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21 May 2021

Our reference: 2480167 Peak District (East) VIP – Great Crested Newt Letter Report

Dear Zoe

Great Crested Newt eDNA and survey results – Peak District (East) VIP

This letter report presents the results of the updated habitat suitability index (HSI) assessments and Great Crested Newt (GCN) eDNA surveys of the 10 waterbodies previously identified within 500 m of the proposed working area. Surveys were carried out during two site visits in April 2021 by Lewis Wright, Claire Hesketh and Daniel Berry.

Methods

Habitat Suitability Index (HSI) Assessment

The HSI assessment uses a scale from 0 (unsuitable) to 1 (optimal) to assess the suitability of a pond for Great Crested Newts. It is derived from information on ten factors e.g. location, water quality, shading etc. (by convention called suitability indices (SIs) and coded as SI1 to SI10). Background Data Search data and field survey results are used with graphs in Oldham et al. 20001 to score each SI individually on a scale from 0.01 to 1, and an overall HSI score for the pond is calculated using the following formula:

HSI = (SI1 × SI2 × SI3 × SI4 × SI5 × SI6 × SI7 × SI8 × SI9 × SI10)1/10.

The resultant score allows the suitability of the pond to be categorised as poor, below average, average, good or excellent. Although the score gives an indication of suitability, it does not confirm presence/ likely absence of GCN and therefore further investigation is required.

eDNA Analysis

This survey technique involves analysing water samples to confirm the presence or absence of Great Crested Newt DNA (which can be naturally shed by newts, through skin secretions, excrement etc., when in water bodies). A total of 20 samples were taken from the pond, whilst being careful not to enter the water and contaminate the sample. The locations of samples were spaced evenly around the water body margin. However, sampling locations targeted the vegetated areas which may be used during egg laying and open water areas which newts may

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use for displaying. The samples were then collected into a single sample bag and gently agitated to homogenize the samples before six sub-samples were taken and preserved in ethanol-based preservative. Samples were then sent to the laboratory for analysis.

Results

A habitat suitability index assessment was carried out for all 10 ponds in April 2021, the results of which are provided in Table 1. Following this, GCN eDNA surveys were undertaken for all ponds, excluding pond 5, and produced negative results for all nine water bodies tested. The laboratory analysis can be found in Appendix A.

Table 1. Updated HSI scores and eDNA results for waterbodies within 500m of the proposed working area

Pond ID	2021 HSI score	2021 Rating	Scoped in/out	eDNA results
Pond 1	0.67	Average	In	Negative
Pond 2	0.53	Below Average	In	Negative
Pond 3	0.75	Good	In	Negative
Pond 4	0.70	Good	In	Negative
Pond 5	N/A - dry	Not suitable	Out	N/A
Pond 6	0.72	Good	In	Negative
Pond 7	0.71	Good	In	Negative
Pond 8	0.69	Average	In	Negative
Pond 9	0.70	Good	In	Negative
Pond 10	0.69	Average	In	Negative

Conclusions

The results of the eDNA analysis demonstrate that Great Crested Newts are not present on the site. Consequently, no further actions in regard to Great Crested Newts are required.

If you have any questions please contact Lewis on 07741 725930 or email Lewis.Wright@rskbiocensus.com.

Yours sincerely,



Claire Hesketh, Assistant Ecologist

Technical and Commercial Review by



Tom Smith, Operations Director
On behalf of RSK Biocensus

APPENDIX A – eDNA results

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Sample ID: ADAS-0668	Condition on Receipt: Good	Volume: Passed
Client Identifier: 1, 2480167	Description: pond water samples in preservative	
Date of Receipt: 19/04/2021	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	20/04/2021
Degradation Control[§]	Within Limits	Real Time PCR	20/04/2021
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	20/04/2021
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:	22/04/2021	Date of issue:	22/04/2021

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.

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[†] 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.

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Sample ID: ADAS-0669	Condition on Receipt: Good	Volume: Passed
Client Identifier: 2, 2480167	Description: pond water samples in preservative	
Date of Receipt: 19/04/2021	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	22/04/2021
Degradation Control[§]	Within Limits	Real Time PCR	22/04/2021
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	22/04/2021
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
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Sample ID: ADAS-0670	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: 3, 2480167	Description: pond water samples in preservative	
Date of Receipt: 19/04/2021	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	21/04/2021
Degradation Control[§]	Within Limits	Real Time PCR	21/04/2021
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	21/04/2021
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
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Sample ID: ADAS-0671	Condition on Receipt: Good	Volume: Passed
Client Identifier: 4, 2480167	Description: pond water samples in preservative	
Date of Receipt: 19/04/2021	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	21/04/2021
Degradation Control[§]	Within Limits	Real Time PCR	21/04/2021
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	21/04/2021
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
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Sample ID: ADAS-0672	Condition on Receipt: Good	Volume: Passed
Client Identifier: 6, 2480167	Description: pond water samples in preservative	
Date of Receipt: 19/04/2021	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	1 of 2	Real Time PCR	21/04/2021
Degradation Control[§]	Within Limits	Real Time PCR	21/04/2021
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	21/04/2021
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

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Sample ID: ADAS-0673	Condition on Receipt: Good	Volume: Passed
Client Identifier: 7, 2480167	Description: pond water samples in preservative	
Date of Receipt: 19/04/2021	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	21/04/2021
Degradation Control[§]	Within Limits	Real Time PCR	21/04/2021
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	21/04/2021
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
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Sample ID: ADAS-0674	Condition on Receipt: Good	Volume: Passed
Client Identifier: 8, 2480167	Description: pond water samples in preservative	
Date of Receipt: 19/04/2021	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	21/04/2021
Degradation Control[§]	Within Limits	Real Time PCR	21/04/2021
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	21/04/2021
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
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Sample ID: ADAS-0675	Condition on Receipt: Good	Volume: Passed
Client Identifier: 9, 2480167	Description: pond water samples in preservative	
Date of Receipt: 19/04/2021	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	21/04/2021
Degradation Control[§]	Within Limits	Real Time PCR	21/04/2021
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	21/04/2021
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Sample ID: ADAS-00676	Condition on Receipt: Good	Volume: Passed
Client Identifier: 10, 2480167	Description: pond water samples in preservative	
Date of Receipt: 19/04/2021	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	20/04/2021
Degradation Control[§]	Within Limits	Real Time PCR	20/04/2021
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	20/04/2021
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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)