Butterfly Solar Farm

on behalf of RWE Renewables UK Limited

Appendix 5-4: Great Crested Newt Presence or Absence (eDNA) Survey Report

avianecology

Document Control	
Project Name:	Butterfly Proposed Solar Farm
Project Number:	AxisL-043-1591
Report Title:	Appendix 5-4 Great Crested Newt Presence or Absence (eDNA) Survey Report

Issue	Date	Notes	Prepared	Reviewed
V1	06/11/2024	Draft for Client Comment	Z Hinchcliffe *MRes BSc (Hons.)* **Senior Ecologist**	J. Stevens *BSc (Hons*) **Principal Ecologist**
V2	13/11/2024	First issue	Z Hinchcliffe *MRes BSc (Hons.)* **Senior Ecologist**	J. Stevens *BSc (Hons*) **Principal Ecologist**
V3	22/08/2025	Amended Site Boundary		J. Stevens *BSc (Hons*) **Principal Ecologist**

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TABLES

ANNEXES

1 INTRODUCTION

1.1 Background

1.1.1 Avian Ecology Ltd. was commissioned by Axis on behalf of RWE Renewables to undertake a great crested newt (GCN) *Triturus cristatus* presence/absence survey, adopting the environmental DNA (eDNA) sampling methodology. The surveys were undertaken in relation to a proposed solar energy generating station and an associated on-site Battery Energy Storage System (BESS) (the 'Proposed Development) located on land to the north of the B5426, Wrexham (the 'Site') as shown on **Figure 7-5.** The Proposed Development also includes the associated infrastructure and connection to the Legacy National Grid substation.

1.1.2 This report presents the survey methodology and results.

1.2 Survey Area

1.2.1 Ponds were identified from aerial images and Ordnance Survey (OS) maps on or within 250m of the main solar site, and within 50m of the grid connection route. Due to the low impact of solar energy developments on GCN habitats, ponds beyond this distance were not considered.

1.2.2 Ponds subject to assessment are identified on **Figure 7-5**.

2 METHODOLOGY

2.1 Pond Selection

2.1.1 Ordnance Survey and aerial mapping were used to search for potential ponds within the Site or within a 250m buffer around the Array Areas or within 50m of the cable route. This identified a total of 91 possible pond features (**Figure 7-5**), of which nine were located within 50m of the cable route only, 43 within 250m of the array areas only and the remaining 39 within both 50m of the cable route and 250m of the array areas.

2.1.2 Western Ecology sampled the Site in June 2022 and June 2023 with 19 ponds in total visited and sampled. The remaining ponds were not viewed at all due to access limitations.

2.1.3 In June 2024, Avian Ecology accessed the Site, with 22 ponds viewed (**Table 2**). Four of the previous ponds accessed by Western Ecology in 2022 (P60, 75, 75 and 89) were also sampled again by Avian Ecology in 2024. The remaining potential ponds could not be viewed at all due to access limitations.

2.1.4 In total, 37 ponds (or potential ponds) were surveyed over three survey seasons by the two separate consultancies with 54 ponds inaccessible.

2.2 Field Sampling

2.2.1 During 2022, field sampling was conducted by ecologists at Western Ecology using suitably qualified ecologists with experience of conducting GCN eDNA surveys.

2.2.2 During 2024, field sampling was conducted by L Quarton and assisted by P Baker and F Wilde BSc (Hons.) from Avian Ecology Ltd; all suitably qualified ecologists with experience of conducting GCN eDNA surveys.

2.2.3 Sampling took place on 30th June 2022 and 14th – 21st June 2024.

2.2.4 A total of 26 ponds were subject to eDNA survey sampling across the two survey years (**Table 2**).

2.2.5 The protocol for sampling followed the technical advice note for field and laboratory sampling of great crested newts (Biggs *et al*. 2014)[1], which required the collection of 20 x 30ml subsamples from each pond, spaced as evenly as possible around the pond margin.

2.2.6 The subsamples were all placed within the same sample bag, which was 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.

2.3 Laboratory Analysis

2.3.1 Laboratory analysis was undertaken by SureScreen Scientifics Ltd, using the methodology outlined by Biggs *et al*. (2014).

2.3.2 DNA was extracted from the sample and then amplified and detected using specific primers and probes within a q-PCR test. Each sample was run in 12 replicates, and the results reported as the

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

proportion of the 12 replicates that were successfully amplified (indicating that GCN DNA is present). Inhibition and degradation checks were also carried out on each sample using a known DNA marker.

2.4 Habitat Suitability Index

2.4.1 The sampled ponds were also assessed according to the Habitat Suitability Index (HSI) as developed by Oldham et al. (2000)[2].

2.4.2 HSI provides a score for the suitability of a pond for GCN, based on selected physical and ecological characteristics (**Table 1**). This methodology is detailed in full within ARG UK guidance (ARG UK, 2010)[3].

Table 1: Description of the categories used for the Habitat Suitability Index (HSI).

Category	Definition
S1. Geographic location	Sites should be scored according to the region in which they occur within the UK.
S2. Pond area	The surface area of the pond when water is at its highest level.
S3. Permanence	How often a pond dries out.
S4. Water quality	Assessment in the field based on invertebrate diversity, the presence of submerged water plants, and knowledge of the water sources feeding the pond.
S5. Shade	Shading of the pond from trees and/or buildings. Not including emergent pond vegetation.
S6. Waterfowl	The presence and estimated density of waterfowl.
S7. Fish	The presence and estimated density of fish.
S8. Pond count	The number of ponds occurring within 1 km of survey pond.
S9. Terrestrial habitat	The quality of the terrestrial habitat surrounding the pond as offering cover and foraging opportunities for GCN.
S10. Macrophytes	The percentage of the pond surface area occupied by macrophyte cover (excluding duckweed).

Table 2: Record of sampling effort for accessible ponds.

Pond ID (Western Ecology Pond ID provided where applicable)	Year Viewed	eDNA Sampling	HSI
P31	2024	Y	Y

[2] 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(4). pp. 143-155.

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

Pond ID (Western Ecology Pond ID provided where applicable)	Year Viewed	eDNA Sampling	HSI
P32 Wrexham Legacy no 6	2022	Y	N
P33 Wrexham Legacy no 5	2022	Y	N
P34 Wrexham Legacy no 4	2022	Y	N
P35 Wrexham Legacy no 2/3	2022	Y	N
P36	2024	N	Y
P37	2024	Y	Y
P46	2024	N	Y
P56 Wrexham pond Central 35	2022	Y	N
P57	2024	Y	N
P59	2024	N	Y
P60 Wrexham pond 8 Central	2022 & 2024	Y	Y
P61	2024	N	Y
P62	2024	N	Y
P73	2024	N	Y
P74 Wrexham pond 3 Central	2022 & 2024	Y	Y
P75 Wrexham pond Central 36	2022	Y	N
P76 Wrexham pond Central 37	2022	Y	N
P77 Wrexham pond Central 39	2022	Y	N
P81 Wrexham pond Central 40	2022	Y	N
P83 Wrexham pond Central 16	2022	Y	N
P84	2024	Y	Y
P85 Wrexham pond Central 17	2022	Y	N
P86 Wrexham pond Central 18	2022	Y	N
P88	2022	Y	N

Pond ID (Western Ecology Pond ID provided where applicable)	Year Viewed	eDNA Sampling	HSI
Wrexham pond Central 42			
P89 Wrexham pond Central 1	2022 & 2024	Y	Y
P90	2024	N	Y
P108 Wrexham Legacy no 8	2022	Y	N
P113 Wrexham Legacy no 11	2022	Y	N
P114	2024	N	Y
P115 Wrexham pond eastern 2	2022 & 2024	Y	Y
P116	2022	N	Y
P120	2024	N	Y
P123	2024	Y	Y
P125	2024	N	Y

3 RESULTS

3.1 Habitat Suitability Index

3.1.1 The results show that seven of the 24 ponds scored 'average', 'good' or 'excellent' in terms of their likely suitability for GCN, with the other ponds scoring lower (**Table 3**).

Commented [LM1]: There are only 7 listed in the table as 'average' / 'good' or 'excellent'?

Table 3: HSI results.

Pond	Geographic Location	Pond Area	Permanence	Water Quality	Shade	Waterfowl	Fish	Pond Count	Terrestrial Habitat	Macrophytes	HSI Score*	Likelihood
P31	1	25	0.1	0.33	95%	1	1	57	0.67	20%	0.44	Poor
P36	1	36	0.1	0.33	100%	0.67	1	55	0.67	0%	0.39	Poor
P37	1	30	0.5	0.33	95%	1	1	55	0.67	0%	0.49	Poor
P46	1	25	0.1	0.33	90%	1	1	18	0.67	0%	0.43	Poor
P50	1	10	0.1	0.33	50%	1	1	18	0.67	0%	0.48	Poor
P51	1	120	0.1	0.33	90%	1	1	18	0.67	0%	0.46	Poor
P57	1	30	0.5	0.33	50%	0.67	0.67	18	0.67	5%	0.53	Below Average

Pond	Geographic Location	Pond Area	Permanence	Water Quality	Shade	Waterfowl	Fish	Pond Count	Terrestrial Habitat	Macrophytes	HSI Score*	Likelihood
P59	1	500	0.9	0.67	40%	1	1	18	1	70%	0.95	Excellent
P60	1	500	0.9	0.33	40%	0.67	0.67	18	0.67	10%	0.72	Good
P61	1	8	0.1	0.33	80%	1	1	18	0.33	0%	0.43	Poor
P62	1	10	0.1	0.33	80%	1	1	18	0.33	0%	0.43	Poor
P69	1	28	0.1	0.01	0%	1	1	18	0.67	0%	0.34	Poor
P73	1	350	0.1	0.33	50%	1	1	18	0.67	0%	0.58	Below Average
P74	1	290	0.9	0.67	50%	0.67	0.33	18	0.67	15%	0.69	Average
P84	1	100	0.5	0.33	100%	1	1	18	0.67	5%	0.52	Below Average
P89	1	450	0.9	0.33	90%	0.67	0.67	18	0.33	5%	0.58	Below Average
P90	1	8	0.1	0.33	80%	1	1	18	0.67	0%	0.46	Poor
P114	1	330	0.9	0.33	80%	1	1	54	0.67	0%	0.68	Average
P115	1	1,100	0.9	0.33	80%	0.67	0.67	54	0.67	5%	0.67	Average
P116	Minimal information provided										0.61	Average
P120	1	200	0.1	0.33	95%	1	1	50	0.67	0%	0.48	Poor
P123	1	10	0.1	0.33	95%	1	1	50	0.67	0%	0.42	Poor
P125	1	1,500	0.1	0.33	0%	1	1	51	0.67	50%	0.66	Average

* The HSI is a geometric mean of ten suitability indices. HSI = (S1 x S2 x S3 x S4 x S5 x S6 x S7 x S8 x S9 x S10)1/10.

3.2 eDNA Survey Results

3.2.1 None of the 26 ponds sampled returned a positive result for GCN DNA. The results are summarised in **Table 4**, and the full laboratory report shown in **Annex 2**.

Table 4: eDNA analysis results.

Pond	Sample Kit	Degradation Check	Inhibition Check	Positive Replicated (/12)	Result
P31	6641	Pass	Pass	0	Negative

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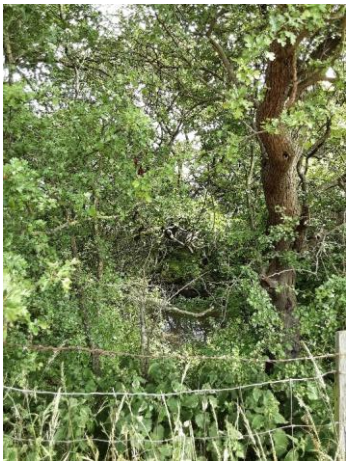
Pond	Sample Kit	Degradation Check	Inhibition Check	Positive Replicated (/12)	Result
P32	5215	Pass	Pass	0	Negative
P33	5216	Pass	Pass	0	Negative
P34	5217	Pass	Pass	0	Negative
P35	5218	Pass	Pass	0	Negative
P37	6642	Pass	Pass	0	Negative
P56	4301	Pass	Pass	0	Negative
P57	GCNR925	Pass	Pass	0	Negative
P59	GCNR928	Pass	Pass	0	Negative
P60	4299 & GCNR927	Pass	Pass	0	Negative
P74	4293 & GCNR693	Pass	Pass	0	Negative
P75	4297	Pass	Pass	0	Negative
P76	4291	Pass	Pass	0	Negative
P77	4295	Pass	Pass	0	Negative
P81	4312	Pass	Pass	0	Negative
P83	4298	Pass	Pass	0	Negative
P84	GCNR926	Pass	Pass	0	Negative
P85	4288	Pass	Pass	0	Negative
P86	4289	Pass	Pass	0	Negative
P88	4300	Pass	Pass	0	Negative
P89	4311 & GCNR703	Pass	Pass	0	Negative
P108	5214	Pass	Pass	0	Negative
P113	5211	Pass	Pass	0	Negative
P114	GCNR701	Pass	Pass	0	Negative
P115	4296 & GCNR686	Pass	Pass	0	Negative
P123	6746	Pass	Pass	0	Negative

Annex 1
Pond Photographs

Sampled ponds	
	
P14	**P31**
	
P36	**P37**

Sampled ponds	
	
P46	**P50**
	
P51	**P57**
	
P59	**P60**

Sampled ponds	
	
P73	**P74**
	
P89a	**P89b**
	
P90	**P114**

Sampled ponds	
	
P115	**P123**

Annex 2
eDNA Laboratory Results

Surveys June 2022

SureScreen Scientifics

Folio No: E14845
Report No: 1
Purchase Order: PO WOR 3135
Client: WESTERN ECOLOGY LTD
Contact: Colin Hicks

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: 04/07/2022
Date Reported: 18/07/2022
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
4288	Wrexham Pond Central 17	SJ34076 46198	Pass	Pass	Pass	Negative	0
4289	Wrexham Pond Central 18	SJ34181 46227	Pass	Pass	Pass	Negative	0
4291	Wrexham Pond Central 37	SJ33593 46196	Pass	Pass	Pass	Negative	0
4293	Wrexham Pond 3 Central	SJ34025 45862	Pass	Pass	Pass	Negative	0
4295	Wrexham Pond Central 39	SJ33651 46310	Pass	Pass	Pass	Negative	0
4296	Wrexham	SJ36511	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

	Pond Eastern 2	46138					
4297	Wrexham Pond Central 36	SJ33520 46157	Pass	Pass	Pass	Negative	0
4298	Wrexham Pond Central 16	SJ34005 46250	Pass	Pass	Pass	Negative	0
4299	Wrexham Pond 8 Central	SJ33907 45674	Pass	Pass	Pass	Negative	0
4300	Wrexham Pond Central 42	SJ34357 46247	Pass	Pass	Pass	Negative	0
4301	Wrexham Pond Central 35	SJ33353 45807	Pass	Pass	Pass	Negative	0
4311	Wrexham Pond Central 1	SJ34137 46022	Pass	Pass	Pass	Negative	0
4312	Wrexham Pond Central 40	SJ33841 46332	Pass	Pass	Pass	Negative	0

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

Reported by: Chris Troth **Approved by:** Esther Strafford

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.

SureScreen Scientifics

Folio No: E18817
Report No: 1
Purchase Order: Wrexham Legacy 11/8/6/5/4/2/3
Client: WESTERN ECOLOGY LTD
Contact: Chris Ayre

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: 06/07/2023
Date Reported: 19/07/2023
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
5211	Wrexham Legacy - No 11	SJ 36197 46094	Pass	Pass	Pass	Negative	0
5214	Wrexham Legacy - No 8	SJ 36029 46029	Pass	Pass	Pass	Negative	0
5215	Wrexham Legacy - No 6	SJ 32065 46738	Pass	Pass	Pass	Negative	0
5216	Wrexham Legacy - No 5	SJ 32064 46665	Pass	Pass	Pass	Negative	0
5217	Wrexham Legacy - No 4	SJ 32067 46543	Pass	Pass	Pass	Negative	0
5218	Wrexham Legacy - No 2/3	SJ 32094 46380	Pass	Pass	Pass	Negative	0

SureScreen Scientifics

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

Reported by: Chris Troth **Approved by:** Jennifer Higginbottom

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]
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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.

Survey June 2024

Folio No: 2162-2024
Purchase Order: AESS-24-020
Contact: Avian Ecology Ltd
Issue Date: 02.07.2024
Received Date: 20.06.2024

GCN Report

Technical Report

SureScreen Scientifics

Folio No: 2162-2024
Purchase Order: AESS-24-020
Contact: Avian Ecology Ltd
Issue Date: 02.07.2024
Received Date: 20.06.2024

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
R928	Legacy, P59	SJ3386645689	Pass	Pass	Negative	0/12
R703	Legacy, P89	SJ3413846017	Pass	Pass	Negative	0/12
R925	Legacy, P57	SJ3368245600	Pass	Pass	Negative	0/12
R701	Legacy, P114	SJ3635146135	Pass	Pass	Negative	0/12
R926	Legacy, P84	SJ3388546152	Pass	Pass	Negative	0/12
R685	Legacy, P115	SJ3656146048	Pass	Pass	Negative	0/12
R927	Legacy, P60	SJ3390345674	Pass	Pass	Negative	0/12

Matters affecting result: none

Reported by: Daisy Chambers

Approved by: Christopher Troth

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Folio No:	2162-2024
Purchase Order:	AESS-24-020
Contact:	Avian Ecology Ltd
Issue Date:	02.07.2024
Received Date:	20.06.2024

SureScreen Scientifics

Methodology

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

If GCN DNA is present, the DNA is amplified up to a detectable level, resulting in positive species detection. If GCN DNA is not present then amplification does not occur, and a negative result is recorded. Analysis of eDNA requires attention to detail to prevent the risk of contamination. True positive controls, negative controls, and spiked synthetic DNA are included in every analysis and these have to be correct before any result is declared and reported. Stages of the DNA analysis are also conducted in different buildings at our premises for added analytical security.

SureScreen Scientifics Ltd is ISO9001 accredited and participates in Natural England's proficiency testing scheme for GCN eDNA testing.

Interpretation of Results

Sample Integrity Check: When samples are received in the laboratory, they are inspected for any tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to inconclusive results. Any samples which fail this test are rejected and eliminated before analysis.

Degradation Check: **Pass/Fail**. Analysis of the spiked DNA marker to see if there has been degradation of the kit or sample between the date it was made to the date of analysis. Degradation of the spiked DNA marker may lead indicate a risk of false negative results.

Inhibition Check: **Pass/Fail.** The presence of inhibitors within a sample is assessed using a DNA marker. If inhibition is detected, samples are purified and re-analyzed. Inhibitors cannot always be removed, if the inhibition check fails, the sample should be re-collected.

Result: **Presence of GCN eDNA (Positive/Negative/Inconclusive)**
Positive: GCN DNA was identified within the sample, indicative of GCN presence within the sampling location at the time the sample was taken or within the recent past at the sampling location.
Positive Replicates: Number of positive qPCR replicates out of a series of 12. If one or more of these are found to be positive the pond is declared positive for GCN presence. It may be assumed that small fractions of positive analyses suggest low level presence, but this cannot currently be used for population studies. In accordance with the WC1067 Natural England protocol, even a score of 1/12 is declared positive. 0/12 indicates negative GCN presence.
Negative: GCN eDNA was not detected or is below the threshold detection level and the test result should be considered as evidence of GCN absence, however, does not exclude the potential for GCN presence below the limit of detection.
Inconclusive: Controls indicate inhibition or degradation of the sample, resulting in the inability to provide conclusive evidence for GCN presence or absence.

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Purchase Order: AESS-24-022
Contact: Avian Ecology Ltd
Issue Date: 08.07.2024
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GCN Report

Technical Report

SureScreen Scientifics

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SureScreen Scientifics

GCN eDNA Analysis

Summary

When great crested newts (GCN), *Triturus cristatus* , inhabit a pond, they continuously release small amounts of their DNA into the environment. By collecting and analyzing water samples, we can detect these small traces of environmental DNA (eDNA) to confirm GCN habitation or establish GCN absence.

Results

Lab ID	Site Name	OS Reference	Degradation Check	Inhibition Check	Result	Positive Replicates
6641	Legacy Pond 31	SJ3152646287	Pass	Pass	Negative	0/12
6642	Legacy Pond 37	SJ3179945986	Pass	Pass	Negative	0/12
6746	Legacy Pond 123	SJ3690946522	Pass	Pass	Negative	0/12
R693	Legacy Pond 74	SJ3402345862	Pass	Pass	Negative	0/12

Matters affecting result: none

Reported by: Daisy Chambers

Approved by: Christopher Troth

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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.
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