27th September 2021

Our reference: CES/105/GB

Sent by email only to: matt.christie@castlegreenhomes.uk

Cheshire Wildlife Trust,
Bickley Hall Farm,
Bickley,
Malpas,
Cheshire,
SY14 8EF

web: www.cheshirewildlifetrust.org.uk

F.A.O. Matt Christie

Dear Matt,

Re: Upper Denbigh Road, St Asaph – Great Crested Newt eDNA Survey Report

Introduction

Cheshire Ecological Services (CES), the commercial arm of the Cheshire Wildlife Trust, was instructed to carry out an eDNA survey for great crested newt in connection with proposed residential development of the above site.

CES has produced a Preliminary Ecological Appraisal (PEA) for the site, which identified one suitable waterbody (Pond 1) for great crested newt (GCN) within 250m of the site (see Appendix A – Site Location Plan). This pond is located on-site and is identified as 'Pond 1'.

The purpose of the survey was to establish whether a positive result for GCN presence would be returned for this pond. If so, this would confirm that GCN are a constraint to the site proposals. Due to the survey being undertaken outside of the GCN survey period of March to June, a negative result could not be relied upon.

Pond Description

Pond 1 (see Plates 1 & 2 below) is a small pond (area approximately 100m²) located within grazing fields. The pond was covered entirely in duckweed (*Lemna minor*) and pondweed (*Potamogeton sp.)* and had a thick mat of floating grass around its perimeter. There was some emergent/floating vegetation within the pond and marginal vegetation that could be suitable for GCN egg laying.

Plates 1 & 2: Pond 1 within the proposed development site

Methodology

GCN eDNA sampling is a survey technique accepted by Natural Resources Wales for the purposes of informing planning and any need for GCN mitigation.

Whilst noting that the site lies in Wales, survey guidelines issued by Natural England are accepted methodologies in Wales. Natural England's Technical Advice Note: *Field and laboratory sampling of great crested newt (Triturus cristatus) environmental DNA* details the methodology required for eDNA survey for GCN. **In summary, a single visit to each target pond should be made between mid-April and June, during the GCN breeding season**. Twenty sub-samples of 30ml are taken from the perimeter of each pond (where accessible), using sterile sampling equipment provided by a laboratory. Samples are then mixed to homogenise the samples, after which twelve 15ml samples are taken, combined with a preservative and thereafter sent to the laboratory for analysis.

The testing laboratory used for analysis for this site was ADAS Ltd, which has met Natural England's proficiency standard for eDNA analysis.

The collection of samples was undertaken on 6th September 2021 by Kyle Mellish BSc (Hons) ACIEEM and Lindsay Overstall BSc (Hons) MSc ACIEEM, who are both trained and experienced in eDNA survey methodologies and fully conversant with the English Nature Great Crested Newt Mitigation Guidelines 2001, GCN survey methodologies and current legislation relating to the species. Kyle and Lindsay also hold Natural England GCN survey licenses (No. 2015-16993-CLS-CLS & No: 2016-24282-CLS-CLS respectively) and as members of CIEEM, they both abide by the institute's code of conduct. N.B. collection of water samples for eDNA survey is not a licensable action and so the surveyors were not required to be licensed by Natural Resources Wales.

The protocol for eDNA testing was followed in full.

The survey was conducted outside the survey window for GCN eDNA survey, which ends 30th June. Therefore positive results only may be relied upon; negative results do not confirm that GCN are absent from the pond. A further negative result from a repeated survey between 15th April – 30th June 2022 would be required to establish the absence of GCN at Pond 1.

Results

Pond water samples were tested by ADAS Ltd on the 17th September 2021. Samples were found to be valid and PCR (Polymerase Chain Reaction) testing found all samples from the pond to be negative for GCN DNA (see Appendix B – eDNA Results).

Conclusions

The purpose of the survey was to establish whether a positive result for GCN presence would be returned for Pond 1. If so, this would confirm that GCN are a constraint to the site proposals. Due to the survey being undertaken outside of the GCN survey period, a negative result could not be relied upon.

The eDNA testing result for Pond 1 was negative, and may therefore not be relied upon.

Should GCN be absent from Pond 1 then a negative result would be expected, however, further survey at appropriate time of year will be required to confirm whether this is the case.

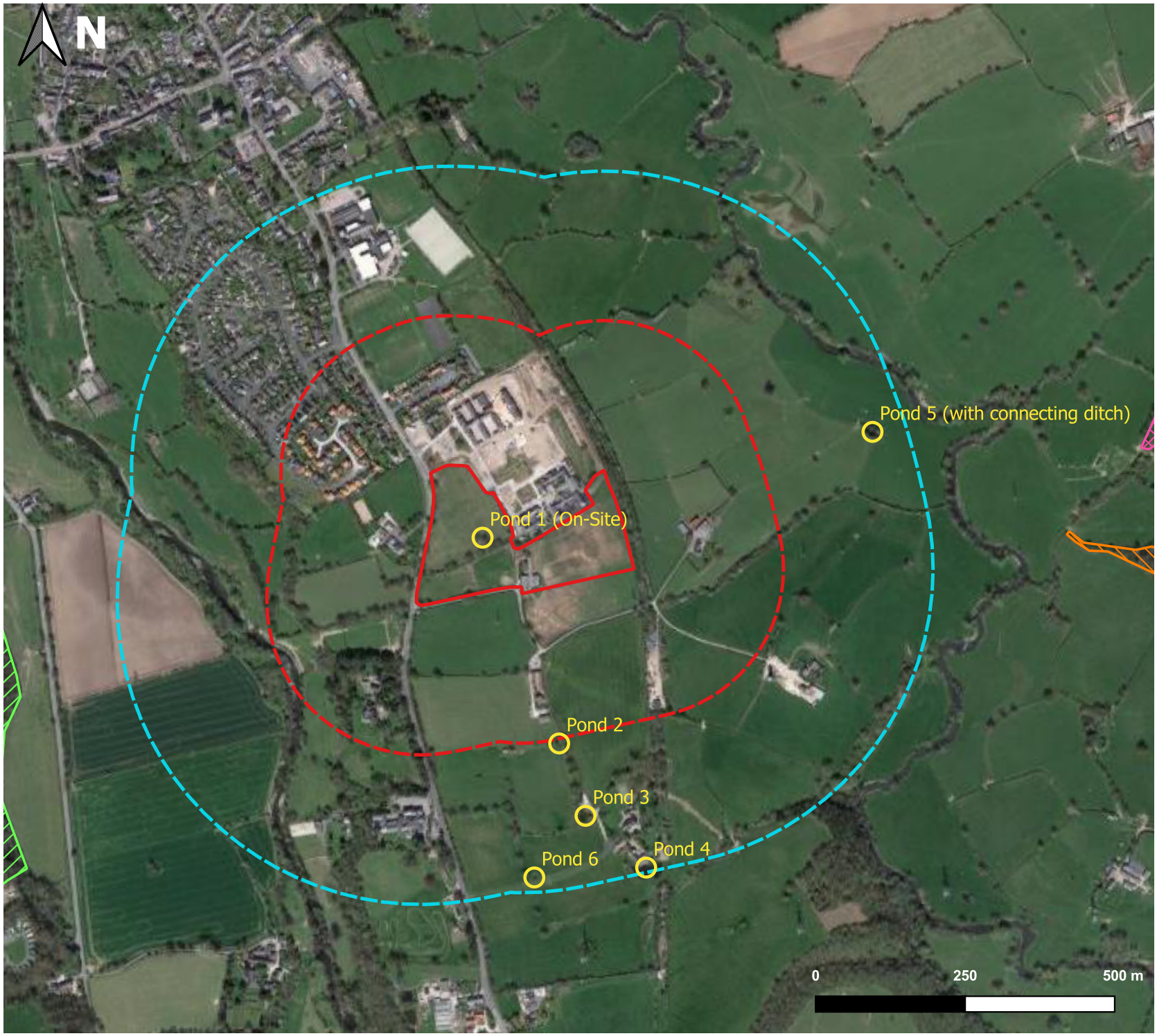
Grace Bishop BSc (Hons) MSc Qualifying CIEEM
Assistant Ecologist
gbishop@cheshirewt.org.uk

References

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, 2014.

Appendices

Appendix A: Site Location Plan



Site Boundary
250m Buffer
500m Buffer
Ponds

Local Wildlife Sites

Coed Fron & Eryl Hall Wood
Fachwen Dingle
Nant Y Waen

Project:	Land off Upper Denbigh Road, St Asaph, Denbighshire
Map:	Site Location Plan
CES Ref:	CES/750.105/09-21/KM
Scale:	Approximate
Date:	September 2021

Cheshire Ecological Services
Bickley Hall Farm
Bickley
Malpas
Cheshire
SY14 8EF

Appendix B: eDNA Survey Results

Client: Grace Bishop
Cheshire Wildlife Trust

ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

Tel: 01159 516747
Email: Helen.Rees@adas.co.uk

www.adas.uk

Sample ID: ADAS-0892	Condition on Receipt: High Sediment	Volume: Passed
Client Identifier: Pond 1, St Asaph, 105	Description: pond water samples in preservative	
Date of Receipt: 16/09/2021	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	17/09/2021
Degradation Control[§]	Within Limits	Real Time PCR	17/09/2021
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	17/09/2021
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)[#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:		Signed:	
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	20/09/2021	Date of issue:	20/09/2021

eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.*

[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#]Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

Appendix 1: Interpretation of results

Sample Condition

Upon sample receipt we score your samples according to quality: good, low sediment, medium sediment, high sediment, white precipitate, and presence of algae.

There are three reasons as to why sediment should be avoided:

1. It is possible for DNA to persist within the sediment for longer than it would if it was floating in the water which could lead to a false positive result i.e. in this case GCN not recently present but present a long time ago
2. In some cases sediment can cause inhibition of the PCR analysis used to detect GCN eDNA within samples which could lead to an indeterminate result.
3. In some cases sediment can interfere with the DNA extraction procedure resulting in poor recovery of the eDNA which in turn can lead to an indeterminate result.

Algae can make the DNA extraction more difficult to perform so if it can be avoided then this is helpful.

Sometimes samples contain a white precipitate which we have found makes the recovery of eDNA very difficult. This precipitate can be present in such high amounts that it interferes with the eDNA extraction process meaning that we cannot recover the degradation control (nor most likely the eDNA itself) at sufficient levels for the control to be within the acceptable limits for the assay, therefore we have to classify these type of samples as indeterminate.

What do my results mean?

A positive result means that great crested newts are present in the water or have been present in the water in the recent past (eDNA degrades over around 7-21 days).

A negative result means that DNA from the great crested newt has not been detected in your sample.

On occasion an inconclusive result will be issued. This occurs where the DNA from the great crested newt has not been detected but the controls have indicated that either: the sample has been degraded and/or the eDNA was not fully extracted (poor recovery); or the PCR inhibited in some way. This may be due to the water chemistry or may be due to the presence of high levels of sediment in samples which can interfere with the DNA extraction process. A re-test could be performed but a fresh sample would need to be obtained. We have successfully performed re-tests on samples which have had high sediment content on the first collection and low sediment content (through improved sample collection) on the re-test. If water chemistry was the cause of the indeterminate then a re-test would most likely also return an inconclusive result.

The results will be recorded as indeterminate if the GCN result is negative and the degradation result is recorded as:

1. evidence of decay - meaning that the degradation control was outside of accepted limits
2. evidence of degradation or residual inhibition - meaning that the degradation control was outside of accepted limits but that this could have been due to inhibitors not being removed sufficiently by the dilution of inhibited samples (according to the technical advice note)

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