EAST PARK ENERGY

East Park Energy

EN010141

Environmental Statement
Volume 2 – Technical Appendices

Appendix 7-5: Great Crested Newt Presence or Absence (eDNA) Survey Report

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Volume 2 – Technical Appendices

Appendix 7-5: Great Crested Newt Presence or Absence (eDNA) Survey Report

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1.0 INTRODUCTION

1.1 Background

1.1.1 This appendix has been prepared to accompany **ES Vol 1 Chapter 7: Ecology and Nature Conservation [EN010141/DR/6.1]** of the Environmental Statement (ES) for the East Park Energy project (the 'Scheme').

1.1.2 The report presents survey methodology and results of great crested newt (GCN) *Triturus cristatus* environmental DNA (eDNA) surveys undertaken in relation to the Scheme.

1.2 Survey Area

1.2.1 Ponds located on or within 250m of the Site were identified from aerial images and Ordnance Survey (OS) maps. Due to the low impact of solar energy developments on GCN habitats, and reflecting guidance published by Natural England[1], ponds beyond 250m from the Site were not considered.

1.2.2 The boundary of the Site has changed since surveys were initially undertaken in 2022. The Site boundary, 250m buffer from the Site and the ponds identified are shown on **ES Vol 3 Figure 7-7 [EN010141/DR/6.3].** Ponds outside 250m of the Site, that may have previously been within 250m of the Site, prior to the change of boundary, have not been considered for GCN; however, where survey data exists, they are included within this report for contextual information.

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

2.0 METHODOLOGY

2.1.1 GCN surveys were undertaken in 2022 and updated in 2025, due to the age of data and changes to the Site boundary.

2.1.2 In 2022 13 ponds were accessed comprising two ponds within the Site, eight ponds within 250m of the Site and three ponds in >250m of the Site.

2.1.3 In 2025, a total of three ponds were identified within the Site boundary, a further 22 ponds were identified within 250m of the Site from OS and aerial mapping.

2.1.4 Of the 25 ponds identified within the survey area in 2025 13 ponds were able to be accessed, comprising three ponds within the Site and ten ponds within 250m of the Site boundary.

2.1.5 A list of all ponds identified within the Site and 250m of the Site is provided within Table 2.1, ponds listed in **bold** were assessed for the survey in either, or both, 2022 and 2025.

2.1.6 Table 2.1 also includes details of ponds that were accessed as part of surveys in 2022 but may now fall outside of the study area.

Table 2.1: Summary of Pond Access

Pond Reference	Access Status		Distance Band from Scheme
	2022	2025	
P3	No access	No access	<250m of the Site
P4	No access	No access	<250m of the Site
P5	No access	No access	<250m of the Site
P6	**Granted**	**Granted**	**<250m of the Site**
P7	No access	No access	<250m of the Site
P8	**Granted**	**No access**	**<250m of the Site**
P9	**Granted**	**Granted**	**<250m of the Site**
P10	No access	No access	<250m of the Site
P11	No access	No access	>250m of the Site
P12	No access	No access	>250m of the Site
P13	**Granted**	**Granted**	**<250m of the Site**

P14	**No access**	**Granted**	**Within the Site**
P17	**Granted**	**No access**	**<250m of the Site**
P19	**Granted**	**No access**	**>250 of the Site**
P20	No access	No access	<250m of the Site
P24	No access	No access	<250m of the Site
P25	**Granted**	**Granted**	**Within the Site**
P28	No access	No access	<250m of the Site
P29	Granted	No access	<250m of the Site
P30	**Granted**	**No access**	**>250 of the Site**
P30a	**Granted**	**No access**	**>250 of the Site**
P31	**Granted**	**Granted**	**Within the Site**
P38	**Granted**	**Granted**	**<250m of the Site**
P39	**Granted**	**Granted**	**<250m of the Site**
P40	No access	No access	<250m of the Site
P41	**No access**	**Granted**	**<250m of the Site**
P42	**No access**	**Granted**	**<250m of the Site**
P43	**No access**	**Granted**	**<250m of the Site**
P44	**No access**	**Granted**	**<250m of the Site**
P45	**No access**	**Granted**	**<250m of the Site**

2.1.7 A total of 13 ponds; P6, P8, P9, P13, P17, P19, P25, P29, P30, P30a, P31, P38 and P39 were accessed during surveys in 2022.

2.1.8 A total of 13 ponds; P6, P9, P13, P14, P25, P31, P38, P39, P41, P42, P43, P44 and P45 were accessed during surveys in 2025 (locations of ponds are shown on **ES Vol 3 Figure 7-7 [EN010141/DR/6.3]**).

2.1.9 During surveys in 2022 and 2025, all ponds accessed were assessed for their suitability to support GCN using the Habitat Suitability Index (HSI) Assessment methodology as developed by Oldham et al. (2000[2]) and as detailed within ARG UK guidance (ARG UK, 2010[3]).

2.1.10 Where the methodology was appropriate (e.g., sufficient access was present and water levels were sufficiently deep), these ponds were also subject to eDNA survey sampling to determine the presence or likely absence of GCN.

[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.*

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 The resulting Habitat Suitability Score is a number between 0.01 and 1; final scores relate to pond suitability for GCN and range from 'poor' to 'excellent'.

2.2.3 The scoring system to define pond suitability is:

- < 0.5 = Poor;
- 0.5 – 0.59 = Below Average;
- 0.6 – 0.69 = Average;
- 0.7 – 0.79 = Good; and
- > 0.8 = Excellent.

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

[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.*

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 GCN 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 likely 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 All ponds, in both 2022 and 2025, were sampled within the specified GCN eDNA survey window (mid-April – 30th June). All samples were collected by GCN licenced surveyors who were accompanied by an additional person for health and safety.

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 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
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 all replicates are negative. 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 sterile environment and the different phases of testing are kept separate to reduce any risk of cross contamination.

2.4 Limitations

2.4.1 Not all ponds located within 250m of the Site could be surveyed due to access constraints.

2.4.2 Ponds P8, P17, P19, P30 and P30a were surveyed in 2022 but access constraints prevented re-survey in 2025.

2.4.3 Some ponds surveyed in 2022 are now outside of the 250m buffer of the Site and so, outside of the study area; results of these ponds have been included for contextual information.

3.0 RESULTS

3.1.1 A description of ponds surveyed and their HSI score is presented in Table 3.1.

3.1.2 Detailed HSI results are present in Table 3.2, and eDNA survey results in Table 3.3.

3.2 HSI

2022

Table 3:1 Pond Information 2022

Pond Reference	Location from Site	Description & Photograph	HSI Score
P6	<250m of the Site	This pond is located in a woodland adjacent to the boundary of East Park Site A. This pond was dry at the time of the survey.	Dry
P8	<250m of the Site	This pond is located east of East Park Site A. This is a rectangular sized pond fed by a drain. With willow *Salix sp* trees on one bankside and mown grassland to the other. Marginal and bankside vegetation consisted of willow herb *Epilobium sp*, and reeds *Phragmites sp*.	Excellent
P9	<250m of the Site	This pond is located south of East Park Site C. This pond was located down a steep bank and was unable to be safely accessed.	Below Average
P13	<250m of the Site	This pond is located within an area of land surrounded by East Park Site A. This large figure of eight shaped pond surrounded by thistle *Cirsium sp*, common hogweed *Heracleum sphondylium*, scattered bramble *Rubus fruticosus agg.*, reeds, yellow flag iris	Excellent

		Iris pseudacorus, teasel *Dipsacus sp.* and a number of oak *Quercus sp* and hawthorn *Crataegus monogyna* trees. 	
P17	<250m of the Site	This pond is located to the south of East Park Site B. This is a small pond located within a woodland coppice of hawthorn, oak and willow. One bank side was fully covered by bramble *Rubus sp* with the other bare ground. 	Below Average
P19	>250m of the Site	This pond is located in a woodland adjacent to the south of East Park Site C. This is a medium sized pond located within a mixed woodland glade. The bankside vegetation consisted of long grasses, sedges, limited scattered scrub, reeds, water mint *metha aquatica*, mares tail *Hippuris vulgaris* and common reed *Phragmites australis*. A film of oil was present on the water at the time of the survey.	Excellent

P25	Within the Site	This pond is located within East Park Site A and was found to be dry at the time of the survey.	Dry
P29	<250m of the Site	This pond is located north of East Park Site B. This is a small pond covered in lily pads within a grassland with scattered trees. The bankside vegetation consists of willow herb, comfrey *Symphytum sp*, nettles *Urtica dioica*, canary reed grass *Phalaris arundinacea*, water mint, bindweed *Convolvulus* and common reed and a grassland margin extending approximately 10m from the bankside.	Good
P30	>250m of the Site	This pond is located within a farmyard to the south of East Park Site C. This is a small pond fully covered in pond weed and with bankside vegetation limited to willow herb. Half the bank was covered in trees including willow and hawthorn.	Below Average

P30a	>250m of the Site	This pond is located within a farmyard to the south of East Park Site C. This is a medium sized rectangular pond fed by a drain. The bankside vegetation consisted of willow herb, bindweed, thistle and mallow *Malva sp.* with a number of trees present including ash *Fraxinus excelsior* and oak. 	Average
P31	Within the Site	This pond is located within the northeastern extent of East Park Site B. This pond was located within a woodland coppice of willow and dense hawthorn limiting access to the pond edge. The marginal and bankside vegetation consisted of bullrush, willow herb, water mint, pendulous sedge *Carex pendula* , thistle, reeds and a long grasses.	Excellent

P38	<250m of the Site	This pond is located to the east of East Park Site A. This is a large pond with water being pumped into it and a brook running adjacent to it. The bankside vegetation was dominated by comfrey and long grasses and a number of willow trees. 	Excellent
P39	Within the Site	This pond is located within a small woodland adjacent to the Site boundary of East Park Site A. This is a small pond at the end of a ditch. The bank was sparsely vegetated limited to grasses, mint and willow herb.	Poor

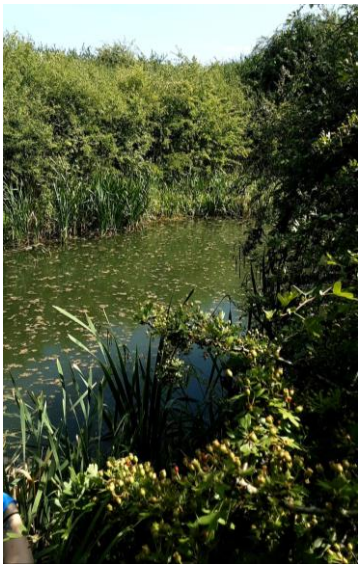
Table 3.2: HSI survey results 2022.

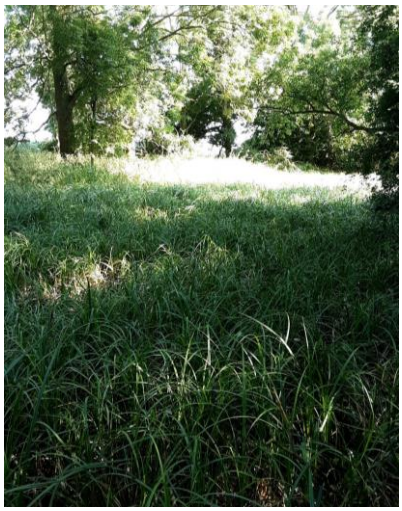
Suitability Indices	P8	P9	P13	P17	P19	P29	P30	P30a	P31	P38	P39
SI1 – Location	1	1	1	1	1	1	1	1	1	1	1
SI2 – Pond area	0.7	0.1	0.7	0.1	0.6	0.2	0.2	0.3	0.4	1	0.05
SI3 – Pond drying	0.9	0.5	1	1	0.9	1	1	0.9	0.9	0.9	0.5
SI4 – Water quality	1	0.33	1	0.33	0.67	1	0.33	0.9	0.9	0.9	0.33
SI5 –Shade	1	1	1	0.4	1	1	0.6	0.6	1	1	0.2
SI6 – Fowl	1	1	1	1	1	1	0.67	1	0.67	1	1
SI7 – Fish	1	1	1	1	1	1	1	1	1	1	1
SI8 – Ponds	0.95	0.8	1	0.38	1	0.95	0.95	0.95	1	0.5	1
SI9 – Terrestrial habitat	0.67	0.67	0.67	0.67	1	1	0.33	0.33	1	1	0.67
SI10 – Macrophytes	0.4	0.3	0.8	0.3	0.35	0.3	0.3	0.3	1	1	0.7
HSI	0.83	0.55	0.91	0.5	0.81	0.75	0.55	0.65	0.83	0.89	0.49
Suitability	**Excellent**	**Below Average**	**Excellent**	**Below Average**	**Excellent**	**Good**	**Below Average**	**Average**	**Excellent**	**Excellent**	**Poor**

2025

Table 3.3 Pond Information 2025.

Pond Reference	Location from Site	Description & Photograph	HSI Score
P6	<250m of the Site	This pond is located in a woodland adjacent to the boundary of East Park Site A. This pond was dry at the time of the survey.	Dry
P9	<250m of the Site	This pond is located within a woodland strip south of East Park Site C. The pond was shallow with little emergent vegetation and poor water quality at the time of the survey.	Below Average
P13	<250m of the Site	This pond is located within an area of land surrounded by East Park Site A. The pond had emergent vegetation, good water quality and supported invertebrates including damselfly.	Average
P14	Within the Site	This pond is located along the southern boundary of East Park Site B. The pond lacked emergent vegetation and had a poor water quality.	Poor

P25	Within the Site	The pond is located within East Park Site A. The pond is well shaded by surrounding vegetation, supports some emergent vegetation and has good water quality. 	Poor
P31	Within the Site	The pond is located within East Park Site C. The pond is partly shaded, with little emergent vegetation and poor water quality. 	Poor

P38	<250m of the Site	The pond is located to the east of East Park Site A. The pond was partly shaded; vegetation was dominated by iris with horsetail. Access to this pond was limited.	Below average.
P39	<250m of the Site	This pond is located within a small woodland adjacent to the Site boundary of East Park Site A. The pond was shallow, mostly shaded and duckweed was present.	Average
P41	<250m of the Site	The pond is located north of East Park Site D. The pond was dry at the time of the Survey. 	Dry
P42	<250m of the Site	This pond is adjacent of the southern boundary of East Park Site D. The pond is large and likely very deep. The pond lacked vegetation suitable for egg-laying.	Good

P43	<250m of the Site	The pond is located adjacent to the grid connection (between East Park Site D and Eaton Socon Substation). The pond is located east of East Park Site D and north of Huntingdon Wood. The pond was large with good water quality and emergent vegetation.	Excellent
P44	>250m of the Site	The pond is located east of East Park Site D. The pond was surrounded by dense vegetation preventing access. Water quality was good with emergent vegetation present.	Average

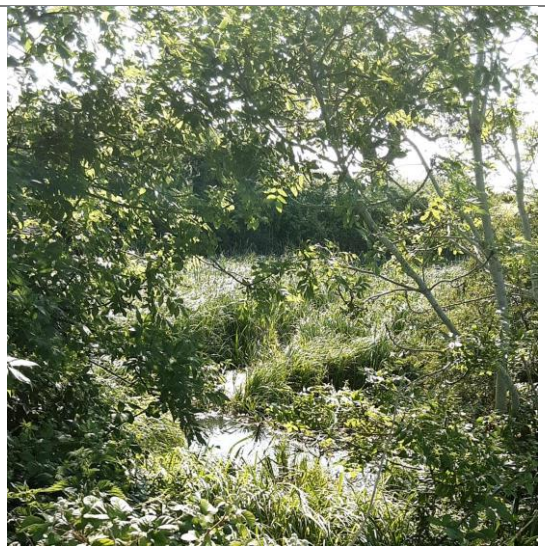
			
P45	>250m of the Site	The pond is adjacent to the boundary of East Park Site D. The pond was found to be dry at the time of the survey. 	Dry

Table 3.4: HSI survey results 2025.

Suitability Indices	P9	P13	P14	P25	P31	P38	P39	P42	P43	P44
SI1 – Location	1	1	1	1	1	1	1	1	1	1
SI2 – Pond area	0.1	0.4	0.1	0.2	0.2	0.4	0.4	0.9	0.92	0.1
SI3 – Pond drying	0.5	0.1	0.1	0.1	0.1	0.1	0.5	0.9	0.9	1
SI4 – Water quality	0.33	1	0.01	1	0.33	0.33	0.33	1	1	1
SI5 –Shade	1	1	0.7	0.4	1	1	0.6	1	1	1
SI6 – Fowl	1	1	1	1	1	1	1	0.67	0.67	0.67
SI7 – Fish	1	1	1	1	1	1	1	1	0.33	1
SI8 – Ponds	0.8	0.8	0.9	0.6	0.8	0.9	0.8	0.45	0.85	1
SI9 – Terrestrial habitat	0.67	0.33	0.33	0.33	0.33	0.33	0.67	0.33	0.67	0.33
SI10 – Macrophytes	0.3	0.9	0.3	0.35	0.4	0.9	0.5	0.4	1	0.9
HSI	0.55	0.63	0.3	0.47	0.48	0.57	0.63	0.71	0.8	0.68
Suitability	**Below Average**	**Average**	**Poor**	**Poor**	**Poor**	**Below Average**	**Average**	**Good**	**Excellent**	**Average**

3.3 eDNA

2022

3.3.1 eDNA surveys were not undertaken on four ponds due to being dry (Ponds P6 and P25) or being inaccessible due to steep banks (Ponds P9 and P39).

3.3.2 Five ponds (P8, P13, P17, P19 and P29) returned positive result for the presence of GCN and four ponds (P30, P30a, P31 and P38) returned negative results for the presence of GCN as summarised in Table 3.3. The laboratory reports are reproduced in Annex 1.

Table 3.5: eDNA survey results 2022

Pond	Sample Ref.	Inhibition Check	Degradation Check	Sample Integrity Score	Result	Result
P8	**4734**	**Pass**	**Pass**	**Pass**	**6/12**	**Positive**
P13	**4736**	**Pass**	**Pass**	**Pass**	**12/12**	**Positive**
P17	**4736**	**Pass**	**Pass**	**Pass**	**4/12**	**Positive**
P19	**4744**	**Pass**	**Pass**	**Pass**	**12/12**	**Positive**
P29	**4097**	**Pass**	**Pass**	**Pass**	**3/12**	**Positive**
P30	4740	Pass	Pass	Pass	0/12	Negative
P30a	4741	Pass	Pass	Pass	0/12	Negative
P31	4089	Pass	Pass	Pass	0/12	Negative
P38	4749	Pass	Pass	Pass	0/12	Negative

2025

3.3.3 eDNA surveys were not undertaken on four ponds due to being dry (Ponds P6, P41 and P45) or inaccessible due to steep banks and dense vegetation (Pond P44).

3.3.4 Three ponds (P13, P14 and P25) returned positive result for the presence of GCN and six ponds (P9, P31, P38, P39, P42 and P43) returned negative results for the presence of GCN as summarised in Table 3.3. The laboratory reports are reproduced in Annex 1.

Table 3.6: eDNA survey results 2025

Pond	Sample Ref.	Inhibition Check	Degradation Check	Positive Replicates	Result
P9	GCN25 8254	Pass	Pass	0/12	Negative
P13	**GCN25 8259**	**Pass**	**Pass**	**12/12**	**Positive**
P14	**GCN25 6798**	**Pass**	**Pass**	**12/12**	**Positive**
P25	**GCN25 8261**	**Pass**	**Pass**	**5/12**	**Positive**
P31	GCN25 8252	Pass	Pass	0/12	Negative
P38	GCN25 8260	Pass	Pass	0/12	Negative
P39	GCN25 8262	Pass	Pass	0/12	Negative
P42	GCN25 8256	Pass	Pass	0/12	Negative
P43	GCN25 9240	Pass	Pass	0/12	Negative

Table 3.7: Summary of eDNA survey results in 2022 and 2025 (positive results in bold)

Pond	Location	2022	2025
P8	**<250m of the Site**	**Positive**	-
P9	<250m of the Site	-	Negative
P13	**<250m of the Site**	**Positive**	**Positive**
P14	**Within the Site**	-	**Positive**
P17	**<250m of the Site**	**Positive**	-
P19	**>250 of the Site**	**Positive**	-
P25	**Within the Site**	-	**Positive**

P29	**<250m of the Site**	**Positive**	**-**
P30	>250 of the Site	Negative	-
P30a	>250 of the Site	Negative	-
P31	Within the Site	Negative	Negative
P38	<250m of the Site	Negative	Negative
P39	<250m of the Site	-	Negative
P42	<250m of the Site	-	Negative
P43	<250m of the Site	-	Negative

Annex 1

eDNA Laboratory Results 2022 (overleaf)

SureScreen Scientifics

Folio No: E14682
Report No: 1
Purchase Order: AE-22-126
Client: AVIAN ECOLOGY LTD
Contact: Joe Stevens

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: 30/06/2022
Date Reported: 11/07/2022
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
4089	Eaton Socon P31		Pass	Pass	Pass	Negative	0
4097	Eaton Socon P29		Pass	Pass	Pass	Positive	3
4734	Eaton Socon P8		Pass	Pass	Pass	Positive	6
4735	Eaton Socon P13		Pass	Pass	Pass	Positive	12
4736	Eaton Socon P17		Pass	Pass	Pass	Positive	4
4740	Eaton Socon P30		Pass	Pass	Pass	Negative	0
4741	Eaton Socon P30a		Pass	Pass	Pass	Negative	0

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

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4744	Eaton Socon P19		Pass	Pass	Pass	Positive	12
4749	Eaton Socon P38		Pass	Pass	Pass	Negative	0

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

Reported by: Chelsea Warner **Approved by:** Gabriela Danickova

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.

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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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eDNA Laboratory Results 2025 (overleaf)

Folio No:	2717-2025
Purchase Order:	AE SS-25-018
Contact:	Avian Ecology Ltd
Issue Date:	04.07.2025
Received Date:	20.06.2025

GCN Report

Technical Report

SureScreen Scientifics

Folio No:	2717-2025
Purchase Order:	AE SS-25-018
Contact:	Avian Ecology Ltd
Issue Date:	04.07.2025
Received Date:	20.06.2025

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 6798	EPEP - P14	TL 08008 62792	Pass	Pass	Positive	12/12
GCN25 8252	EPEP - P31	TL 10293 64144	Pass	Pass	Negative	0/12
GCN25 8254	EPEP - P9	TL 13033 62851	Pass	Pass	Negative	0/12
GCN25 8256	EPEP - P42	TL 14157 62478	Pass	Pass	Negative	0/12
GCN25 8259	EPEP - P13	TL 06739 64573	Pass	Pass	Positive	12/12
GCN25 8260	EPEP - P38	TL 07931 65395	Pass	Pass	Negative	0/12
GCN25 8261	EPEP - P25	TL 06572 64491	Pass	Pass	Positive	5/12
GCN25 8262	EPEP - P39	TL 06985 65080	Pass	Pass	Negative	0/12

Matters affecting result: none

Reported by: Amy Bermudez

Approved by: Consuela Sopronyi

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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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Folio No:	3590-2025
Purchase Order:	AE-SS-25-025
Contact:	Avian Ecology Ltd
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GCN Report

Technical Report

SureScreen Scientifics

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Results

Lab ID	Site Name	OS Reference	Degradation Check	Inhibition Check	Result	Positive Replicates
GCN25 9240	Eaton Socon - P43	TL 15175 62344	Pass	Pass	Negative	0/12

Matters affecting result: none

Reported by: Amy Bermudez

Approved by: Jennifer Higginbottom

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