Mylen Leah Solar Farm

on behalf of Statkraft UK Ltd

Great Crested Newt Presence or Absence (eDNA) Survey Report

avianecology

Document Control				
Project Name:	Mylen Leah Solar Farm			
Project Number:	Statk-713-003380			
Report Title	Great Crested Newt Presence or Absence (eDNA) Survey Report			
Issue	**Date**	**Notes**	**Prepared**	**Reviewed**
V1	12/09/2024	Draft for Client Comment	C. Dean *PhD* Assistant Ecologist	J. Stevens *BSc (Hons)* Principal Ecologist
V2	15/10/2024	Draft for consultee comment	C. Dean *PhD* Assistant Ecologist	J. Stevens *BSc (Hons)* Principal Ecologist
V3	16/12/2024	Amended Red Line Boundary		J. Stevens *BSc (Hons)* Principal Ecologist
V4	20/12/2024	Final for Issue		J. Stevens *BSc (Hons)* Principal Ecologist

CONTENTS

TABLES

FIGURES

ANNEXES

1 INTRODUCTION

1.1 Background

1.1.1 Avian Ecology Ltd. was commissioned by Statkraft UK 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 the proposed Mylen Leah Solar Farm (the 'Proposed Development) on land south of the village of Melbourne in East Yorkshire ('the Site').

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 Site. Due to the low impact of solar energy developments on GCN habitats, and reflecting guidance published by Natural England, ponds beyond 250m from the Site were not considered.

1.2.2 Ponds subject to assessment are identified on **Figure 1**.

2 METHODOLOGY

2.1 Pond Selection

2.1.1 OS and aerial mapping were used to search for potential ponds within the Site or within a 250m buffer around the Site. This identified a total of 89 possible pond features (**Figure 1**).

2.1.2 Avian Ecology accessed the Site over two subsequent field seasons, with 21 ponds viewed in June 2023 and nine viewed in June 2024 (**Table 2**). One pond (P17) was viewed in both years. Of the remaining potential ponds, three features were found not to be ponds when viewed in the field and 56 features could not be viewed at all due to access limitations.

2.1.3 Note that due to iterative Site boundary changes, some ponds scoped in during surveys in 2023 and 2024 are now beyond the 250m survey area, and some ponds previously outside the survey area are now included. Of note, P39 was previously located within the 250m buffer, but is now within the Site, P40 and 41 were previously beyond the scope of surveys, but are now within the 250m buffer, P55 is now beyond the 250m buffer and P56 - 58 were located inside the draft application boundary and are now located beyond the 250m buffer. The survey areas in relation to the Site, including any areas not subject to survey, are shown on **Figure 1.**

2.2 Field Sampling

2.2.1 Field sampling was conducted by A. Tomlinson (licence ref: 2018-3465-CLS-CLS) A. Crone (licence ref: 2015-18548-CLS-CLS) and L. Quarton (assisting) from Avian Ecology Ltd; all suitably qualified ecologists with experience of conducting GCN eDNA surveys.

2.2.2 Sampling took place on 6th - 7th June 2023 and 4th - 5th June 2024.

2.2.3 Sixteen ponds were subject to eDNA survey sampling. The remaining 14 viewed ponds were not suitable for sampling, either because they were too dry, or because the water's edge could not be accessed (**Table 2**).

2.2.4 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.

2.2.5 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	Year Viewed	eDNA Sampling	HSI
P16	2023	N	N
P18	2023	N	N

[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	Year Viewed	eDNA Sampling	HSI
P19	2023	N	N
P20	2023	N	N
P23	**2023**	**Y**	**Y**
P27	2023	N	N
P36	2023	N	N
P42	2023	N	N
P43	**2023**	**Y**	**Y**
P45	**2023**	**Y**	**Y**
P49	**2023**	**Y**	**Y**
P50	2023	N	N
P54	2023	N	N
P56	**2023**	**Y**	**Y**
P57	**2023**	**Y**	**Y**
P58	2023	N	N
P59	2023	N	N
P60	**2023**	**Y**	**Y**
P62	2023	N	N
P71	**2023**	**Y**	**Y**
P3	**2024**	**Y**	**Y**
P4	2024	N	N
P7	**2024**	**Y**	**Y**
P8	**2024**	**Y**	**Y**
P13	**2024**	**Y**	**Y**
P17	**2024**	**Y**	**Y**
P24	**2024**	**Y**	**Y**
P25	**2024**	**Y**	**Y**
P26	**2024**	**Y**	**Y**

3 RESULTS

3.1 Pond Descriptions

3.1.1 Descriptions of the sampled ponds are listed below. Photographs are shown in **Appendix 1.**

P3: A long narrow pond with shallow banks. Mature willow and oak around the margins.

P7: A medium sized pond within woodland (oak, hawthorn, elder). The water surface completely covered with duckweed.

P8: A circular pond with steep sided banks. Some flag iris. Oak, willow, hawthorn, and ash around the margins.

P13: A small pond that appears to be used by cattle. Some hawthorn trees with bases submerged in the water.

P17: A circular pond dominated by mature/veteran willow.

P23: A medium sized, relatively deep pond with earthen vegetated banks of varying gradient. Water clarity murky, no notable inverts observed. Emergent vegetation includes flag iris, reed and rush. Marginal terrestrial vegetation includes, herbs, long sward grasses, scrub and overhanging trees.

P24: An enlarged ditch with mature willow and oak along the margins.

P25: A small, shallow pond with hawthorn and willow standing in the water.

P26: A long shallow pond with willow and hawthorn standing in the water.

P43: A medium sized circular pond with steep earthen banks, enclosed within a line of trees adjoining the hedgerow. The banks are vegetated with long sward grasses and tall herbs typical of enriched margins.

P45: A circular pond with steep banks. The water is murky but there are invertebrates, and emergent aquatic vegetation is present. Bankside there is grass/forb vegetation, overhanging willow, and bramble scrub. Located within an arable field and linked to an adjacent hedgerow.

P49: A small pond with steep banks. Water relatively clear. Partially dry in shallower areas and drying out along the. Emergent bulrush prominent. Some scrub, bramble, and large overhanging willow around the margins. Located within an arable crop but enclosed by grass/herb vegetation.

P56: A circular pond with gentle bank margins, vegetated with grasses and forbs. Water murky and pond scum present on the water surface. Emergent vegetation limited to rushes. Shaded by an overhanging oak. Located in a long sward pasture, adjacent to a hedgerow and road.

P57: A large circular pond. Drying out, with water present only at the centre of the depression. No emergent vegetation, but rush is present at the boundary of the pond footprint. Located in the centre of pasture/unmanaged grassland (pasture).

P60: A moat with gently sloping banks, circling an island, enclosed within a woodland parcel. The water murky and drying but many inverts observed. Completely shaded by mixed broadleaved woodland.

P71: A large C-shaped pond within a woodland parcel. Relatively gentle earth banks. Willow and pondweed extends across the water surface with some flag iris. The pond is entirely shaded by surrounding overhanging trees.

3.1.2 The rationale for ponds unable to be surveyed is given below, with photographs shown in Appendix 1.

P4: Junction of two wet ditches with limited survey access

P16: No pond present at this location

P18 & 19: Dry ditch present at this location, however no ponds

P20: Dry pond described as seasonally wet by landowner.

P27: enclosed pond with barbed wire fencing and large embankment. No access

P36: Pond currently dry but presence of rush and willow suggests may be seasonally wet

P42: Currently waterlogged ground but water levels insufficient to allow survey.

P50: Near dry at time of survey

P58: Dry depression with rushes present suggesting the area may hold water on occasion

P59: Pond not present or dried

P62: No access due to fencing

3.2 Habitat Suitability Index

3.2.1 The results show that 12 of the 16 ponds scored 'average' or 'good' in terms of their likely suitability for GCN, with the other ponds scoring lower (**Table 3**).

Table 3: HSI results.

Pond	Geographic Location	Pond Area	Permanence	Water Quality	Shade	Waterfowl	Fish	Pond Count	Terrestrial Habitat	Macrophytes	HSI Score*	Likelihood
P3	1	0.6	1	0.67	0.9	1	1	1	0.67	0.4	0.79	Good
P7	1	0.5	0.9	0.33	0.4	1	1	1	1	0.8	0.74	Good
P8	1	0.45	0.9	0.67	0.8	0.67	1	1	0.67	0.45	0.73	Good
P13	1	0.4	1	0.67	0.3	1	1	1	0.67	0.4	0.68	Average
P17	1	0.8	1	0.67	1	0.67	0.67	0.9	0.01	0.4	0.49	Poor
P23	1	0.2	0.9	0.67	0.5	0.67	0.67	0.85	0.67	0.3	0.58	Below Average
P24	1	0.2	1	0.67	0.5	1	1	1	0.67	0.3	0.65	Average
P25	1	0.15	1	0.67	0.2	1	1	1	0.67	0.3	0.58	Below Average

Pond	Geographic Location	Pond Area	Permanence	Water Quality	Shade	Waterfowl	Fish	Pond Count	Terrestrial Habitat	Macrophytes	HSI Score*	Likelihood
P26	1	0.25	1	0.33	0.2	1	1	0.95	1	0.3	0.59	Below Average
P43	1	0.5	1	0.67	0.7	1	0.67	1	0.67	0.3	0.71	Good
P45	1	0.75	0.9	0.67	0.5	1	0.67	1	0.67	0.4	0.73	Good
P49	1	0.25	1	0.67	0.3	1	1	0.9	0.67	0.8	0.69	Average
P56	1	0.2	0.5	0.67	0.7	1	1	0.85	1	0.3	0.64	Average
P57	1	0.5	0.5	0.67	1	1	1	0.85	0.67	0.3	0.70	Good
P60	1	0.85	0.5	0.67	1	1	1	0.9	1	0.3	0.77	Good
P71	1	0.97	0.9	0.67	1	1	0.67	0.95	0.67	0.4	0.79	Good

* 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.

3.3 eDNA Survey Results

3.3.1 Of the ponds sampled, three ponds (P49, P57, P60) 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.

P23	4904	Pass	Pass	0	Negative
P43	4907	Pass	Pass	0	Negative
P45	4906	Pass	Pass	0	Negative
P49	**4908**	**Pass**	**Pass**	**1**	**Positive**
P56	4915	Pass	Pass	0	Negative
P57	**4911**	**Pass**	**Pass**	**8**	**Positive**
P60	**4905**	**Pass**	**Pass**	**1**	**Positive**
P71	4909	Pass	Pass	0	Negative
P3	5473	Pass	Pass	0	Negative
P7	5467	Pass	Pass	0	Negative

P8	5474	Pass	Pass	0	Negative
P13	5472	Pass	Pass	0	Negative
P17	5476	Pass	Pass	0	Negative
P24	5465	Pass	Pass	0	Negative
P25	5475	Pass	Pass	0	Negative
P26	5470	Pass	Pass	0	Negative

FIGURE 1A: POND LOCATION PLAN

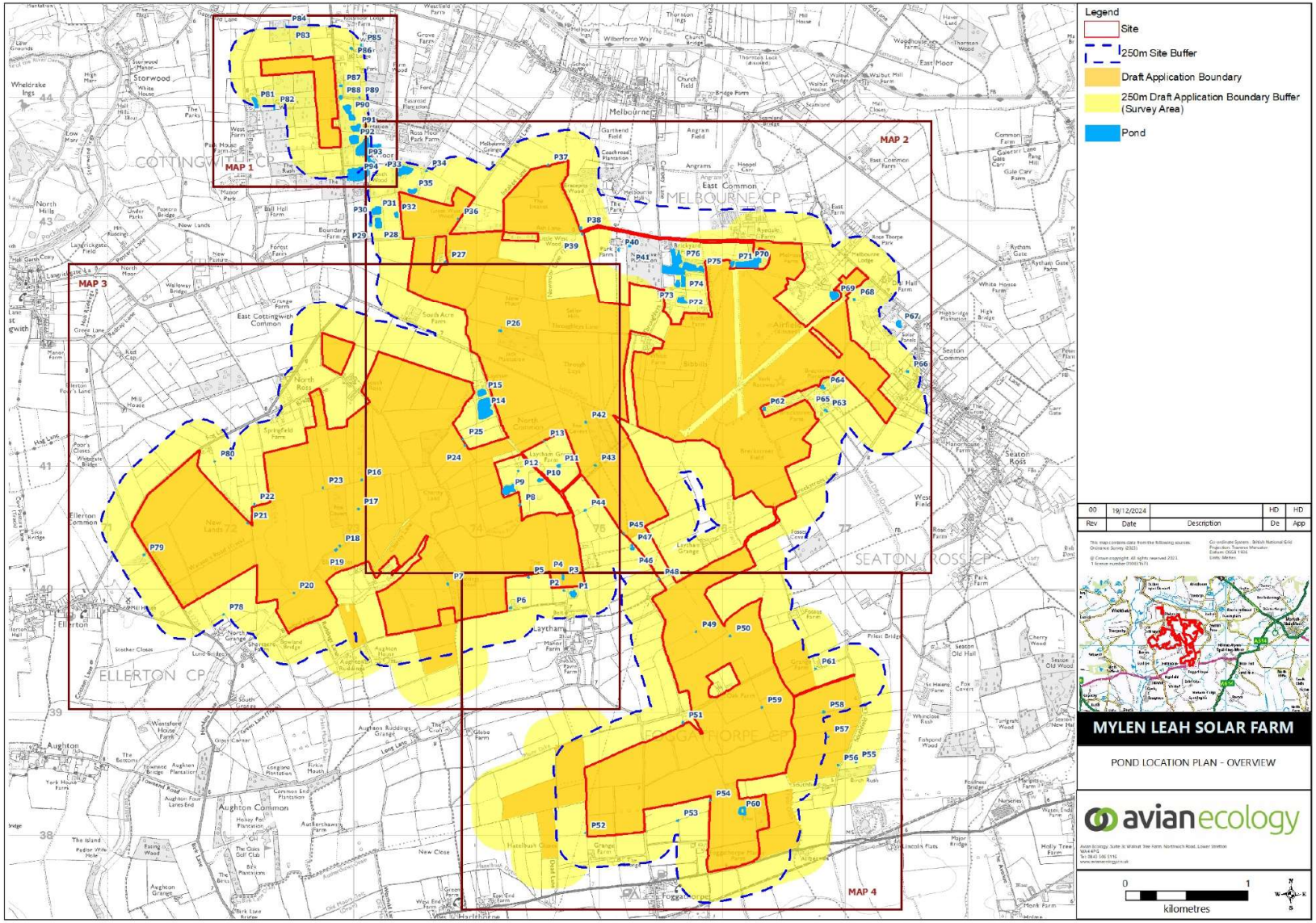


FIGURE 1B: POND LOCATION PLAN

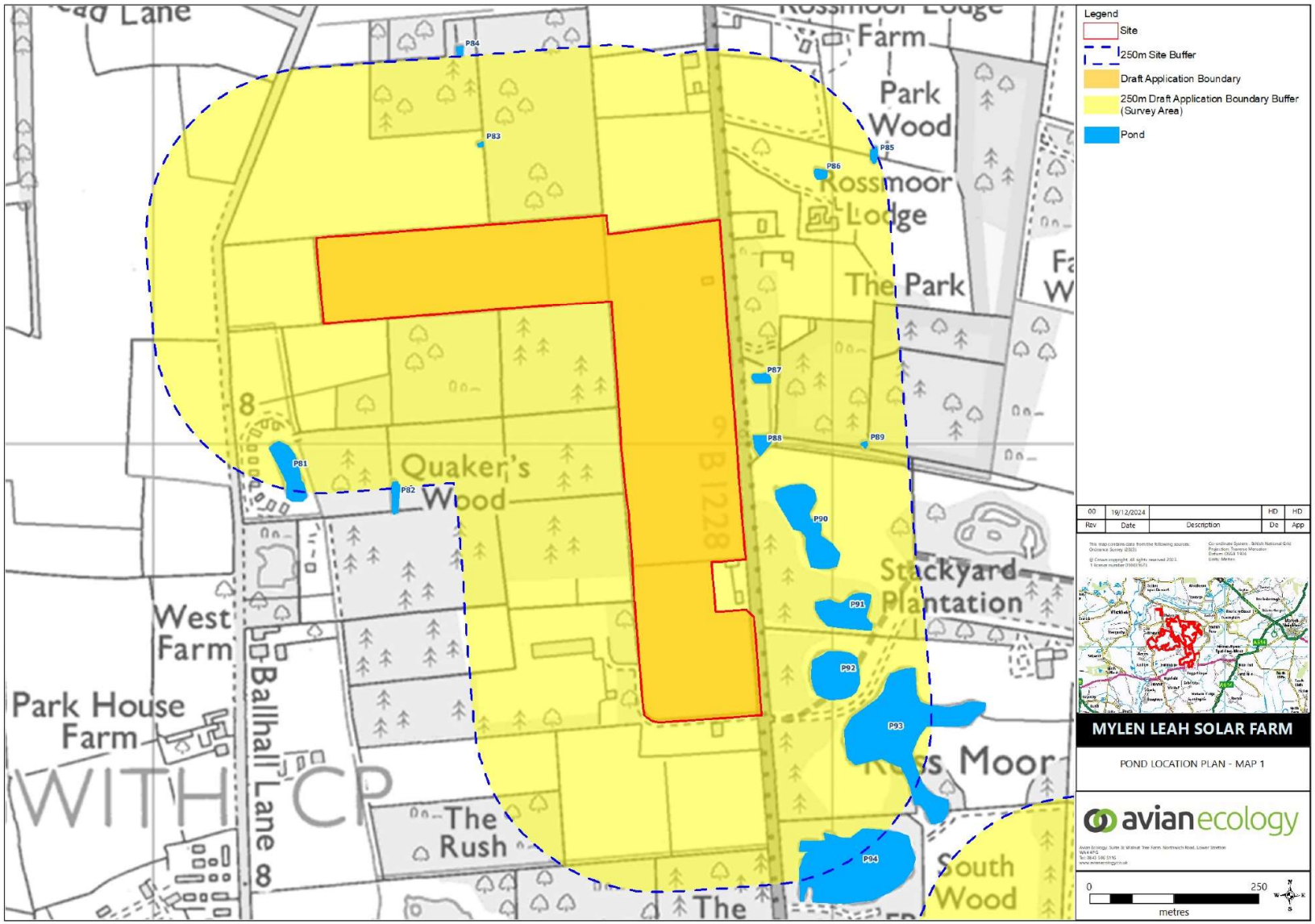


FIGURE 1C: POND LOCATION PLAN

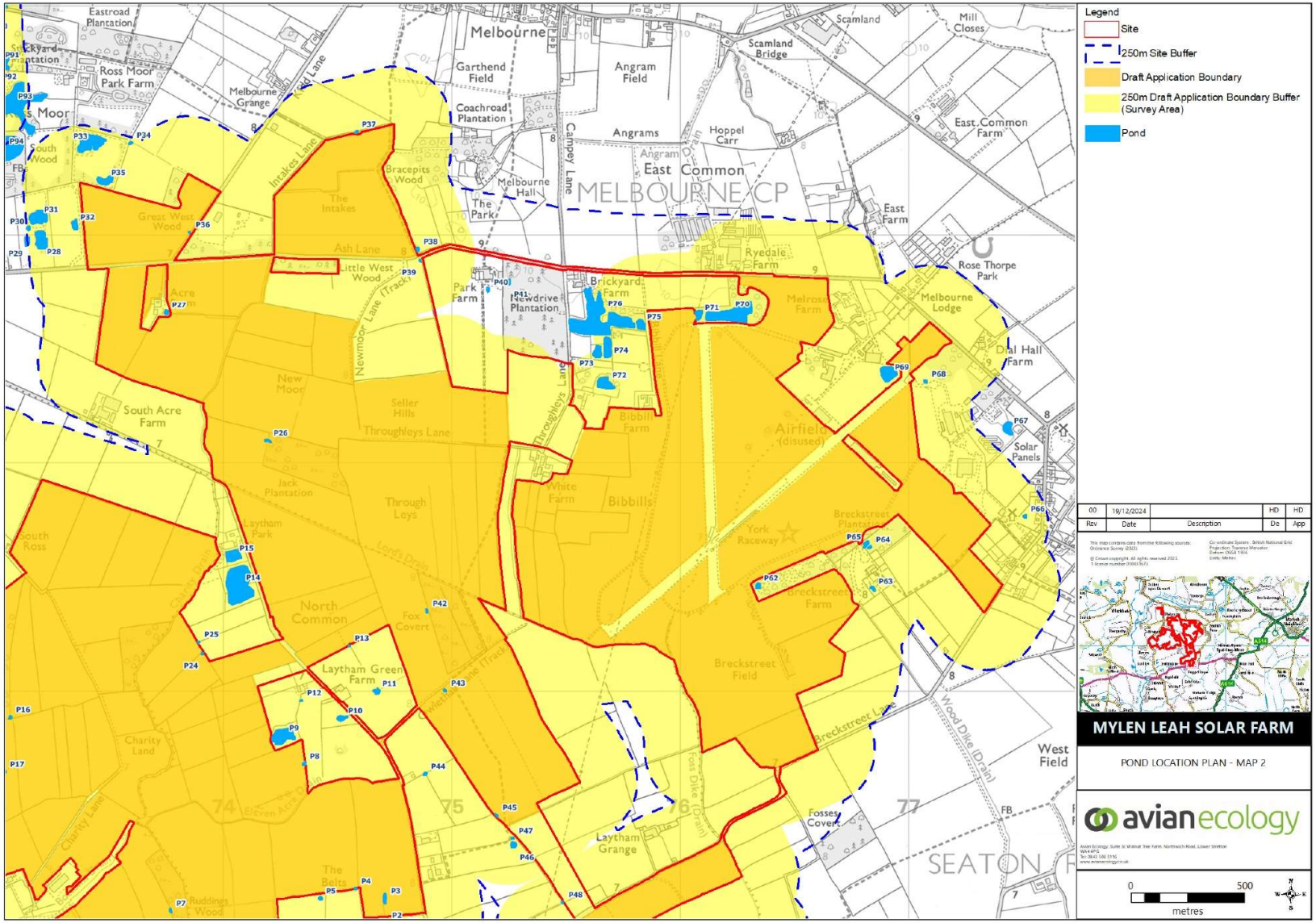


FIGURE 1C: POND LOCATION PLAN

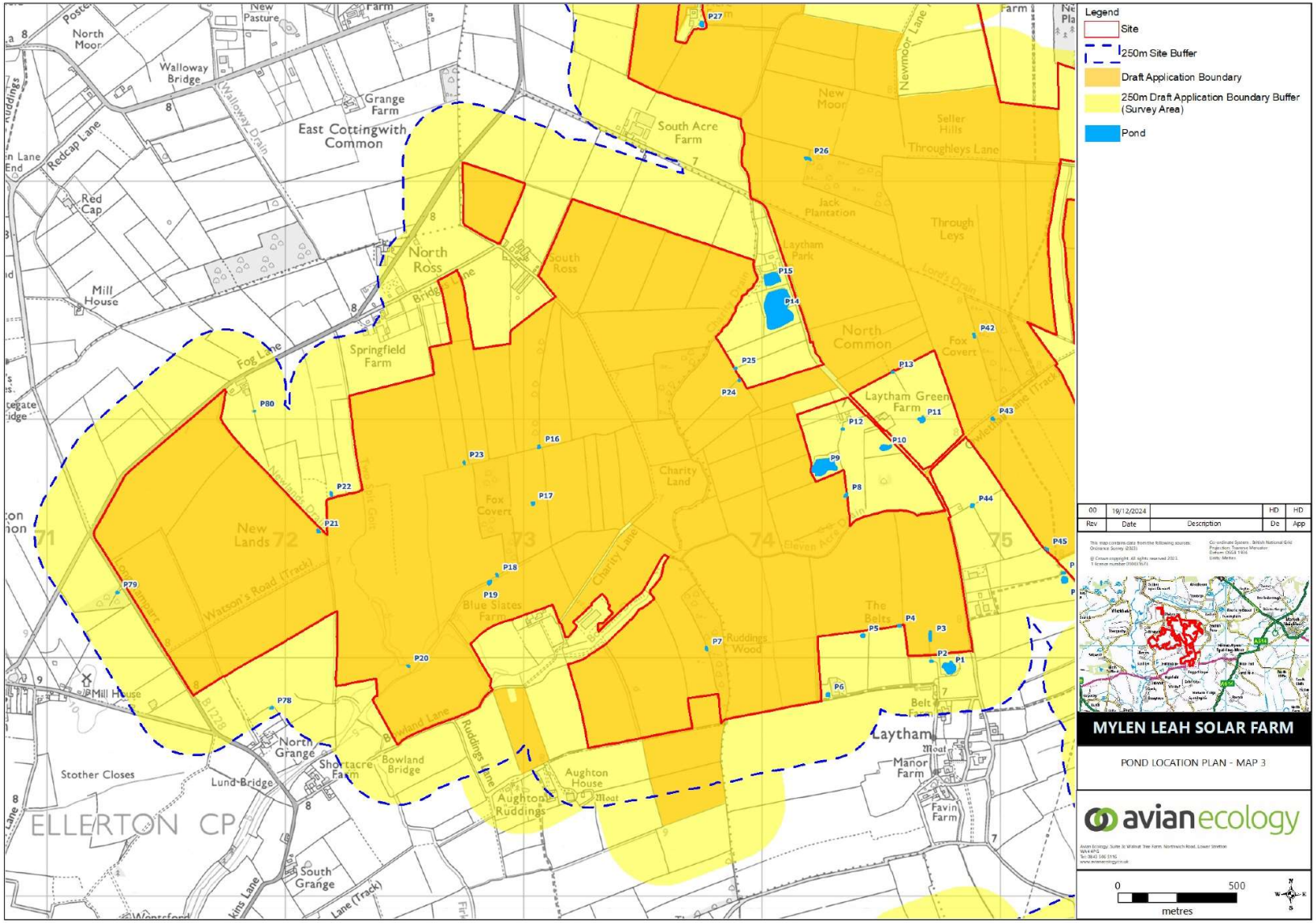
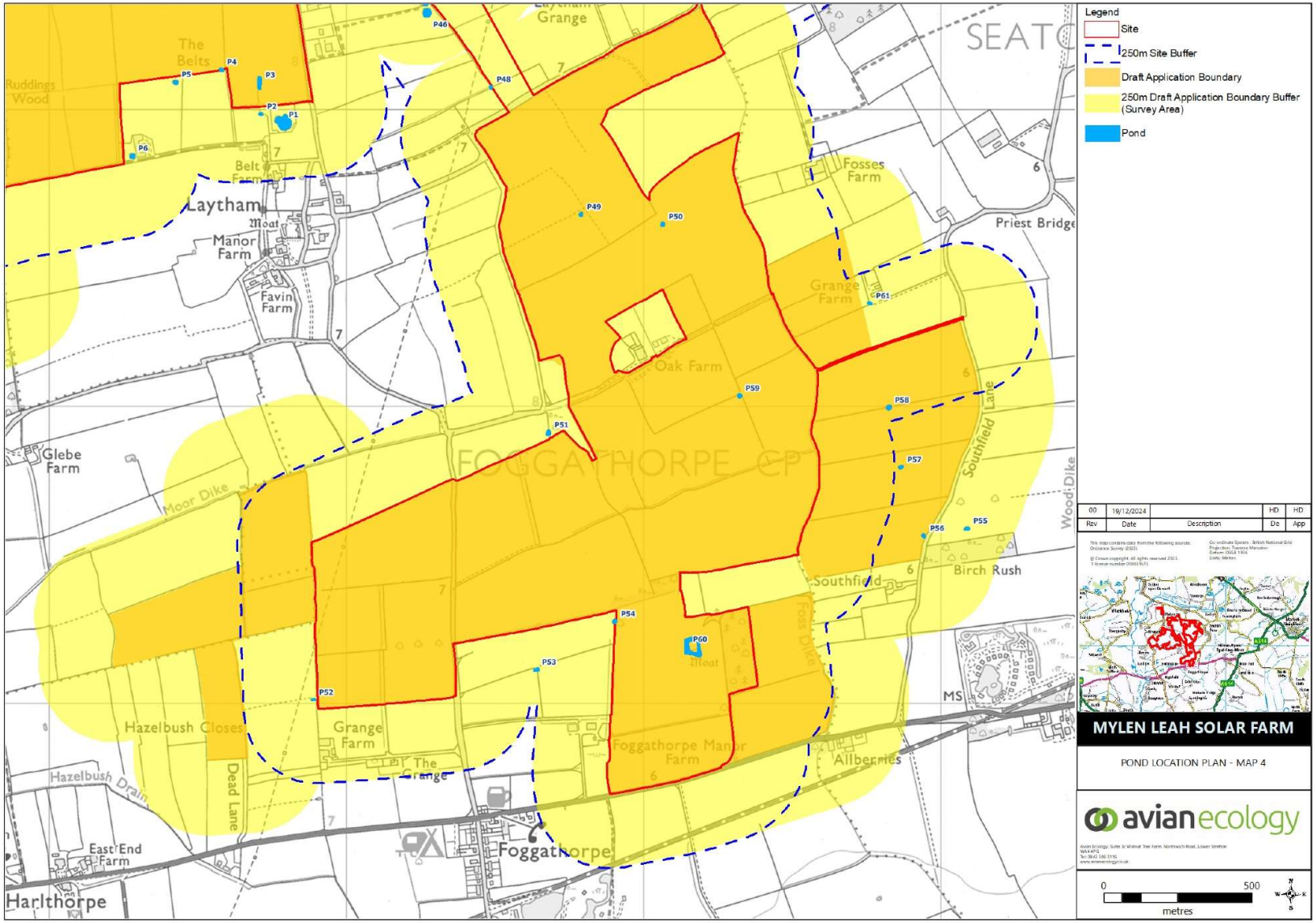


FIGURE 1E: POND LOCATION PLAN



Annex 1
Pond Photographs

Sampled ponds	
	
P3	P7
	
P8	P13
	
P17	P23

Sampled ponds	
	
P24	P25
	
P26	P43
	
P45	P49

Sampled ponds	
	
P56	P57
	
P60	P71

Ponds unable to be surveyed	
	
P4	P16

Ponds unable to be surveyed	
	
P18	P19
	
P20	P27
	
P36	P42
	
P50	P54

Ponds unable to be surveyed	
	
P58	P59
	
P62	

Annex 2

eDNA Laboratory Results

Survey 2023

SureScreen Scientifics

Folio No: E17975
Report No: 1
Purchase Order: AESS-23-036
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: 13/06/2023
Date Reported: 21/06/2023
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
4904	Melbourne - P23	SE 72757 40822	Pass	Pass	Pass	Negative	0
4905	Melbourne - P60	SE 76182 38199	Pass	Pass	Pass	Positive	1
4906	Melbourne - P45	SE 75203 40460	Pass	Pass	Pass	Negative	0
4907	Melbourne - P43	SE 74963 41019	Pass	Pass	Pass	Negative	0
4908	Melbourne - P49	SE 75802 39664	Pass	Pass	Pass	Positive	1
4909	Melbourne - P71	SE 76084 42648	Pass	Pass	Pass	Negative	0
4911	Melbourne - P57	SE 76870 38798	Pass	Pass	Pass	Positive	8

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

4915	Melbourne - P56	SE 76948 38577	Pass	Pass	Pass	Negative	0

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

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

METHODOLOGY

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

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

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

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

INTERPRETATION OF RESULTS

SIC: **Sample Integrity Check** [Pass/Fail]
When samples are received in the laboratory, they are inspected for any tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to inconclusive results.

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

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

Result: **Presence of GCN eDNA** [Positive/Negative/Inconclusive]
Positive: GCN DNA was identified within the sample, indicative of GCN presence within the sampling

location at the time the sample was taken or within the recent past at the sampling location.

Positive Replicates: Number of positive qPCR replicates out of a series of 12. If one or more of these are found to be positive the pond is declared positive for GCN presence. It may be assumed that small fractions of positive analyses suggest low level presence, but this cannot currently be used for population studies. In accordance with Natural England protocol, even a score of 1/12 is declared positive. 0/12 indicates negative GCN presence.

Negative: GCN eDNA was not detected or is below the threshold detection level and the test result should be considered as evidence of GCN absence, however, does not exclude the potential for GCN presence below the limit of detection.

Survey 2024

Folio No: 1760-2024
Purchase Order: AESS-24-014
Contact: Avian Ecology Ltd
Issue Date: 14.06.2024

GCN Report

Technical Report

SureScreen Scientifics

Folio No: 1760-2024
Purchase Order: AESS-24-014
Contact: Avian Ecology Ltd
Issue Date: 14.06.2024

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
5465	Melbourne, P24		Pass	Pass	Negative	0/12
5467	Melbourne, P7		Pass	Pass	Negative	0/12
5470	Melbourne, P26		Pass	Pass	Negative	0/12
5472	Melbourne, P13		Pass	Pass	Negative	0/12
5473	Melbourne, P3		Pass	Pass	Negative	0/12
5474	Melbourne, P8		Pass	Pass	Negative	0/12
5475	Melbourne, P25		Pass	Pass	Negative	0/12
5476	Melbourne, P17		Pass	Pass	Negative	0/12

Matters affecting result: none

Reported by: Daisy Chambers

Approved by: Lauryn Jewkes

SureScreen Scientifics Ltd, Morley Retreat, Church Lane, Morley, Derbyshire, DE7 6DE, UK
+44 (0)1332 292003 | scientifics@surescreen.com | surescreenscientifics.com

Folio No:	1760-2024
Purchase Order:	AESS-24-014
Contact:	Aviar Ecology Ltd
Issue Date:	14.06.2024

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.

SureScreen Scientifics Ltd, Morley Retreat, Church Lane, Morley, Derbyshire, DE7 6DE, UK
+44 (0)1332 292003 | scientifics@surescreen.com | surescreenscientifics.com