



Preliminary Environmental Information Report

Chapter 8D: Great Crested Newts Survey Results

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Future Energy Llanwern Limited

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1.0 Great Crested Newts Survey Results

1.1 Introduction

1.1.1 This Appendix presents the results of amphibian surveys at Future Energy Llanwern in relation to an application for a Development Consent Order (DCO) for the installation of solar photovoltaic panels. The surveys were commissioned by Future Energy Llanwern Ltd.

1.1.2 The area within the PEIR assessment boundary is hereafter referred to as the 'Site'.

Aims

1.1.3 The aims of this report are to:

- Describe the methods used for collecting ecological baseline data;
- Identify the presence or likely absence of great crested newts (GCN) and other amphibians, based on desk study data, field data or assumptions based on the information available.

1.2 Legislation

1.2.1 Great crested newts *Triturus cristatus*, their breeding sites and resting places are protected under UK and European legislation; The Conservation of Habitats and Species Regulations 2017 (as amended), and The Wildlife and Countryside Act (W&CA) 1981 (as amended). It is an offence for anyone to intentionally capture, kill, disturb, or take GCN or GCN eggs. It is also an offence to damage, destroy or obstruct access to any place or structure used for shelter or protection by GCN.

1.2.2 The great crested newt is also listed on Annexes II and IV of the EC Habitats Directive and Appendix II of the Bern Convention. If a development activity is likely to result in disturbance or killing of a GCN, damage to its habitat etc., then a licence will usually be required from Natural Resources Wales.

1.2.3 Great crested newts are listed as Species of Principal Importance under Section 7 of the Environment (Wales) Act 2016.

- 1.2.4 Other amphibian species (common frog *Rana temporaria*, common toad *Bufo bufo*, smooth newt *Lissotriton vulgaris*, and palmate newt *L. helveticus*) are afforded partial legal protection under the Wildlife and Countryside Act 1981 (as amended), and the Countryside and Rights of Way (CROW) Act 2000. This legislation prohibits sale, transportation, and advertising for sale.
- 1.2.5 Common toad is listed as a Species of Principal Importance under Section 7 of the Environment (Wales) Act 2016.

1.3 Methods

Desk Study

- 1.3.1 The South East Wales Biodiversity Records Centre (SEWBRc) were consulted in January 2024 which included a search for records of protected and notable species within 2km of the Site. Full details are provided separately in **Appendix 8A**.

Pond/ Waterbody Scoping

- 1.3.2 A review of OS maps and aerial imagery identified five ponds within 250m of the Site. In addition, the Site is bound and dissected by a number of slow moving reens. As reens are interconnected, multiple sections of reen were sampled, with an even distribution throughout the Site to provide a site-wide representative survey area.

Habitat Suitability Index

- 1.3.3 An exercise was undertaken to assess the suitability of each pond and a representative sample of reens/ditches to support a breeding population of great crested newts (GCN). This was carried out on the 20 - 21 May 2019 by Declan Murphy BSc (Hons) MRes ACIEEM (Natural England Level 1 Class licence number: 2018-36042-CLS-CLS), an agent acting under the licence of Faye Midmore (NRW GCN Licence S085430/1) and Alex Maynard BSc QCIEEM.
- 1.3.4 This assessment followed the Habitat Suitability Index (HSI) (Oldham et al., 2000) methodology described in the ARG UK Guidance Note 5 (ARG UK 2010). The scores translate into a breeding suitability as shown in **Table 8D-1**.

Table 1D-1 Scoring system for GCN breeding suitability using HSI

HSI Score	Suitability for breeding GCN
<0.5	Poor
0.5 – 0.59	Below average
0.6 – 0.69	Average
0.7 – 0.79	Good
> 0.8	Excellent

Environmental DNA (eDNA) Survey

- 1.3.5 This method involved taking water samples from each water body within Natural England’s accepted sampling period of 15th April to 30th June following the methodology described in the Defra Report WC1067 (Biggs *et al.* 2014a) and the subsequent Technical Advice Note (Biggs *et al.*, 2014b).
- 1.3.6 Water samples were taken from locations identified as suitable during the HSI assessment on 20 - 21 May 2019 by Declan Murphy BSc (Hons) MRes ACIEEM (Natural England Level 1 Class licence number: 2018-36042-CLS-CLS), an agent acting under the licence of Faye Midmore (NRW GCN Licence S085430/1) and Alex Maynard BSc QCIEEM. The samples were analysed for eDNA by SureScreen Scientifics.
- 1.3.7 Additional surveys were undertaken in 2023, with samples taken at seven new locations on reens distributed evenly around the Site, and three ponds newly identified by aerial imagery on the 6th June 2023 by Hazel Greenland BSc, Ken Neal BSc (Hons) PhD. Ponds 5 and 6 were not surveyed in 2023 due to being identified as unsuitable at the time of the survey.
- 1.3.8 Additional surveys were undertaken in April-May 2025 by Noctua Ecology Ltd. This involved sampling seven new locations on reens, chosen to provide an even sampling distribution across the Site, when considered with the previous survey effort, an update survey on a reen location previously surveyed in 2019 and two ponds identified by aerial imagery within the Site boundary.
- 1.3.9 Twenty 30ml water samples were taken from each location using sterile field equipment supplied by SureScreen Scientifics. Sample locations were selected based on accessibility and suitable GCN egg laying and displaying areas, with as much of the margin being sampled as possible. The surveyor wore sterile gloves whilst taking the sample and did not enter the water. The water column was mixed

gently before taking the sample, with care taken not to disturb the sediment at the bottom of the pond. The samples from each pond were mixed and 15ml added to six sterile tubes containing 35ml ethanol.

1.3.10 Samples were immediately sent to SureScreen Scientifics for analysis. The methodology for laboratory analysis can be provided by SureScreen Scientifics.

Great Crested Newt Population Size Class Surveys

1.3.11 Traditional great crested newt surveys were undertaken by licensed ecologist Craig Stenson (NRW GCN level 1 licence number - SO85467/1) between 2 April 2020 and 7 May 2020 on Location 1a in accordance with methods stated within the Great Crested Newt Mitigation Guidelines (English Nature, 2001).

1.3.12 In 2025 the surveys on Location 1a were updated, and additional surveys were undertaken at Location 15 and Pond 4 which returned positive eDNA results when tested in 2023. Surveys took place between 14 April 2025 and 6 May 2025 by Sunny Jones an Accredited Agent under Lewis Hillier (NRW GCN level 2 licence number - S093302/2).

1.3.13 This involved a combination of at least three of the following methods: bottle trapping, torchlight survey, egg searching and netting, over six visits within the recommended survey season; between mid-March and mid-June (with at least half the surveys undertaken between mid-April and mid-May).

1.3.14 Survey details are shown in **Table 8D-2** below.

Table 1D-2 Great Crested Newt survey dates and weather conditions

Visit	Date	Minimum overnight Temperature	Weather
1	02/04/2020	5°C	Dry, cloudy, calm
2	09/04/2020	9°C	Dry, clear, calm
3	20/04/2020	7°C	Dry, clear, light winds
4	07/05/2020	11°C	Dry, patchy cloud, calm
5	16/05/2020	7°C	Dry, cloudy, calm
6	24/05/2020	7°C	Dry, clear, calm
1	14/04/2025	7°C	Light rain, overcast, light winds
2	22/04/2025	8°C	Light rain, overcast, windy
3	23/04/2025	7°C	Dry, clear, calm
4	28/04/2025	7°C	Dry, clear, calm

5	30/04/2025	10°C	Dry, clear, calm
6	06/05/2025	5°C	Dry, clear, light wind

1.3.15 The maximum count of adult GCN, using either torch surveying or bottle trapping on any one survey visit was used to calculate a population estimate. The categories used are as follows:

- 'Small' for maximum counts up to 10;
- 'Medium' for maximum counts between 11 and 100; or
- 'Large' for maximum counts over 100.

1.3.16 It should be noted that survey techniques are thought to reveal only between 2% and 30% of the total population (English Nature 2001) and therefore, results should be regarded as an estimate only.

Limitations

1.3.17 Care has been taken to ensure that balanced advice is provided on the information available and collected during the study period(s), and within the resources available for the project. However, the possibility of important ecological features being missed due to survey timings, absence during surveys or the year of survey cannot be ruled out. In addition, the lack of evidence or records of protected species on Site does not preclude their presence from Site.

1.3.18 2023 eDNA sampling was not possible for Pond 6 as it was dry at the time of the survey.

1.3.19 2023 eDNA sampling was not possible for Pond 5 as access was not granted.

1.3.20 2025 eDNA sampling was not undertaken at Pond 5 as it was deemed unsuitable for great crested newt as it was a cattle slurry pit at the time of survey.

1.4 Results

Desk Study

1.4.1 There were 24 records of GCN within 2km of the Site returned by SEWBRcC

between 1993 and 2023, the nearest of these was within the Site boundary and corresponds to three field records from 1993 by Mason Pittendrigh on behalf of Cardiff Bay Development Corporation. The records are given a four-figure grid reference, which corresponds to a 1km square, so the exact location is not known. The four-figure grid reference lies within the Site and does not include any of the locations where GCN were identified within this report. The next nearest was approximately 850m west of the Site.

HSI Results

2019 Survey Results

- 1.4.2 In 2019 14 reed/ ditch locations were selected as a representative sample of the Site to assess GCN presence within the Site. These locations were assessed using HSI, as shown in **Table 8D-3**. Two ponds were not assessed as they were dry at the time of survey.

Table 1D-3 Pond HSI Survey Results

SI No	SI Description	Location 1a	Location 1	Location 2	Location 3	Location 4	Location 5	Location 6
1	Geographic location	0.50	0.50	0.50	0.50	0.5	0.5	0.5
2	Pond area	0.65	0.65	0.95	0.95	0.1	0.1	0.2
3	Pond permanence	1.00	1.00	0.50	1.00	0.5	0.5	1
4	Water quality	0.33	0.33	0.33	0.33	0.33	0.33	0.67
5	Shade	1.00	1.00	1.00	1.00	0.8	1	1
6	Water fowl effect	1.00	1.00	1.00	1.00	1	1	1
7	Fish presence	0.67	0.67	0.67	0.67	0.67	0.67	0.67
8	Pond Density	1.00	1.00	1.00	1.00	1	1	1
9	Terrestrial habitat	0.67	0.67	1.00	0.67	0.67	1	0.67
10	Macrophyte cover	0.32	0.32	0.40	0.32	1	0.3	0.35
HSI Score		0.66	0.66	0.68	0.66	0.56	0.53	0.63
Pond suitability		Average	Average	Average	Average	Below average	Below average	Average

SI No	SI Description	Location 7	Location 8	Location 9	Location 10	Location 11	Location 12	Location 13
1	Geographic location	0.5	0.50	0.50	0.50	0.50	0.5	0.5
2	Pond area	0.3	0.10	0.10	0.80	0.40	0.2	0.2
3	Pond permanence	0.9	0.50	0.50	0.90	1.00	1	1
4	Water quality	0.33	0.33	0.33	0.67	0.67	0.67	0.33
5	Shade	1	0.80	1.00	1.00	1.00	0.8	1
6	Water fowl effect	1	1.00	1.00	1.00	0.67	1	1
7	Fish presence	0.67	0.67	0.67	0.33	0.33	0.33	0.67
8	Pond Density	1	1.00	1.00	1.00	1.00	1	1
9	Terrestrial habitat	1	0.67	0.67	1.00	0.67	1	0.67
10	Macrophyte cover	0.85	1.00	0.40	0.60	0.32	0.6	0.35
HIS Score		0.69	0.56	0.52	0.74	0.60	0.63	0.59
Pond Suitability		Good	Below average	Below average	Good	Average	Average	Average

eDNA Results

2019 Survey Results

- 1.4.3 Samples of eDNA were taken from all locations suitable for this method. Location 1a tested positive for great crested newt DNA. Refer to **Annex 8D-1** at the end of this report for laboratory reports and **Table 8-3** for all results.

2023 Survey Results

- 1.4.4 During additional surveys in 2023 a total of seven new reen locations and three ponds (Pond 2, 3 & 4) were sampled for eDNA. Pond 6 was not sampled due to being dry at the time of the survey and access to Pond 5 was not permitted.
- 1.4.5 Of the locations surveyed, only Pond 4 and Location 15 returned positive eDNA results in 2023. Refer to **Annex 8D-2** at the end of this report for laboratory reports and **Table 8-3** for all results.

2025 Survey Results

- 1.4.6 Water samples were collected from eight new reen locations, Location 1a and Pond 6, of which none returned with positive eDNA results. Pond 5 was deemed unsuitable for water sampling at the time of survey, as it is a slurry pit associated with a cattle farm.

1.4.7 Refer to **Annex 8D-3** at the end of this report for laboratory reports

1.4.8 The eDNA results are summarised in **Table 8D-4**. Pond locations are shown in **Figure 8.4.1**.

Table 1D-4 Great Crested Newt eDNA Survey Results

Location	eDNA Results		
	2019	2023	2025
Location 1a	Positive		Negative
Pond 2		Negative	
Pond 3		Negative	
Pond 4		Positive	
Pond 5		No Access	No Sample – Slurry
Pond 6		No Sample – Dry	Negative
Location 7a			Negative
Location 1	Negative		
Location 2	Negative		
Location 3	Negative		
Location 4	Negative		Negative
Location 5	Negative		
Location 6	Negative		
Location 7	Negative		
Location 8	Negative		
Location 9	Negative		
Location 10	Negative		
Location 11	Negative		
Location 12	Negative		
Location 13	Negative		
Location 14		Negative	
Location 15		Positive	
Location 16		Negative	
Location 17		Negative	
Location 18		Negative	
Location 19		Negative	
Location 20		Negative	
Location 22			Negative
Location 23			Negative
Location 24			Negative
Location 25			Negative
Location 26			Negative
Location 27			Negative

Population Size Class Assessment

2023

- 1.4.9 No GCN were recorded during the six visits within Location 1a. A maximum of 2 male, 1 female and 2 sub adults of smooth newt *Lissotriton vulgaris* and 20 three-spined sticklebacks *Gasterosteus aculeatus* were caught in bottle traps.

2025

- 1.4.10 A peak count of 4 adult great crested newt were recorded during the population size class assessment surveys within Pond 4. No GCN were recorded within Location 1a and Location 15. The detailed survey results are below in **Table 8-5** .

Table 1-5 Traditional Survey Results – Pond 4

Pond 4						
Visit	GCN					
	Male	Female	Subadult	Juvenile	Eggs	Other Species
1	1 (trap)	1 (trap)	-	-	-	Lvul, Lhel
2	1 (trap)	1 (trap)	-	-	-	Lvul
3	3 (trap)	1 (trap)				
1 (torch)	-	-	-	Lvul		
4	-	1 (trap)	-	-	-	Lvul
5	1 (trap)	-	-	-	-	Lvul
6	-	-	-	-	-	-
Peak Count	3	1	-	-	-	-

Lvul = Smooth newt *Lissotriton vulgaris*; **Lhel** = Palmate newt *Lissotriton helveticus*

1.5 Summary and Conclusions

- 1.5.1 eDNA surveys of the reens and ponds on Site returned 31 negative water samples and 3 positive water samples for great crested newt, two of which were reens and therefore likely to be moving water.
- 1.5.2 Following a positive eDNA survey for Location 1a in 2023, further surveys were undertaken but returned no evidence of great crested newts. The eDNA result from Location 1a could be explained as either a very small population that did not materialise in the traditional presence / absence survey or as a false positive result originating from an offsite location, with moving water relocating the eDNA, from the accidental transference of GCN DNA to the reen e.g. by wildfowl or sample contamination in the laboratory. The presence of high numbers of fish observed

within the reens, clearly makes these locations generally unsuitable for GCN as eggs, and young would be predated by them.

- 1.5.3 A small population of GCN were recorded within Pond 4 offsite during traditional survey methods in 2025 with a peak count of 4 individuals.

1.6 References

[ARG UK \(2010\). ARG UK Advice Note 5: Great Crested Newt Habitat Suitability Index. ARGUK.](#)

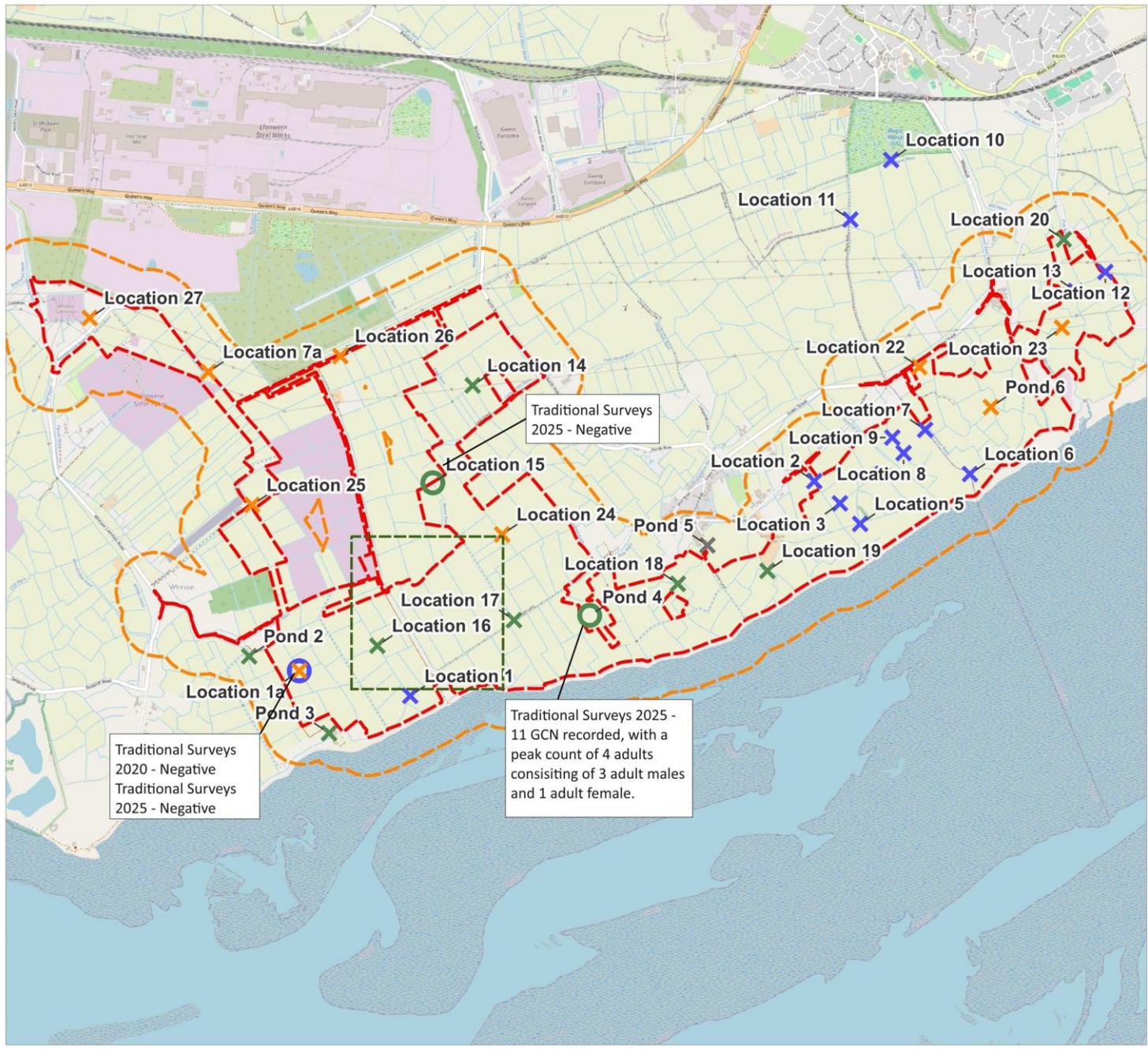
Biggs J, Ewald N, Valentini A, Gaboriaud C, Griffiths RA, Foster J, Wilkinson J, Arnett A, Williams P and Dunn F (2014a) Analytical and methodological development for improved surveillance of the Great Crested Newt. Defra Project WC1067. Freshwater Habitats Trust: Oxford.

Biggs J, Ewald N, Valentini A, Gaboriaud C, Griffiths RA, Foster J, Wilkinson J, Arnett A, Williams P and Dunn F (2014b) Analytical and methodological development for improved surveillance of the Great Crested Newt. Appendix 5. Technical advice note for field and laboratory sampling of great crested newt (*Triturus cristatus*) environmental DNA. Freshwater Habitats Trust, Oxford.

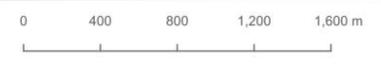
English Nature (2001) *Great Crested Newt Mitigation Guidelines*. English Nature, Peterborough.

Oldham R.S., Keeble J., Swan M.J.S. & Jeffcote M. (2000) *Evaluating the Suitability of Habitat for the Great Crested Newt (Triturus Cristatus)*. Herpetological Journal, 10: 143-155.

Annex A: Figures



- Key:**
- Site Boundary Oct 2024
 - GCN Data Search Onsite Result
 - 250m Buffer
 - Positive eDNA 2019
 - × Negative eDNA 2019
 - Positive eDNA 2023
 - × Negative eDNA 2023
 - × Negative eDNA 2025
 - × Unsuitable for eDNA



(c) Crown copyright and database rights 2025 Ordnance Survey 0100031673.

Figure 8.4.1:
Great Crested Newt Survey Results

Project:
Future Energy Llanwern

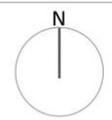
Client:
Future Energy Llanwern Limited

Date:
5/11/2025

Drawn:
JH

Ref:
0840-ETA-8.4.1

Revision:
-



Annex B: GCN eDNA results 2019



Folio No: E5288
Report No: 1
Order No: 0840
Client: GREEN ECOLOGY
Contact: Mark Witherall
Contact Details: markwitherall@green-ecology.co.uk
Date: 04/06/2019

TECHNICAL REPORT

ANALYSIS OF ENVIRONMENTAL DNA IN POND WATER FOR THE DETECTION OF GREAT CRESTED NEWTS

Pond 1

Date sample received at Laboratory: 28/05/2019
Date Reported: 04/06/2019
Matters Affecting Results: None

RESULTS

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
0392	Location 2	ST 369 852	Pass	Pass	Pass	Negative	0
0396	Location 5	ST 369 852	Pass	Pass	Pass	Negative	0
0397	Pond 5	ST 369 852	Pass	Pass	Pass	Negative	0
0399	Location 3	ST 369 852	Pass	Pass	Pass	Negative	0
0401	Location 1a	ST 369 852	Pass	Pass	Pass	Positive	1
0402	Location 6	ST 369 852	Pass	Pass	Pass	Negative	0

Forensic Scientists and Consultant Engineers
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0405	Location 7	ST 369 852	Pass	Pass	Pass	Negative	0
0408	Location 11	ST 369 852	Pass	Pass	Pass	Negative	0
0411	Location 8	ST 369 852	Pass	Pass	Pass	Negative	0
0414	Location 13	ST 369 852	Pass	Pass	Pass	Negative	0
0415	Unknown	ST 369 852	Pass	Pass	Pass	Negative	0
0416	Location 1	ST 369 852	Pass	Pass	Pass	Negative	0
0417	Location 10	ST 369 852	Pass	Pass	Pass	Negative	0
0418	Location 9	ST 369 852	Pass	Pass	Pass	Negative	0
0419	Location 12	ST 369 852	Pass	Pass	Pass	Negative	0
0420	Location 4	ST 369 852	Pass	Pass	Pass	Negative	0

SUMMARY

When Great Crested Newts (GCN); *Triturus cristatus* inhabit a pond, they deposit traces of their DNA in the water as evidence of their presence. By sampling the water, we can analyse these small environmental DNA (eDNA) traces to confirm GCN habitation, or establish GCN absence.

The water samples detailed below were submitted for eDNA analysis to the protocol stated in DEFRA WC1067 (Latest Amendments). Details on the sample submission form were used as the unique sample identity.

RESULTS INTERPRETATION

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Lab Sample No.- When a kit is made it is given a unique sample number. When the pond samples have been taken and the kit has been received back in to the laboratory, this sample number is tracked throughout the laboratory.

Site Name- Information on the pond.

O/S Reference - Location/co-ordinates of pond.

SIC- Sample Integrity Check. Refers to quality of packaging, absence of tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to results errors. Inspection upon receipt of sample at the laboratory. To check if the Sample is of adequate integrity when received. Pass or Fail.

DC- Degradation Check. Analysis of the spiked DNA marker to see if there has been degradation of the kit since made in the laboratory to sampling to analysis. Pass or Fail.

IC- Inhibition Check- PCR inhibitors can cause false results. Inhibitors are analysed to check the quality of the result. Every effort is made to clean the sample pre-analysis however some inhibitors cannot be extracted. An unacceptable inhibition check will cause an indeterminate sample and must be sampled again.

Result- NEGATIVE means that GCN eDNA was not detected or is below the threshold detection level and the test result should be considered as no evidence of GCN presence. POSITIVE means that GCN eDNA was found at or above the threshold level and the presence of GCN at this location at the time of sampling or in the recent past is confirmed. Positive or Negative.

Positive Replicates- To generate the results all of the tubes from each pond are combined to produce one eDNA extract. Then twelve separate analyses are undertaken. If one or more of these analyses are positive the pond 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 used for population studies. In accordance with Natural England protocol, even a score of 1/12 is declared positive.

METHODOLOGY

The laboratory testing adheres to strict guidelines laid down in WC1067 Analytical and Methodological Development for Improved Surveillance of The Great Crested Newt, Version 1.1

The analysis is conducted in two phases. The sample first goes through an extraction process where all six tubes are pooled together to acquire as much eDNA as possible. The pooled sample is then tested via real time PCR (also called q-PCR). This process amplifies select part of DNA allowing it to be detected and measured in 'real time' as the analytical process develops. qPCR combines PCR amplification and detection into a single step. This eliminates the need to detect products using gel electrophoresis. With qPCR, fluorescent dyes specific to the target sequence are used to label PCR products during thermal cycling. The accumulation of fluorescent signals during the exponential phase of the reaction is measured for fast and objective data analysis. The point at which amplification begins (the Ct value) is an indicator of the quality of the sample. True positive controls, negatives and blanks as well as spiked synthetic DNA are included in every analysis and these have to be correct before any result is declared so they act as additional quality control measures.

The primers used in this process are specific to a part of mitochondrial DNA only found in GCN ensuring no DNA from other species present in the water is amplified. The unique sequence appropriate for GCN analysis is quoted in DEFRA WC 1067 and means there should be no detection of closely related species. We have tested our system exhaustively to ensure this is the case in our laboratory. We can offer eDNA analysis for most other species including other newts.

Analysis of eDNA requires scrupulous attention to detail to prevent risk of contamination. Kits are manufactured by SureScreen Scientifics to strict quality procedures in a separate building and with separate staff, adopting best practice from WC1067 and

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WC1067 Appendix 5. Kits contain a 'spiked' DNA marker used as a quality control tracer (SureScreen patent pending) to ensure any DNA contained in the sampled water has not deteriorated in transit. Stages of the DNA analysis are also conducted in different buildings at our premises for added security.

SureScreen Scientifics Ltd also participate in Natural England's proficiency testing scheme and we also carry out inter-laboratory checks on accuracy of results as part of our quality procedures.

Reported by: Sarah Evans

Approved by: Chris Troth

End Of Report

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Annex C: GCN eDNA results 2023

Folio No: E18101
 Report No: 1
 Purchase Order: 0840
 Client: GE CONSULTING
 Contact: Ken Neal

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: 16/06/2023
Date Reported: 27/06/2023
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
1588	Location 19	ST 41745 83775	Pass	Pass	Pass	Negative	0
4652	Location 18	ST 41154 83690	Pass	Pass	Pass	Negative	0
4657	Pond 4	ST 40572 83483	Pass	Pass	Pass	Positive	12

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

Reported by: Chris Troth

Approved by: Jennifer Higginbottom



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METHODOLOGY

The samples detailed above have been analysed for the presence of GCN eDNA following the protocol stated in DEFRA WC1067 'Analytical and methodological development for improved surveillance of the Great Crested Newt, Appendix 5.' (Biggs et al. 2014). Each of the 6 sub-sample tubes are first centrifuged and pooled together into a single sample which then undergoes DNA extraction. The extracted sample is then analysed using real time PCR (qPCR), which uses species-specific molecular markers to amplify GCN DNA within a sample. These markers are unique to GCN DNA, meaning that there should be no detection of closely related species.

If GCN DNA is present, the DNA is amplified up to a detectable level, resulting in positive species detection. If GCN DNA is not present then amplification does not occur, and a negative result is recorded.

Analysis of eDNA requires scrupulous attention to detail to prevent risk of contamination. True positive controls, negative controls and spiked synthetic DNA are included in every analysis and these have to be correct before any result is declared and reported. Stages of the DNA analysis are also conducted in different buildings at our premises for added security.

SureScreen Scientifics Ltd is ISO9001 accredited and participate in Natural England's proficiency testing scheme for GCN eDNA testing. We also carry out regular inter-laboratory checks on accuracy of results as part of our quality control procedures.

INTERPRETATION OF RESULTS

- SIC:** **Sample Integrity Check** [Pass/Fail]
When samples are received in the laboratory, they are inspected for any tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to inconclusive results.
- DC:** **Degradation Check** [Pass/Fail]
Analysis of the spiked DNA marker to see if there has been degradation of the kit or sample between the date it was made to the date of analysis. Degradation of the spiked DNA marker may lead indicate a risk of false negative results.
- IC:** **Inhibition Check** [Pass/Fail]
The presence of inhibitors within a sample are assessed using a DNA marker. If inhibition is detected, samples are purified and re-analysed. Inhibitors cannot always be removed, if the inhibition check fails, the sample should be re-collected.
- Result:** **Presence of GCN eDNA** [Positive/Negative/Inconclusive]
Positive: GCN DNA was identified within the sample, indicative of GCN presence within the sampling location at the time the sample was taken or within the recent past at the sampling location.
Positive Replicates: Number of positive qPCR replicates out of a series of 12. If one or more of these are found to be positive the pond is declared positive for GCN presence. It may be assumed that small fractions of positive analyses suggest low level presence, but this cannot currently be used for population studies. In accordance with Natural England protocol, even a score of 1/12 is declared positive. 0/12 indicates negative GCN presence.
Negative: GCN eDNA was not detected or is below the threshold detection level and the test result should be considered as evidence of GCN absence, however, does not exclude the potential for GCN presence below the limit of detection.



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Page 2 of 2

Folio No: E18009
 Report No: 1
 Purchase Order: 0840
 Client: GE CONSULTING
 Contact: Ken Neal

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: 14/06/2023
Date Reported: 21/06/2023
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
1584	Location 14	ST 39744 84956	Pass	Pass	Pass	Negative	0
3443	Location 15	ST 39613 84256	Pass	Pass	Pass	Positive	11
3444	Location 16	ST 3900 83050	Pass	Pass	Pass	Negative	0
3445	Pond 2	ST 38316 83268	Pass	Pass	Pass	Negative	0
4651	Location 17	ST 436 859	Pass	Pass	Pass	Negative	0
4653	Pond 3	ST 38859 82717	Pass	Pass	Pass	Negative	0
4655	Location 20	st 40635 83851	Pass	Pass	Pass	Negative	0



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

Reported by: Chris Troth

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.

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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



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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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Annex D: GCN eDNA results 2025

Folio No: 379-2025
Purchase Order: GE005-1_7
Contact: Noctua Ecology Ltd
Issue Date: 06.05.2025
Received Date: 17.04.2025

GCN Report

Technical Report



Folio No: 379-2025
Purchase Order: GE005-1_7
Contact: Noctua Ecology Ltd
Issue Date: 06.05.2025
Received Date: 17.04.2025



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.

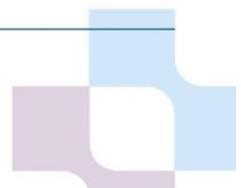
Results

Lab ID	Site Name	OS Reference	Degradation Check	Inhibition Check	Result	Positive Replicates
GCN25 1741	Location 23	ST 43678 85372	Pass	Pass	Negative	0/12
GCN25 1747	Pond 6	ST 43227 84851	Pass	Pass	Negative	0/12
GCN25 1749	Location 1a	ST 38650 83126	Pass	Pass	Negative	0/12

Matters affecting result: none

Reported by: Lauryn Jewkes

Approved by: Lauryn Jewkes



Folio No: 379-2025
Purchase Order: GE005-1_7
Contact: Noctua Ecology Ltd
Issue Date: 06.05.2025
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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: 980-2025
Purchase Order: GE005-2-8
Contact: Noctua Ecology Ltd
Issue Date: 15.05.2025
Received Date: 30.04.2025

GCN Report

Technical Report



Folio No: 980-2025
Purchase Order: GE005-2-8
Contact: Noctua Ecology Ltd
Issue Date: 15.05.2025
Received Date: 30.04.2025



GCN eDNA Analysis

Summary

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Results

Lab ID	Site Name	OS Reference	Degradation Check	Inhibition Check	Result	Positive Replicates
GCN25 1738	Location 22	51.5612130, -2.8292980	Pass	Pass	Negative	0/12
GCN25 1739	Location 24	ST 40176 83943	Pass	Pass	Negative	0/12
GCN25 1740	Location 26	ST 38969 85211	Pass	Pass	Negative	0/12
GCN25 1744	Location 25	ST 38322 84205	Pass	Pass	Negative	0/12
GCN25 1745	Location 27	ST 37249 85486	Pass	Pass	Negative	0/12
GCN25 1746	Location 7a	ST 38024 85098	Pass	Pass	Negative	0/12
GCN25 1750	Location 4	51.355621, -2.8906636	Pass	Pass	Negative	0/12

Matters affecting result: none

Reported by: Consuela Sopronyi

Approved by: Lauryn Jewkes

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