Candwr Solar Farm

Pre Application Consultation

Environmental Statement

Appendix 6.5 Great Crested Newt Survey Report

2 July 2026

TotalEnergies

Candwr Solar Farm

Appendix 6.5: Great Crested Newt Presence or Absence Survey Report

avianecology

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V1	11/09/2025	First issue for Client comment	A Crone BSc (Hons.)	B. Gray BSc (Hons.) MCIEEM
V2	09/10/2025	Amendments following Client comments	B. Gray BSc (Hons.) MCIEEM	B. Gray BSc (Hons.) MCIEEM
V3	05/06/2026	Amendments following changes to the Site boundary	B. Gray BSc (Hons.) MCIEEM ***Principal Ecologist***	J. Whittick BSc (Hons.) MCIEEM ***Managing Director***

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Annex 3: Avian Ecology (2023) Great Crested Newt Presence or Absence (eDNA) Survey Report

1 INTRODUCTION

1.1 Background

1.1.1 Avian Ecology Limited (AEL) was commissioned by Pegasus Planning Group to undertake habitat suitability index surveys (HSI) and environmental DNA (eDNA) presence/absence surveys for great crested newt (GCN) *Triturus cristatus* in relation to a proposed solar development (the 'Proposed Development') located within land north of Ponthir, Monmouthshire (hereafter referred to as the 'Site').

1.1.2 The purpose of this report is to present the results of the GCN desk study and field surveys undertaken in June 2025, the results of which will inform the Developments of National significance (DNS) submission, Environmental Impact Assessment (EIA), any required mitigation requirements and enhancement measures.

1.2 Site description

1.2.1 The Site is dominated by cattle and sheep grazed pasture within a rolling pastoral farmland landscape. Pockets of broadleaved woodland are connected via hawthorn *Crataegus monogyna* dominated hedgerows. Within the wider surrounding landscape, habitats include further agricultural fields and pockets of woodland. The Afon Lwyd flows between the pockets of land located within the Site, before flowing into the River Usk.

1.3 Previous survey work

1.3.1 In 2023 eDNA samples were taken on the 5th of June by a suitably experienced and licensed ecologist, Z Hinchcliffe *MRes* (2019-44230-CLS-CLS) assisted by S Viles *BSc (Hons.).* Four ponds were subject to HSI and eDNA. All ponds sampled returned negative results for the presence of GCN eDNA. The 2023 survey report is included as an annex (**Annex 3**) to this report.

1.4 Quality assurance

1.4.1 All surveys and assessments were undertaken by suitably experienced ecologists as per CIEEMs competency framework (CIEEM, 2021) 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).

1.4.2 As per the advice note from CIEEM (2019) *On the Lifespan of Ecological Reports & Surveys;* the findings presented in this report are valid for up to 18 months from the date of survey. Following this, the survey data should be reviewed and, if appropriate, updated to ensure any assessment and mitigation approach remains valid.

2 METHODOLOGY

2.1 Zone of influence

2.1.1 The current guidance on ecological impact assessments (EcIA) (CIEEM, 2018) recommends that all ecological features that occur within a 'zone of influence' (ZoI) for a proposed development are investigated. The ZoI includes:

- Areas directly within the land take for the Proposed Development and access;
- Areas which will be temporarily affected during construction;
- Areas likely to be impacted by hydrological disruption; and
- Areas where there is a risk of pollution and noise disturbance during construction and/or operation.

2.1.2 The ZoI is variable depending on the ecological receptors affected. The size of study areas are determined by considering the potential ZoI for each receptor.

2.1.3 Two separate areas have been defined for the purposes of this report. These include the 'Study Area', which describes the geographical extent subject to desk based study (2km) and the 'Survey Area', which describes the area of land subject to GCN field surveys (Site boundary plus 250m). The extent of the Study Area and the Survey Area were selected to ensure data was collected for the Site boundary and the surroundings that may support GCN and may reasonably be affected by the proposals.

2.2 Desk study

2.2.1 A desk study was undertaken in June 2025 in accordance with the current guidelines for Preliminary Ecological Appraisal (CIEEM, 2017) to determine the presence of any GCN records within the ZoI of the Site. Data was obtained from South-East Wales Biodiversity Centre (SEWBC) database and from survey work carried out in 2023.

2.3 Identification of waterbodies

2.3.1 OS mapping, aerial imagery and survey data from 2023 was utilised to identify waterbodies within the Survey Area which may have suitability for GCN.

2.3.2 A total of 26 ponds were identified either within the Site, or within 250m of the Site boundary. Four waterbodies are located within the Site boundary, these are ponds 10, 11, 12 and 14. A further three ponds are located on the Site boundary, these are ponds 4, 5 and 7. The remaining waterbodies (ponds 1, 2, 3, 6, 8, 9, 13, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 and 26) are all located within 250m of the Site boundary.

2.3.3 The four ponds within the Site boundary were all accessible for assessment, however, ponds 11 and 12 were considered to have been dry for a significant period, and Pond 14 was considered to no longer exist. Of the ponds located at the Site boundaries, or within 250m, access was available to ponds 5, 17, 24 and 26. Of these waterbodies pond 5 was found to be dry. Pond 25 was scoped out of the assessment as being unsuitable to support GCN due to being a large reservoir.

2.3.4 No access was available to ponds 1, 2, 3, 4, 6, 7, 8, 9, 13, 16, 18, 19, 20, 21, 22, 23 due to landowners' permission and the presence of a bull on arable land near P7.

2.3.5 A layout plan of the 26 identified ponds is shown in Figure 1.

2.4 Habitat suitability index

2.4.1 All accessible waterbodies (P5, P10, P11, P12, P17, P24 and P26) within the GCN Survey Area were assessed for their suitability to support GCN using the HSI assessment methodology. The HSI methodology followed the UK ARG's Advice Note 5 (ARGUK, 2010), which is an updated version of the original HSI methodology described in Oldham et al., (2000).

2.4.2 The HSI assessment is a standard approach adopted for assessing a pond's potential to support great crested newt. The HSI is a measure of habitat suitability and incorporates 10 indices (all of which are environmental variables), which are thought to affect great crested newt:

- Geographic location (SI1);
- Pond area (SI2);
- Pond permanence (SI3);
- Pond quality (SI4);
- Pond shading (SI5);
- Number of waterfowl (SI6);
- Occurrence of fish (SI7);
- Pond density (SI8);
- Proportion of suitable habitat in the Study Area (SI9); and,
- Macrophyte cover of the target pond (SI10).

2.4.3 The HSI score is the geometric mean of the 10 habitat variables listed above, obtained by multiplying each of the variable scores together, and then taking the 10th root of the product. The resulting HSI scores were cross-referenced with the National Amphibian and Reptile Recording Scheme (NARRS) (2008) guidance (as shown in Table 2.1) to estimate the suitability of each waterbody to support a breeding population of great crested newt

Table 2.1: HSI and Suitability for GCN

HSI Score	Suitability for GCN
0.00 – 0.49	Poor
0.50 – 0.59	Below Average
0.60 – 0.69	Average
0.70 – 0.79	Good
0.80 – 1.00	Excellent

2.4.4 The index produces a score of between 0 and 1 (in increments of 0.1), with 0 being unsuitable habitat and 1 representing optimal habitat. In general, waterbodies with high HSI scores (>0.6) are more likely

to support great crested newt than those with low scores (<0.5) but the method is not sufficiently precise to allow the conclusion that any particular waterbody with a high score will support newts, or that waterbody with a low score will not do so. However, the HSI score can be used as an aid in decision whether to undertake great crested newt presence/absence surveys on a waterbody.

2.4.5 Where waterbodies were visited but were recorded as dry a HSI survey was still undertaken. Environmental variables of the waterbody were assessed, and high waterline was used to determine size.

2.4.6 The HSI survey was undertaken alongside the environmental DNA (eDNA) survey conducted on 26th and 27th June 2025 by experienced ecologists A Littlechild MRes BSc (Hons.) and B Gray BSc (Hons.) MCIEEM.

2.5 eDNA

2.5.1 eDNA surveys were completed of all accessible ponds within the Survey Area which were holding water at the time of the assessment (P10, P17, P24 and P26). 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[1]). The technique for determining presence/absence of GCN uses Polymerase Chain Reaction (PCR) laboratory techniques to detect the species eDNA within water samples.

2.5.2 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).

2.5.3 Natural Resources Wales (NRW) 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). NRW will only accept eDNA survey results undertaken between 15th April and 30th June, in strict accordance with the published technical advice note, by suitably trained, experienced and licensed GCN surveyors[2].

Field sampling technique

2.5.4 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 waterbody, spaced as evenly as possible around the waterbody margin.

2.5.5 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.

[1] 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 A survey licence is not required to take the water samples, however, for mitigation licence applications NRW require evidence and confirmation that experienced, licensed GCN surveyor/s collected the water samples.

2.5.6 The ponds were sampled on 26th and 27th June 2025. Samples were collected by a suitably experienced and licensed ecologists, A Littlechild MRes BSc (Hons.) (2022-10636-CL08-GCN) and B Gray BSc (Hons) (2020-50776-CLS-CLS) MCIEEM[3].

Laboratory analysis

2.5.7 Laboratory analysis was undertaken by SureScreen Scientifics:

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Church Lane,
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2.5.8 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.

2.5.9 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.

2.5.10 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.

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

2.6 Limitations

2.6.1 Upon completion of the eDNA sampling undertaken and reported within this baseline report the Site boundary was amended to include additional land for a substation and haul road. However, no additional ponds fall within the Survey area, as a result of this change to the Site boundary. Therefore, this is not deemed to be a constraint.

2.6.2 Biological records obtained from third parties and presented in this desk study do not represent a full and complete species list for the area. They are mostly provided by individuals on an ad-hoc basis, meaning while they give a good representation there may be errors or deficiencies in the data.

2.6.3 Access to eight of the 26 ponds within the Survey Area was granted by landowners. This is not deemed to be a significant constraint when considering the negative results of eDNA surveys undertaken in 2023 and that during consultation with Ecology Officers from Monmouthshire, Newport and Torafen Local Planning Authorities it was advised that there was no knowledge of local GCN populations.

[3] Both surveyors hold Natural England class licences for great crested newt.

3 RESULTS

3.1 Desk study

3.1.1 No records of GCN were returned from the biological record data from within the Study Area, within the last 10 years. Additionally, no positive GCN results were returned from field surveys undertaken for the Proposed Development in 2023.

3.2 Habitat suitability index

3.2.1 Access was granted to eight (P5, P10, P11, P12, P14, P17, P24 and P26[4]) of the 26 ponds within the Survey Area. Pond 14 (P14) was deemed to no longer exist as its location was situated within a hedgerow and no water was recorded during the field survey. Therefore, seven ponds (P5, 10, 11, 12, 17, 24 and 26) were assessed for their suitability to support GCN, in line with the HSI methodology. Results of the HSI survey are detailed below in Table 3.1 and photographs of the ponds can be found in **Annex 1**.

[4] For pond locations refer to **Figure 1**.

Table 3.1: Habitat Suitability Index Assessment

Pond	Zone	Area	Drying	Water Quality	Shade	Waterfowl presence	Fish presence	Pond count	Terrestrial habitat	Macrophytes	HSI	GCN habitat suitability
P5	B	0.05	0.10	0.33	0.2	1.00	1.00	0.71	1.00	0.30	0.36	**Poor**
P10	B	0.80	0.10	0.33	1.00	1.00	1.00	0.83	1.00	0.35	0.57	**Below Average**
P11	B	0.98	0.10	0.33	1.00	1.00	1.00	0.83	1.00	0.3	0.58	**Below Average**
P12	B	0.95	0.10	0.33	1.00	1.00	1.00	0.83	1.00	0.60	0.62	**Average**
P17	B	0.10	0.5	1.00	0.7	1.00	1.00	0.55	0.67	0.80	0.59	**Below Average**
P24	B	0.05	1.00	0.01	1.00	0.01	1.00	0.83	0.67	0.30	0.22	**Poor**
P26	B	0.05	1.00	0.67	0.4	1.00	0.33	0.85	1.00	0.30	0.47	**Below Average**

3.3 eDNA

3.3.1 P5, P11 and P12 were dry at the time of survey, therefore, it was not possible to take water samples for eDNA analysis from these waterbodies.

3.3.2 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 2**.

Table 3.2: eDNA survey results.

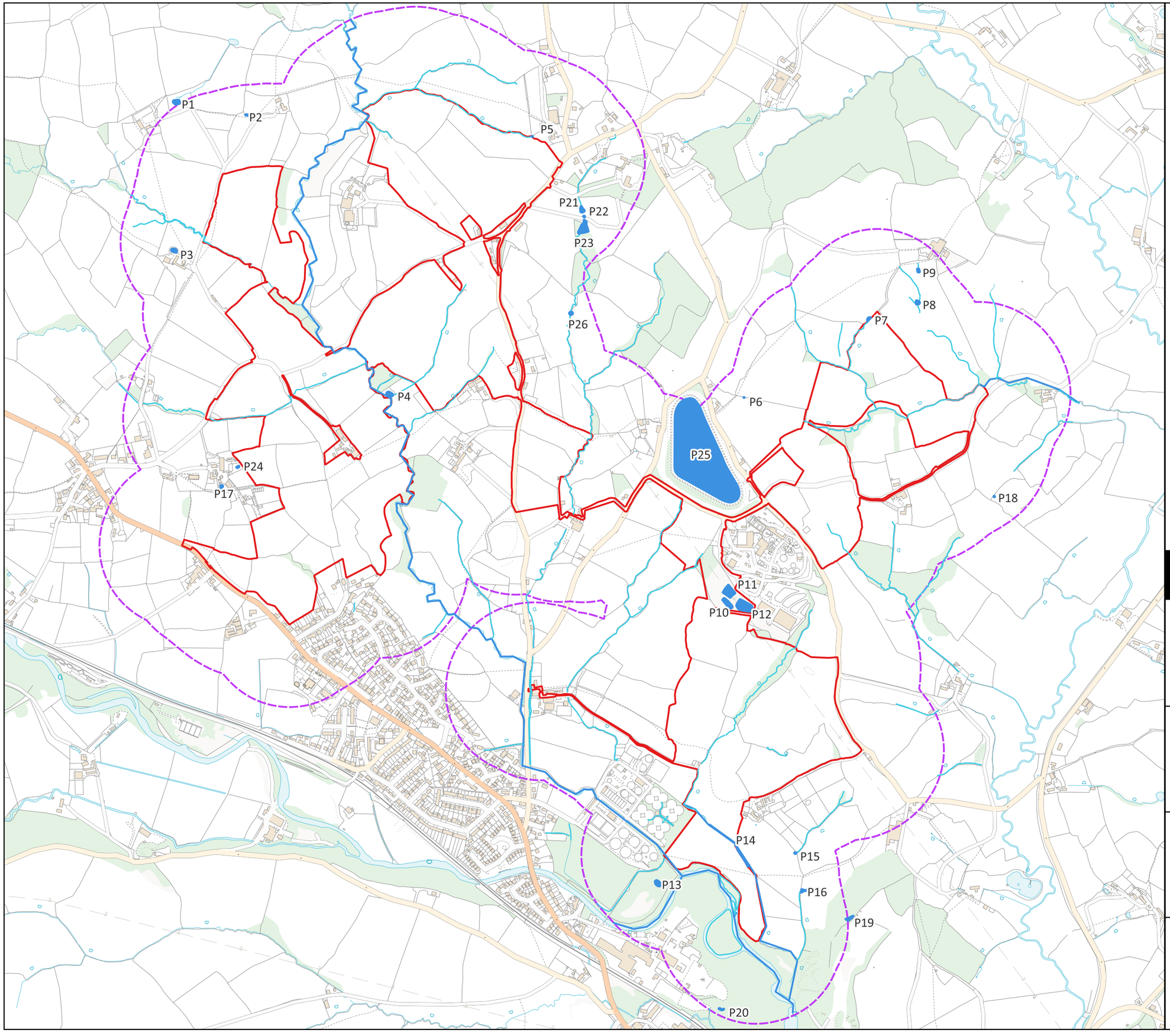
Pond	Lab ID.	Inhibition Check	Degradation Check	Result
P10	GCN259426	Pass	Pass	Negative 0/12
P17	GCN259423	Pass	Pass	Negative 0/12
P24	GCN259424	Pass	Pass	Negative 0/12
P26	GCN259425	Pass	Pass	Negative 0/12

4 CONCLUSIONS

4.1.1 The eDNA sampling and analysis returned a negative result for all four ponds containing water located within the Survey Area.

4.1.2 With all ponds affected from the Proposed Development not being sampled the presence of GCN on Site cannot be completely ruled out, however, in conjunction with the results of the 2023 surveys and absence of GCN recorded during these surveys, lack of historical records returned and no local knowledge of GCN populations from the local authorities GCN presence is discounted from further assessment. Precautionary measures in respect of amphibians will form part of the standard mitigation measures for the Proposed Development.

Figure 1: Waterbodies Location Plan



Site
250m Site Buffer
Ditch
Main river
Waterbodies

CANDWR SOLAR FARM

Waterbodies Plan

Version: 01

Date: 27/05/2026

avian ecology

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0 200 400 m

Co-ordinate System : British National Grid
Projection: Traverse Mercator
Datum: OSGB 1936
Units: Metres

N

This map contains data from the following sources:

Annex 1: Pond Photographs

Pond	Description
	**P5** This is no longer a waterbody it was previously a well that has been covered by the landowner and is now overgrown with nettles this area will no longer be suitable for GCN.
 	**P10** Small waterbody within the redline boundary and close to a hedgerow. With patches of willow growing in the middle of the waterbody and some parts of the bank. With the bank being dominated by brambles grasses and other areas of willow.
	**P11** This waterbody was dry at the time of the survey located to the north of pond 10 and within the red line boundary. This waterbody returned a negative result and a below average HSI score. The area is now dominated by grasses and common arable weeds.

	**P12** This waterbody is located to the northeast of pond 10. No samples were taken at the time of the survey as this pond was dry. With willow growing within the centre of what would have been the waterbody and the banks being dominated by brambles grasses willow herb and other arable weeds.
 	**P17** This small waterbody is located 85m west from the red line boundary. This waterbody retuned a negative eDNA result and a below average HSI result. With several aquatic flowing plants present within the waterbody.
	**P24** This waterbody is located 10m west of the redline boundary and northeast of P17. A small oval shaped pond that its main use is a duck pond in the animal sanctuary All Creatures Great and Small.

P26 (including photograph of stream running into it)

This waterbody is located 200m east of the redline boundary within a patch of woodland that borders arable land. This pond/pool is stream fed (pictured below left) with a dam being built to create the body of water. This waterbody returned a negative result from GCN and a below average HSI score.

Annex 2: eDNA Laboratory Results

Folio No: 3593-2025
Purchase Order: AE SS-25-029
Contact: Avian Ecology Ltd
Issue Date: 18.07.2025
Received Date: 03.07.2025

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 9423	Court Farm - P17	ST 32111 93590	Pass	Pass	Negative	0/12
GCN25 9424	Court Farm - P24	ST 3217 93659	Pass	Pass	Negative	0/12
GCN25 9425	Court Farm - P26	ST 33184 94135	Pass	Pass	Negative	0/12
GCN25 9426	Court Farm - P10	ST 33653 93246	Pass	Pass	Negative	0/12

Matters affecting result: none

Reported by: Amy Bermudez

Approved by: Consuela Sopronyi

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Received Date:	03.07.2025

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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Annex 3: Avian Ecology (2023) Great Crested Newt Presence or Absence (eDNA) Survey Report

Court Farm Solar DNS

on behalf of Pegasus Planning Group

Great Crested Newt Presence or Absence (eDNA) Survey Report

avianecology

Document Control				
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Figure 1: Pond Survey Plan

ANNEXES

Annex 1: e-DNA Laboratory Results

1 INTRODUCTION

1.1 Background

1.1.1 Avian Ecology Ltd. was commissioned by Pegasus Planning Group to undertake a great crested newt (GCN) *Triturus cristatus* environmental DNA (eDNA) presence/absence survey. The survey was undertaken in relation to a proposed solar development (hereafter referred to as the 'Site') located within land north of Ponthir, Newport, Monmouthshire.

1.1.2 This report subsequently provides detailed survey methodology and results.

1.2 Site Description

1.2.1 The Site is dominated by cattle and sheep grazed pasture within a rolling arable farmland landscape. Pockets of broadleaved woodland are connected via hawthorn *Crataegus monogyna* dominated hedgerows.

1.2.2 Within the wider surrounding landscape, habitats include further pastoral fields and pockets of woodland. The Afon Lwyd flows between the pockets of land located within the Site, before flowing into the River Usk.

2 METHODOLOGY

2.1.1 No waterbodies are present within or immediately adjacent to the Site. A total of 16 ponds were identified on aerial imagery and using OS maps within 250m of the Site boundary. Access to all 16 ponds was possible during the visit. At the time of survey, Ponds 2, 5, 6, 7 and 16 were considered either no longer be ponds or have been dry for a significant period of time.

2.1.2 A layout plan of the 16 identified ponds is shown in **Figure 1**.

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'.

2.2.3 The HSI survey was undertaken alongside the environmental DNA (eDNA) survey conducted on 5th June 2023 by experienced ecologist Z Hinchcliffe MRes BSc (Hons.). Assessments were carried out on four (P1, 3, 9 and 13) of the 16 ponds that were considered wet at the time of survey.

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[1]). 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 Lagoons were sampled on 5th June 2023. Samples were collected by a suitable experienced and licensed ecologist, Z Hinchcliffe *MRes* (2019-44230-CLS-CLS) and S Viles *BSc (Hons.)*.

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 waterbody, spaced as evenly as possible around the waterbody 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
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

[1] 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.

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 Habitat Suitability Index

3.1.1 Four of the 16 visited ponds were wet and HSI was possible. These four ponds (P1, 3, 9 and 13) were assessed for their suitability for GCN during the survey. Within the Survey Area, Ponds P1 and P3 were assessed as Average (0.65 and 0.68 HSI score) with the other two ponds (9 and 13) within the Survey Area assessed as Poor (<0.5).

3.1.2 HSI results are presented in **Table 3.1**.

Table 3.1: Habitat Suitability Index Assessment

Pond	Zone	Area (m^2)	Drying	Water Quality	Shade	Fowl present?	Fish present?	Pond count	Terrestrial habitat	Macrophytes	HSI	GCN habitat suitability
P1	B	110	Rarely	Moderate	10%	Absent	Possible	7	Poor	70	0.65	**Average**
P3	B	270	Never	Good	40%	Minor	Possible	7	Poor	50	0.68	**Average**
P9	B	90	Annually	Bad	0%	Absent	Absent	9	Poor	10	0.38	**Poor**
P13	B	700	Sometimes	Poor	100%	Minor	Absent	6	Moderate	0	0.45	**Poor**

3.2 eDNA

3.2.1 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
P1	4869	Pass	Pass	Pass	Negative 0/12
P3	4867	Pass	Pass	Pass	Negative 0/12
P9	4868	Pass	Pass	Pass	Negative 0/12
P13	4870	Pass	Pass	Pass	Negative 0/12

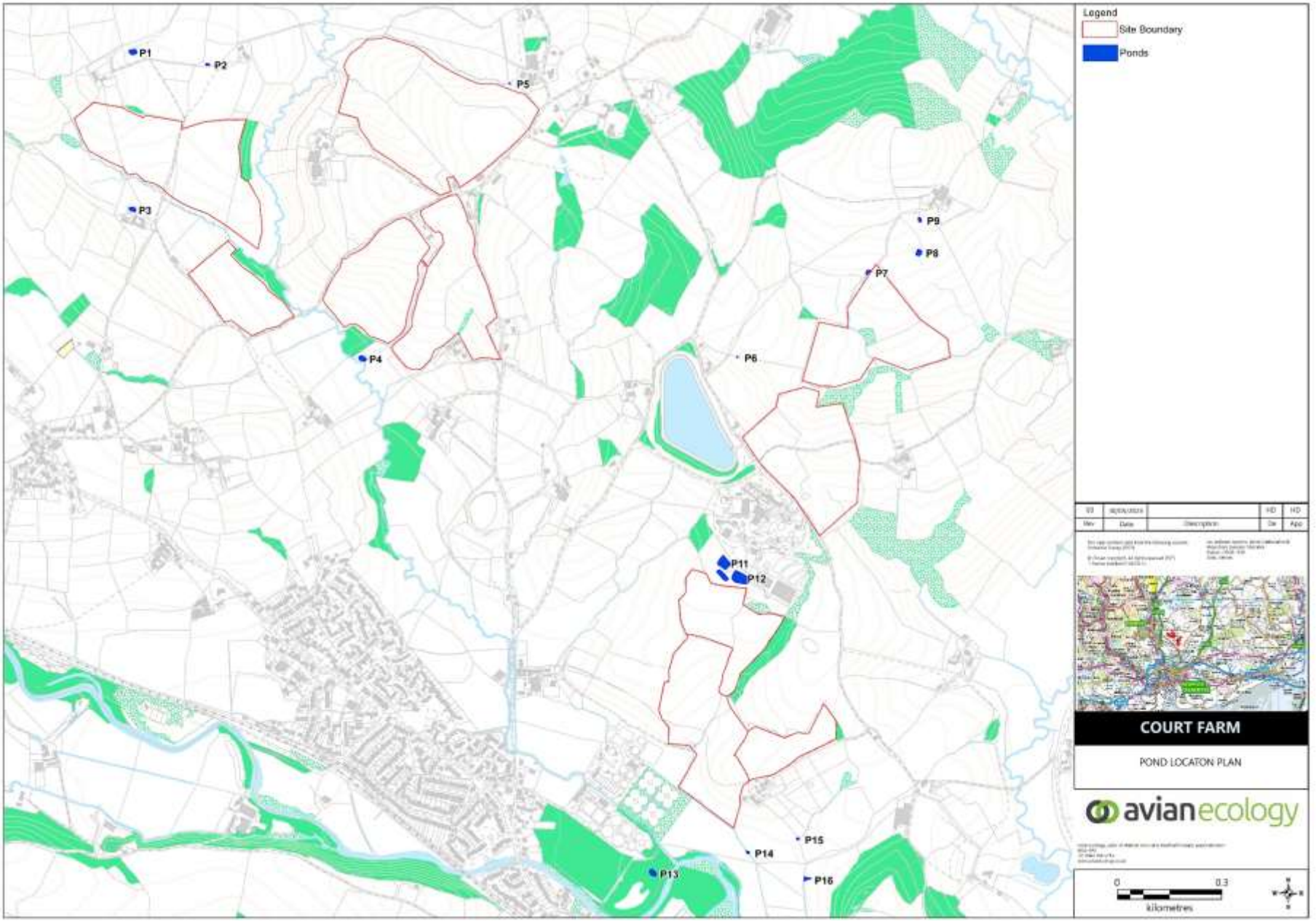
4 CONCLUSIONS

4.1.1 The eDNA sampling and analysis returned a negative result for all for wet ponds located within 250m of the Site boundary.

4.1.2 To generate the e-DNA results, all of the samples from each waterbody 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 waterbody 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.

Figure 1:

Pond Survey Plan


Legend
Site Boundary
Ponds
P1
P2
P3
P4
P5
P6
P7
P8
P9
P11
P12
P13
P14
P15
P16
COURT FARM
POND LOCATON PLAN
avianecology
0
0.3
kilometres

Annex 1 – e-DNA Laboratory Results

SureScreen Scientifics

Folio No: E17977
Report No: 1
Purchase Order: AESS-23-037
Client: AVIAN ECOLOGY LTD
Contact: Dan Foy

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: 13/06/2023
Date Reported: 21/06/2023
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
4867	Court Farm - Pond 3	ST 31978 94315	Pass	Pass	Pass	Negative	0
4868	Court Farm - Pond 9	ST 34236 94252	Pass	Pass	Pass	Negative	0
4869	Court Farm - Pond 1	ST 31984 94767	Pass	Pass	Pass	Negative	0
4870	Court Farm - Pond 13	ST 33421 92389	Pass	Pass	Pass	Negative	0

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

Reported by: Jennifer Higginbottom **Approved by:** Jackson Young

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.