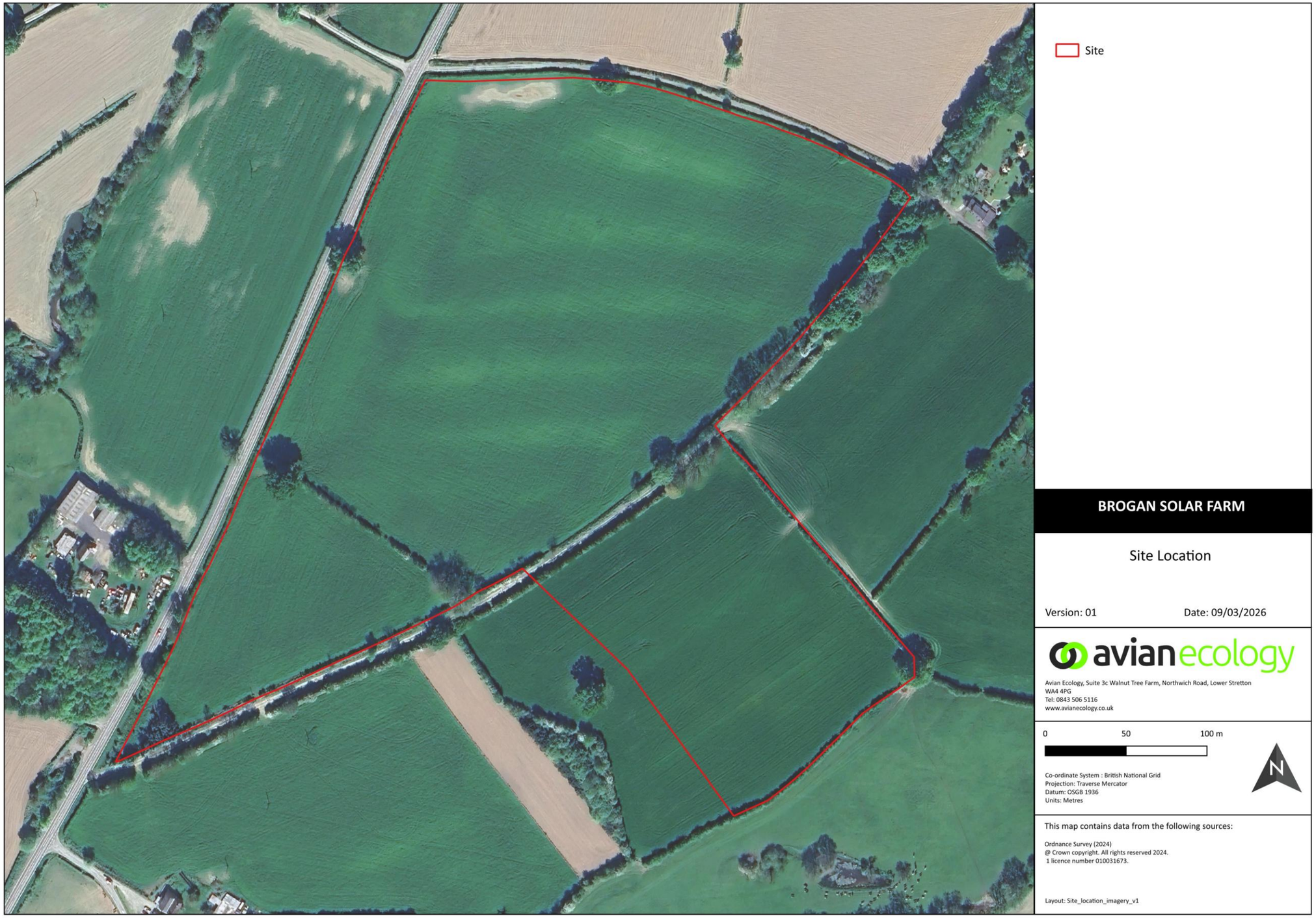
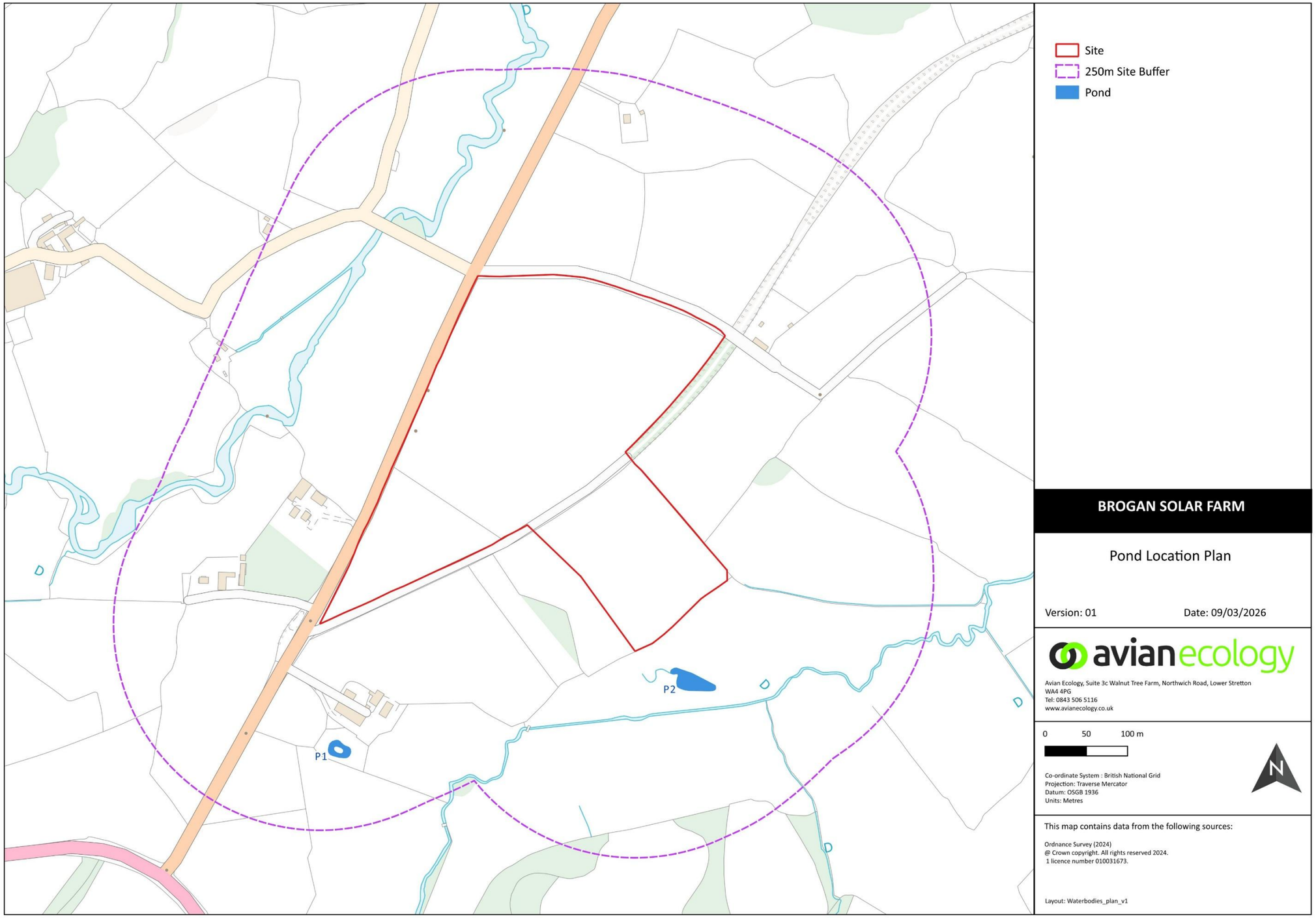


Figure 4.2: Pond Location Plan



Annex 4.1: Photographs

Photograph Reference	Photograph
Photograph 1: Pond P1	
Photograph 2: Pond P2	

Annex 4.2: SureScreen Scientifics - GCN eDNA Analysis Report

Folio No:	2917-2025
Purchase Order:	AE SS-25-022
Contact:	Avian Ecology Ltd
Issue Date:	09.07.2025
Received Date:	25.06.2025

GCN Report

Technical Report

SureScreen Scientifics

Folio No:	2917-2025
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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 5698	Bryngwyn - P1	SJ 17422 18547	Pass	Pass	Positive	12/12
GCN25 5699	Bryngwyn - P2	SJ 17852 18628	Pass	Pass	Positive	2/12

Matters affecting result: none

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SureScreen Scientifics

Methodology

The samples detailed above have been analyzed 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 tube which then undergoes DNA extraction. The extracted sample is then analyzed 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 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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