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Client Name: **GA Petfoods Ltd**

Site Name: **Asland Walks Energy Park**

Project Ref: **GAPet-794-3213**

Date: **10th August 2026**

Great Crested Newt Presence or Likely Absence eDNA Survey Report

Background

Avian Ecology Ltd. was commissioned by GA Petfoods Ltd. to undertake Great Crested Newt (GCN) *Triturus cristatus* presence/absence surveys adopting the environmental DNA (eDNA) sampling methodology.

The survey was in relation to the proposed installation of a solar and wind energy park with associated infrastructure ('the Main Site'), plus two required cable routes (the 'Cable Routes'), all together the 'Site' as shown in **Figure 1.**

This report subsequently provides detailed survey methodology and results, with reference to the 2025 Ecological Assessment Report[1] (EAR) produced to support the solar and wind energy park development.

Desk Study Results

The desk study[1] undertaken within 2km of Site returned 33 records of GCN. No records were returned within the Site itself, with the closest comprising two GCN records approximately 210 m east of one of the proposed Cable Routes from 2021.

Two Natural England GCN licence applications were granted within 2 km of the Site. These licence applications (case reference: EPSM2013-6287 and EPSM2010-2283) were identified in two locations 60 m north and 1.42 km south-east of the Site's proposed North-East Cable Route respectively. The licences permitted the destruction of GCN resting locations between 2013 to 2020 and between 2010 and 2011 respectively.

Four locations of GCN class survey licence returns were also identified in the wider 2 km Site buffer area. One related to a ditch surveyed in 2017 (located 375 m north-west of the Site's proposed North-East Cable Route) which all recorded positive GCN results. The three other locations were located in a cluster of ponds approximately 1.82 km north-west of the Site's proposed North Cable Route. Each comprised two surveys during 2017, which all confirmed GCN presence.

Three ponds within 2 km of the Site were subject to Natural England pond surveys between 2017 and 2019. Of these, one was recorded with GCN presence approximately 1.25 km north of the Site's proposed North-East Cable Route.

[1]Avian Ecology, 2025. Ecological Assessment Report- Asland Walk Energy Park.

Survey Area

Ponds were identified from aerial images, Ordnance Survey (OS) maps and Site visits. No ponds are located on Site, although four are located within 250 m of the Main Site and five were located within 50 m of the two Cable Routes, as shown on **Figure 1**.

Due to the low impact of solar energy, single wind turbine and cable route developments on GCN habitats, and reflecting guidance published by Natural England, ponds beyond 250 m from the Main Site and 50 m from the Cable Routes were not considered.

Ponds 1-4 are segregated from the Site by major watercourse carriers and as such not considered further. Through a review of recent aerial imagery and following client confirmation, it was also confirmed that Pond 5 is no longer present. As such, surveys were conducted on remaining ponds associated with the Cable Routes: Ponds 6–9.

Methodology

Ponds 6, 7 and 9 were accessed on 15th June 2026, whilst Pond 8 was accessed on 24th June 2026. All were subject to both Habitat Suitability Index (HSI) assessments and eDNA survey sampling to determine the presence or likely absence of GCN.

HSI

Ponds that were accessed during the survey were assessed for their suitability to support great crested newt using the HSI Assessment methodology as developed by Oldham *et al.* (2000[2]) and as detailed within ARG UK guidance (ARG UK, 2010[3]).

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.

Final scores relate to pond suitability for great crested newt and range from 'poor' to 'excellent', with those scoring average or above more likely to support GCN.

eDNA

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[4]). The technique for determining presence/absence of GCN uses Polymerase Chain Reaction (PCR) laboratory techniques to detect the species eDNA within water samples.

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

[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.
[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.

for determining whether GCN are present or absent during the breeding season, even where eDNA is present in very low concentrations (Biggs *et al.,* 2014).

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

The eDNA surveys were undertaken on 15th and 24th June 2026 in suitable weather conditions by a suitably experienced and licensed GCN surveyors. This is within the time period accepted by Natural England.

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 30ml subsamples from each pond, spaced as evenly as possible around the pond margin.

Each sample was then placed within a sample 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

Laboratory analysis was undertaken by SureScreen Scientifics, an approved laboratory for eDNA testing.

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.

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.

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.

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

Limitations

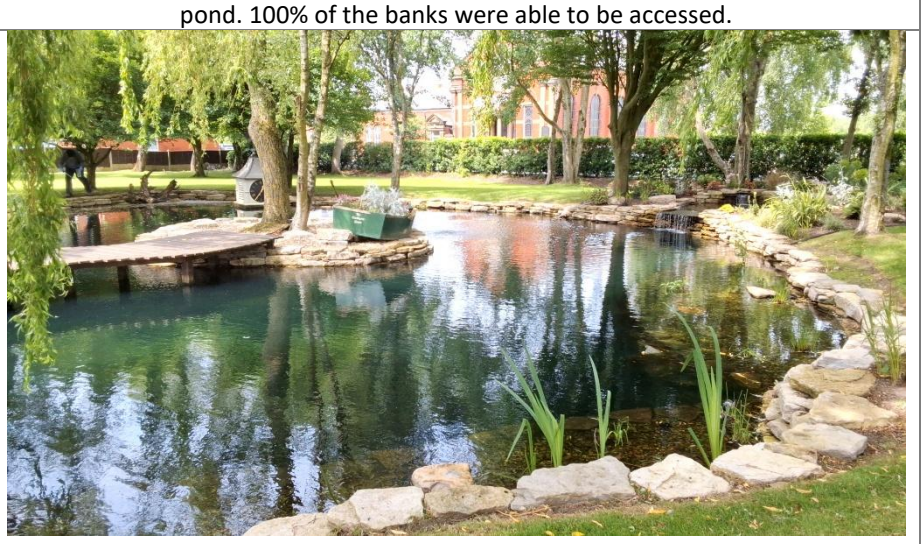
Only approximately 50% of the pond edges were accessible to take water samples for Pond 7 due to the presence of woodland/scrub around the pond margins. This is not considered a significant constraint as water samples were still successfully taken and analysed.

Results

Photographs and brief descriptions of the ponds surveyed are provided in **Table 1** below, with locations shown in **Figure 1.**

Table 1: Pond Information

Pond Ref.	Approximate Location in relation to Cable Routes	Photograph and description
P6	5 m south	 Natural appearing pond within area of willow/ oak woodland, drying out at the time of survey. Banks surrounded by nettle. 100% of the banks were able to be accessed.
P7	17 m west	 Established residential pond with rocky banks and enclosed by mature trees on the northern aspect. Only *c*. 50% of the banks were able to be accessed due to scrub and tree presence limiting access.

P8	35 m east	 Ornamental garden pond, surrounded largely by short mown grassland but a hedgerow feature close to the southern aspect. Varied depth with good quality, clear water and invertebrates present, however fish (koi carp) are also kept in the pond. 100% of the banks were able to be accessed.
P9	15 m south	 Large ornamental residential pond surrounded by short mown grassland and mature trees. Ducks present using the waterbody. 100% of the banks were able to be accessed.

<u>HSI</u>

HSI results for all ponds surveyed are shown in **Table 2** below.

Pond 8 had a score <0.50, which indicates the waterbody has 'poor' habitat suitability for GCN, Pond 6 and Pond 7 had a score between 0.50-0.59, which indicates these waterbodies have 'below average' habitat suitability for GCN, whilst Pond 9 had a HSI score of >0.6 indicating 'average' habitat suitability.

Table 2: HSI survey results

Pond No.	Location	Area (m2)	Pond Permeance	Water Quality	Shade (%)	Waterfowl	Fish	Pond Density	Terrestrial Habitat Quality	Macro Cover	Final HSI Score	Suitability
P6	1	0.4	0.5	0.33	0.2	1	1	1	0.67	0.3	0.55	**Below Average**
P7	1	0.6	0.9	1	0.9	0.67	0.01	1	1	0.3	0.50	**Below Average**
P8	1	0.2	0.9	0.67	1	1	0.01	1	0.67	0.3	0.43	**Poor**
P9	1	0.6	0.9	0.67	0.6	0.67	0.67	1	0.33	0.45	0.65	**Average**

eDNA

A summary of the eDNA survey results is shown below in **Table 3**, with the original laboratory reports reproduced in **Annex 1 and Annex 2**.

All surveyed ponds returned a 'negative' result for the presence of GCN.

Table 3: eDNA survey results

Pond	Sample Ref.	Degradation Check	Inhibition Check	Result
P6	GCN26 07601	Pass	Pass	Negative 0/12
P7	GCN26 07603	Pass	Pass	Negative 0/12
P8	GCN26 07605	Pass	Pass	Negative 0/12
P9	GCN26 03316	Pass	Pass	Negative 0/12

Conclusion

A total of four ponds identified within a 50m buffer of the Cable Routes were surveyed.

Pond 8 retuned a 'poor' HSI habitat suitability score for GCN, Pond 6 and Pond 7 'below average' and Pond 9 'average'.

The eDNA sampling and analysis returned a negative result for all four ponds, indicating that GCN are likely absent from these waterbodies.

The desk study however revealed numerous GCN results within 2km of Site, the closest of which was c. 210 m east of the Cable Routes, which indicates that GCN are locally present.

The habitats present within the Cable Routes have mixed suitability for GCN in their terrestrial phase, with arable land and existing roads of negligible value but higher value habitats such as hedgerows and longer grassland present in small areas within the working area.

The vast majority of GCN suitable habitat is to be retained, with cable works largely restricted to existing roads, road margins and tracks. However, as GCN are known to be in the wider area, a precautionary approach should be taken to Site works.

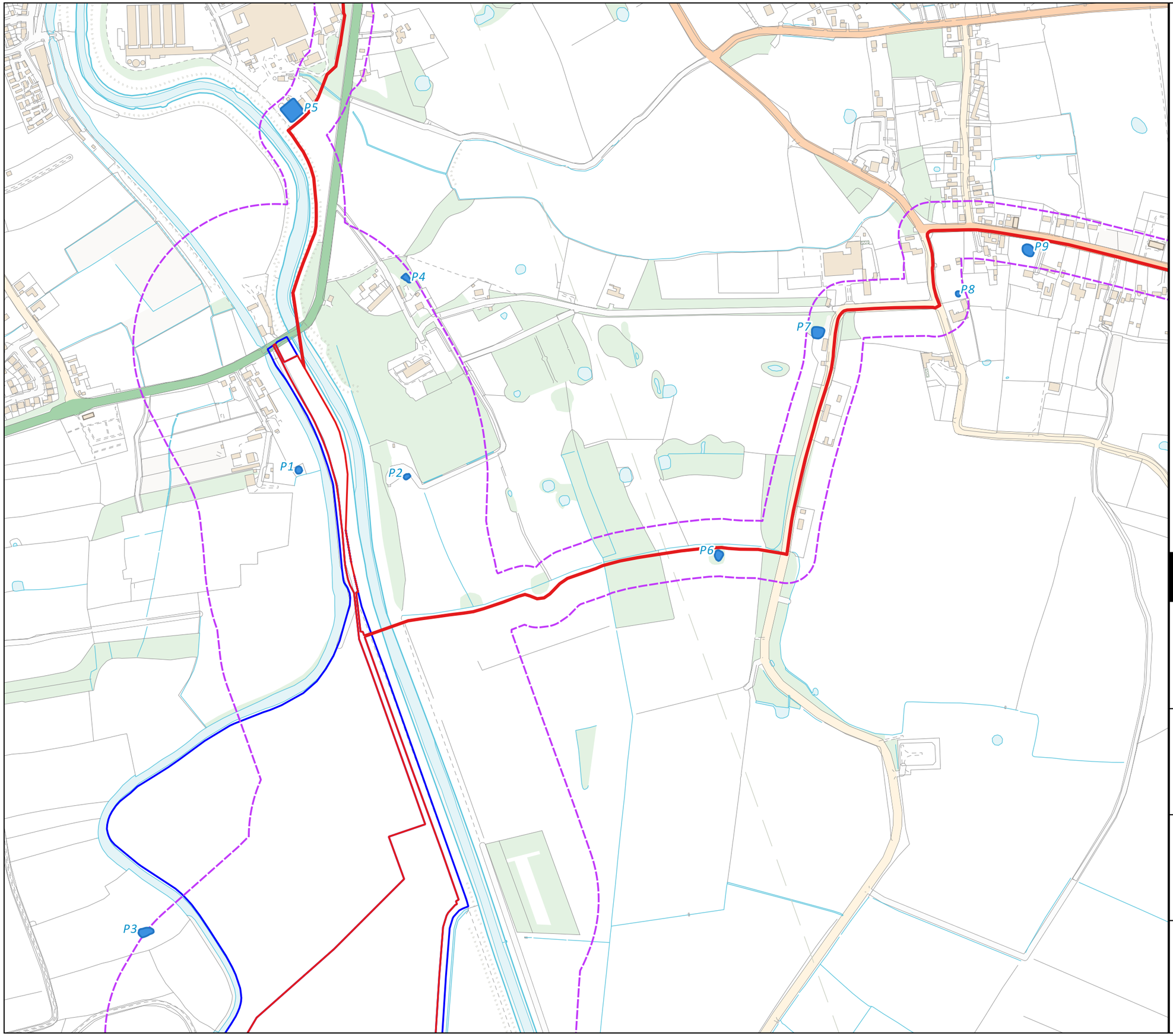
No further survey work is considered necessary but, as already recommended within the EAR, Reasonable Avoidance Measures (RAM's) should be implemented, to safeguard any GCN in the event they are present on site. Please refer to the EAR for further details.

Should GCN be found on Site during works, all works must stop immediately and the Site ecologist be consulted. A license to continue works will then likely be required.

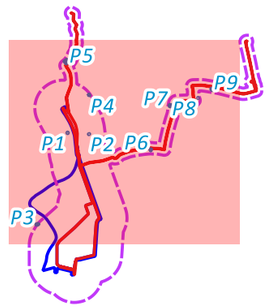
Prepared by: A. Littlechild *BSc (Hons)*

Reviewed by: T Goater *MSc, BSC (Hons) MCIEEM*

Figure 1: Pond Plan



Site
Land Ownership Area
250m Site and 50m Cable Route Buffer
Pond



ASLAND WALKS

Pond Location Plan

Version: 01

Date: 04/06/2026

avian ecology

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0 50 100 m

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

N

This map contains data from the following sources:

Appendix 1: GCN eDNA Results- Ponds 6, 7 and 9

Folio No:	2609-2026
Purchase Order:	AEPO25422
Contact:	Avian Ecology Ltd
Issue Date:	30.06.2026
Received Date:	16.06.2026

GCN Report

Technical Report

SureScreen Scientifics

Folio No: 2609-2026
Purchase Order: AEPO25422
Contact: Avian Ecology Ltd
Issue Date: 30.06.2026
Received Date: 16.06.2026

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
GCN26 03316	Asland Walks - Pond 9		Pass	Pass	Negative	0/12
GCN26 07601	Asland Walks - Pond 6		Pass	Pass	Negative	0/12
GCN26 07603	Asland Walks - Pond 7		Pass	Pass	Negative	0/12

Matters affecting result: none

Reported by: Ruby Fielding

Approved by: Jennifer Higginbottom

Folio No:	2609-2026
Purchase Order:	AEPO25422
Contact:	Avian Ecology Ltd
Issue Date:	30.06.2026
Received Date:	16.06.2026

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.

Appendix 2: GCN eDNA Results- Pond 8

Folio No:	3315-2026
Purchase Order:	AEPO-25-451
Contact:	Avian Ecology Ltd
Issue Date:	14.07.2026
Received Date:	30.06.2026

GCN Report

Technical Report

SureScreen Scientifics

Folio No: 3315-2026
Purchase Order: AEPO-25-451
Contact: Avian Ecology Ltd
Issue Date: 14.07.2026
Received Date: 30.06.2026

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
GCN26 07605	Aslad Walk P8	SD47226 20348	Pass	Pass	Negative	0/12

Matters affecting result: none

Reported by: Chelsea Warner

Approved by: Vanessa Hind

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.