

CARLTON MAIN BRICKWORKS

Appendi 7.1 - Great Crested Newt eDNA Survey Report

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1.0 Introduction

SLR Consulting Limited (SLR) was commissioned by Michelmersh Brick Holdings Plc in May 2020 to carry out a Great Crested Newt (GCN) (*Triturus cristatus*) environmental DNA (eDNA) survey at Carlton Main Brick Works, which is situated off Clayburn Road, on the southern edge of Grimethorpe, South Yorkshire (approximate Central OS Grid Reference: SE 413 082).

The survey was commissioned in order to inform the Ecology Chapter of an Environmental Impact Assessment (EIA) Report to accompany a planning application for a proposed southern extension to quarry workings.

The need for this survey was identified following a review of desk study information and OS mapping and following an ecological walkover survey undertaken by SLR Consulting in May 2020.

An amphibian survey was also requested by Barnsley Metropolitan Borough Council's Biodiversity Officer in a Scoping Opinion response dated January 2020.

This report presents the findings of GCN eDNA surveys undertaken in June 2020 and updated in May 2022.

The above surveys involved collecting and analysing water samples from ponds/waterbodies within the active quarry and proposed extension area (herein referred to as the 'Site') and a 250m radius (where evident and accessible, and where no major barriers to dispersal exist). This report also presents the results of Habitat Suitability Index (HSI) assessments of the ponds.

The aim of the survey was to determine the presence or absence of GCN within the ponds, and therefore determine whether GCN are likely to occur within the Site, in order to inform the development of an appropriate mitigation strategy, if required.

The assessment of impacts resulting from the proposed development, and the development of mitigation measures, if required, are beyond the scope of this report and will be covered in the Ecology Chapter of the EIA.

2.0 Relevant Legislation¹

GCN is protected under the Conservation of Habitats and Species Regulations 2017 (the Habitats Regulations)² and the Wildlife and Countryside Act 1981 (as amended)³, making it an offence to deliberately kill, injure or take a GCN; to possess, control, transport, sell or exchange a GCN/any part of a GCN; to damage, destroy or obstruct access to any place that a GCN uses for shelter or protection; or to intentionally or recklessly disturb⁴ GCN while they are occupying a structure or place that they use for shelter or protection.

GCN is listed as a 'species of principal importance for the purpose of conserving biodiversity in England' under Section 41 of the Natural Environment and Rural Communities (NERC) Act 2006⁵. GCN is also a local priority species under the Barnsley Biodiversity Action Plan (second edition)⁶.

¹ Please note that this legal information is a summary and intended for general guidance only. The original legal documents should be consulted for definitive information.

² <http://www.legislation.gov.uk/ukxi/2017/1012/contents/made>

³ www.opsi.gov.uk/RevisedStatutes/Acts/ukpga/1981/cukpga_19810069_en_1

⁴ Disturbance, as defined by the Conservation of Habitats and Species Regulations 2017, includes in particular any action which impairs the ability of animals to survive, breed, rear their young, hibernate or migrate (where relevant); or which affects significantly the local distribution or abundance of the species.

⁵ http://www.opsi.gov.uk/acts/acts2006/ukpga_20060016_en_1

⁶ <http://www.barnsleybiodiversity.org.uk/biodiversityplan.html>

3.0 Methodology

3.1 Desk Study

Existing amphibian records (in addition to records of other protected and notable species) for the Site and a 2km radius were obtained from Barnsley Biological Records Centre (BBRC) in May 2020 and were updated in May 2022.

3.2 Field Survey

3.2.1 Habitat Suitability Index (HSI) Assessment

Ordnance Survey maps were reviewed in order to identify off-Site ponds within a 250m radius of the Site boundary. Evident and accessible ponds within the Site and a 250m radius, where there was an absence of a major barrier to dispersal between the pond and the Site, were assessed for their potential to support GCN using standard Habitat Suitability Index (HSI) assessment criteria⁷, on the 22nd May 2020. Pond locations are shown in Drawing 1.

GCN HSI scores are calculated using ten parameters, to assess the likely value of any given waterbody to support breeding GCN. The parameters used for the assessment are: site location; pond area; frequency of pond drying; water quality; shade; waterfowl; fish; presence of other ponds in the area; terrestrial habitat; and macrophyte communities. Each parameter scores a value of between 0.01 and 1. These scores are then multiplied and then 'ten rooted' to produce a geometric mean score, of between 0 and 1. The following categorical scale is then used to estimate the overall suitability of the waterbody concerned:

HSI score	Pond suitability for GCN
<0.5	Poor
0.5-0.59	Below average
0.6-0.69	Average
0.7-0.79	Good
>0.8	Excellent

3.2.2 Great Crested Newt eDNA Survey

A total of four ponds/lagoons were sampled for the presence of GCN eDNA; three within the active quarry area (P1-P3 in Drawing 1) and one located c. 250m south of the extension area (P4 in Drawing 1).

A further two ponds were identified from OS mapping within 250m of the Site, however, these were found to be dry when inspected in May and June 2020 and May 2022 (P5 and P6 in Drawing 1). These ponds were therefore scoped out from survey. There are also a number of ditches within the extension area and in the surrounding New Park Springs, however, these were also found to be dry and were scoped out from survey.

Ponds P1-P4 were initially sampled for the presence of GCN eDNA on the 4th June 2020, following the Natural England approved protocol⁸. Twenty samples were collected from the ponds using sterile equipment provided by ADAS, at points evenly spread out along the pond perimeter, such that a minimum of 80% of the margins were sampled. The water at each sampling area was gently stirred using a sterile ladle before samples were

⁷ Oldham, R.S., Keeble, J., Swan, M.J.S., and Jeffcote, M. (2000) Evaluating the Suitability of Habitat for the Great Crested Newt (*Triturus cristatus*). *Herpetological Journal* 10: 143-155.

⁸ Biggs J, Ewald N, Valentini A, Gaboriaud C, Griffiths RA, Foster J, Wilkinson J, Arnett A, Williams P and Dunn F 2014. *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.

taken, to mix up DNA which tends to sink, whilst ensuring that sediment on the pond bottom was not disturbed, where historical DNA can persist.

The samples were then fixed in an ethanol preserving solution, and sent to ADAS laboratory for analysis, using the Natural England approved method⁸. According to Biggs *et al.* (2014)⁸ GCN DNA can be detected within the pond water for up to 21 days after a GCN (including efts) has left the water; a 99.3 % detection rate is achieved when 80 – 90 % of the waterbody margin is sampled.

In order to avoid contamination, the surveyors avoided entering the water. Latex gloves were worn when sampling and only sterile equipment came into contact with the water.

ADAS laboratory reported that the samples from Ponds P1-P3 had formed a white precipitate, which inhibited the DNA analysis process and led to an indeterminate result. This is most likely due to the polluted nature of the water within the brickworks lagoons, due to their use in the quarrying process and the adjacent former landfill. Following advice from ADAS laboratory, the three quarry ponds/lagoons (P1-P3) were further sampled on the 25th June 2020. The same methodology was used, with the exception that the water samples were passed through a filter which filtered out contaminants from the water that were inhibiting the DNA extraction process.

The GCN eDNA survey was updated on 4th May 2022. Ponds P1-P3 were sampled, however, pond P4 was dry and could not be sampled. The same methodology was used, including the use of filters to reduce contaminants from the water.

3.3 Personnel

Surveys were undertaken by SLR Senior Field Ecologist Dr Dan Alexander PhD PgCert BSc (Hons) ACIEEM, SLR Consultant Ecologist Ms Julia Kozłowska BSc (Hons) MCIEEM, and Assistant Ecologist Mr Harry Sunter MSc.

This report was produced by Dr Dan Alexander and Ms Julia Kozłowska, who hold Natural England Survey Class 1 licences for great crested newts. The report has been peer reviewed by SLR Associate Ecologist Hazel Douglas MBiolSci. Hazel has over seven years' experience within ecological consultancy and is experienced in GCN survey. The 2022 updated report has been peer reviewed by SLR Principal Ecologist Andy Law CEcol MCIEEM who has over 30 years professional experience.

3.4 Limitations

The use of a filter in the water sampling process deviates from the Natural England approved methodology⁸, as it is not possible to undertake a degradation control test, which tests for the degradation of eDNA between taking the sample and analysing the samples. However, ADAS states that the filter methodology is a frequently used practice within analytical laboratories. The study on the efficacy of eDNA testing⁸, on which the Natural England approved method is derived from, did not have any instances of the eDNA within the samples degrading; nonetheless a degradation control is included in the approved methodology as a precaution.

The samples collected using the filter method were preserved using an ADAS issued preservative, and stored in a fridge prior to analysis, to minimise the chance for DNA degradation.

Given the results of the eDNA study⁸ and the fact that the samples were kept cool, and a preservative was added, the likelihood of obtaining a false negative result due to degradation in the sample is very low. As such, it is considered that the re-sampling provides a valid result on the presence or absence of GCN eDNA within the ponds.

4.0 Results

4.1 Desk Study

The desk study from BBRC returned a record of c.100 GCN (i.e. a large population) in four years between 2000 and 2010, in pitfall traps during open casting at Cudworth, located c. 1.2km to the west of the Site. In addition to this a further 21 GCN records were returned dated between 2000 and 2020 with the closest of these located c.1.6km west of Site.

Forty four toad (*Bufo bufo*) records, 62 smooth newt (*Lissotriton vulgaris*) records, one palmate newt (*Lissotriton helveticus*) record and 36 common frog (*Rana temporaria*) records were also provided, dating from between 1989 and 2020, from various locations, with the closest records from the Site being from a pond c. 0.3km to the west on the far side of the A6195.

4.2 Field Survey

4.2.1 Habitat Suitability Index Assessment of Ponds

A summary of the results of the HSI assessments, along with a description and photograph of each pond, are provided in Table 4-1. The full HSI calculations are provided in Appendix 1.

Table 4-1: Pond Descriptions and HSI Assessments

Pond Ref. No.	Description	Photograph	HSI Score*	Suitability Assessment
P1	Settlement lagoon within active quarry area measuring c. 32 x 10m; poor water quality with an absence of aquatic and marginal vegetation.		0.53	Below average
P2	Settlement lagoon within active quarry area measuring c. 130 x 7m; poor water quality with very little aquatic and marginal vegetation.		0.54	Below average

Pond Ref. No.	Description	Photograph	HSI Score*	Suitability Assessment
P3	Settlement lagoon within active quarry area measuring c. 130 x 10m; poor water quality with very little aquatic and marginal vegetation.		0.54	Below average
P4	Located c. 250m south of the extension area. Man-made channel with culvert at one end, with concrete sides and bottom, measuring c. 15 x 2m, shaded with no aquatic vegetation.		0.55	Below average
P5	Dry pond within reedbed c. 240m south of the extension area which has dried up.		0.58	Below average
P6	Dry pond within reedbed c. 220m south-west of the quarry and 650m west of the extension area, which has dried up.		0.68	Average

* The definition of pond suitability using HSI scores is categorised as follows: <0.51 = poor, 0.5-0.59 = below average, 0.6-0.69 = average, 0.7-0.79 = good, >0.8 = excellent

4.2.2 Great Crested Newt Presence / Absence eDNA Surveys

The results of the GCN eDNA surveys are summarised in Table 4-2 and a copy of the reports produced by ADAS laboratory are provided in Appendix 2. GCN eDNA was not detected in any of the ponds sampled.

A dead smooth newt was recorded incidentally within P1 during water sampling on 4th June 2020.

Table 4-2: Results of eDNA Survey

Pond Ref. No.	Results of eDNA analysis		
	First test: 4 th June 2020	Re-test using filter: 25 th June 2020	Updated test : 4 th May 2022
P1	Indeterminate	Negative	Negative
P2	Indeterminate	Negative	Negative
P3	Indeterminate	Negative	Negative
P4	Negative	N/A	Dry

5.0 Summary and Conclusion

Desk study results from BBRC returned a record of a large GCN population c. 1.2km west of the Site. Dispersal from this population onto the Site is very unlikely due to the separation distance involved and the separation from the Site by the A6195 road which presents a major barrier to dispersal.

Six waterbodies identified within a 250m radius of the Site were subject to HSI assessments in May 2020, and four of these (i.e. those containing water) were subject to GCN eDNA presence / absence surveys in June 2020. Three ponds which contained water in 2022 (P1-P3) were subject to an updated GCN eDNA presence / absence survey in May 2022.

The ponds were assessed as having below average or average suitability for GCN using HSI parameters.

Off-Site pond P4 tested negative for GCN eDNA on 4th June 2020. The initial analysis of samples taken on 4th June 2020 failed for on-Site ponds P1-P3, due to a white precipitate within the samples that inhibited the analysis process. Samples were re-taken on 25th June 2020 and 4th of May 2022 and passed through a filter before re-analysis; these samples tested negative for GCN eDNA on both occasions. Although the re-analysis did not include a degradation control test, the likelihood of degradation of the samples is considered to be low, based on the Biggs *et al.* (2014)⁸ eDNA efficacy study upon which the Natural England approved method is derived. As such, the results are considered to be sufficient to determine GCN absence.

The two ponds that were found to be dry may periodically contain water, however the fact that the ponds were found to be dry as early in the year as May of both survey years, indicates that they are unlikely to be capable of supporting a breeding population of GCN, which require ponds to retain water until August for eft survival.

Overall, given the lack of suitable ponds testing positive for GCN within the Site and 250m radius, it is highly unlikely that GCN occur on the Site.

The assessment of impacts on GCN resulting from the proposed development and the development of appropriate mitigation measures, will be covered separately in the Ecology Chapter of the EIA.

DRAWINGS

Drawing 1: Locations of Ponds

441000 441500 442000

408500

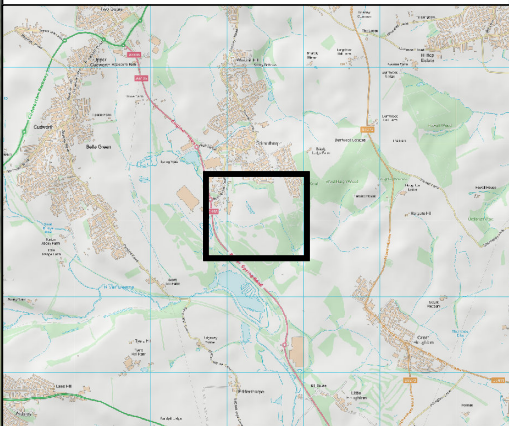
408000

03563.00037.21.02.0 Figure 1 Locations of Ponds



LEGEND

- Site Boundary
- Pond Surveyed: Negative for Great Crested Newt
- Dry Pond







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CARLTON MAIN BRICKWORKS
GREAT CRESTED NEWT EDNA
SURVEY REPORT
LOCATIONS OF PONDS
DRAWING 1

Scale 1:4,000 @ A3

Date
APRIL 2021

Appendix 1: Results of HSI Assessments of Ponds

Pond number/reference		P1		
OS Grid reference		SE 41080 08080		
Location details		Immediately adjacent to the site, within the active quarry area.		
Habitat Suitability Index				
SI1.	Map location	A/B/C	A	SI value 1.00
SI2.	Surface area	rectangle/ellipse/irregular	irregular	
		length (m)		
		width (m)		
		OR estimate (m ²) if irregular	420	
		area (m ²) =	420	0.84
SI3.	Desiccation rate	never/rarely/sometimes/frequently	rarely	1.00
SI4.	Water quality	good/moderate/poor/bad	bad	0.01
SI5.	Shade	% of margin shaded 1m from bank	0	1.00
SI6.	Waterfowl	absent/major/minor	absent	1.00
SI7.	Fish population	absent/possible/minor/major	absent	1.00
SI8.	Pond density	number of ponds within 1km	8	1.00
SI9.	Terrestrial habitat	good/moderate/poor/isolated	moderate	0.67
SI10.	Macrophyte cover	%	0	0.31
			HSI =	0.53
			provisional HSI =	0.49
Use provisional HSI value if above 0.75				
			Date undertaken	22.5.20

Pond number/reference	P2		
OS Grid reference	SE 41285 08138		
Location details	Within the site, within the active quarry area.		
Habitat Suitability Index			
SI1. Map location	A/B/C	A	SI value 1.00
SI2. Surface area	rectangle/ellipse/irregular	irregular	
	length (m)		
	width (m)		
	OR estimate (m ²) if irregular	1040	
	area (m ²) =	1040	0.94
SI3. Desiccation rate	never/rarely/sometimes/frequently	rarely	1.00
SI4. Water quality	good/moderate/poor/bad	bad	0.01
SI5. Shade	% of margin shaded 1m from bank	0	1.00
SI6. Waterfowl	absent/major/minor	absent	1.00
SI7. Fish population	absent/possible/minor/major	absent	1.00

SI8. Pond density	number of ponds within 1km	8	1.00
SI9. Terrestrial habitat	good/moderate/poor/isolated	moderate	0.67
SI10. Macrophyte cover	%	1	0.32
			HSI = 0.54
Use provisional HSI value if above 0.75			provisional HSI = 0.50
			Date undertaken 22.5.20

Pond number/reference	P3		
OS Grid reference	SE 41318 08141		
Location details	Within the site, within the active quarry area.		
Habitat Suitability Index			
SI1. Map location	A/B/C	A	SI value 1.00
SI2. Surface area	rectangle/ellipse/irregular	irregular	
	length (m)		
	width (m)		
	OR estimate (m ²) if irregular	1310	
	<i>area (m²) =</i>	1310	0.90
SI3. Desiccation rate	never/rarely/sometimes/frequently	rarely	1.00
SI4. Water quality	good/moderate/poor/bad	bad	0.01
SI5. Shade	% of margin shaded 1m from bank	0	1.00
SI6. Waterfowl	absent/major/minor	absent	1.00
SI7. Fish population	absent/possible/minor/major	absent	1.00
SI8. Pond density	number of ponds within 1km	8	1.00
SI9. Terrestrial habitat	good/moderate/poor/isolated	moderate	0.67
SI10. Macrophyte cover	%	1	0.32
		HSI =	0.54
		provisional HSI	
<i>Use provisional HSI value if above 0.75</i>		=	0.50
		Date undertaken	22.5.20

Pond number/reference	P4		
OS Grid reference	SE 41892 07601		
Location details	Approx. 250m south of the extension area.		
Habitat Suitability Index			
SI1. Map location	A/B/C	A	SI value 1.00
SI2. Surface area	rectangle/ellipse/irregular	rectangle	
	length (m)	15	
	width (m)	2	
	OR estimate (m ²) if irregular		
	area (m ²) =	30	0.06

SI3. Desiccation rate	never/rarely/sometimes/frequently	rarely	1.00
SI4. Water quality	good/moderate/poor/bad	moderate	0.67
SI5. Shade	% of margin shaded 1m from bank	100	0.20
SI6. Waterfowl	absent/major/minor	absent	1.00
SI7. Fish population	absent/possible/minor/major	absent	1.00
SI8. Pond density	number of ponds within 1km	8	1.00
SI9. Terrestrial habitat	good/moderate/poor/isolated	good	1.00
SI10. Macrophyte cover	%	0	0.31
			HSI = 0.55
Use provisional HSI value if above 0.75			provisional HSI = 0.51
			Date undertaken 22.5.20

Pond number/reference	P5		
OS Grid reference	SE 41876 07617		
Location details	Approx. 240m south of the extension area.		
Habitat Suitability Index			
SI1. Map location	A/B/C	A	SI value 1.00
SI2. Surface area	rectangle/ellipse/irregular	rectangle	
	length (m)		
	width (m)		
	OR estimate (m ²) if irregular	340	
	<i>area (m²) =</i>	340	0.68
SI3. Desiccation rate	never/rarely/sometimes/frequently	frequently	0.10
SI4. Water quality	good/moderate/poor/bad	moderate	0.67
SI5. Shade	% of margin shaded 1m from bank	100	0.20
SI6. Waterfowl	absent/major/minor	absent	1.00
SI7. Fish population	absent/possible/minor/major	absent	1.00
SI8. Pond density	number of ponds within 1km	8	1.00
SI9. Terrestrial habitat	good/moderate/poor/isolated	good	1.00
SI10. Macrophyte cover	%	20	0.51
		HSI =	0.58
<i>Use provisional HSI value if above 0.75</i>		provisional HSI =	0.55
		Date undertaken	22.5.20

Pond number/reference	P6		
OS Grid reference	SE 40867 07878		
Location details	Approx. 220m south-west of the quarry and 650m west of the extension area.		
Habitat Suitability Index			
SI1. Map location	A/B/C	A	SI value 1.00

SI2.	Surface area	rectangle/ellipse/irregular	irregular	
		length (m)		
		width (m)		
		OR estimate (m ²) if irregular	850	
		<i>area (m²) =</i>	850	0.97
SI3.	Desiccation rate	never/rarely/sometimes/frequently	frequently	0.10
SI4.	Water quality	good/moderate/poor/bad	moderate	0.67
SI5.	Shade	% of margin shaded 1m from bank	80	0.60
SI6.	Waterfowl	absent/major/minor	absent	1.00
SI7.	Fish population	absent/possible/minor/major	absent	1.00
SI8.	Pond density	number of ponds within 1km	8	1.00
SI9.	Terrestrial habitat	good/moderate/poor/isolated	good	1.00
SI10.	Macrophyte cover	%	20	0.51
				HSI = 0.68
				provisional HSI = 0.65
				Date undertaken 22.5.20

Use provisional HSI value if above 0.75

Appendix 2: Reports of eDNA Survey Results from ADAS Laboratory

Client: Gary Oliver,
SLR Consulting Ltd.



ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

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

www.adas.uk

Sample ID: 2020-1369 Condition on Receipt: White Precipitate Volume: Passed
Client Identifier: G, Carlton Main, 403.03563.00037 Description: pond water samples in preservative
Date of Receipt: 12/06/2020 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	0 of 2	Real Time PCR	15/06/2020
Degradation Control [§]	Evidence of degradation or residual inhibition	Real Time PCR	15/06/2020
Great Crested Newt*	Indeterminate	Real Time PCR	15/06/2020
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: 16/06/2020 Date of issue: 16/06/2020

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.

Client: Gary Oliver,
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Tel: 01159 516747
Email: Helen.Rees@adas.co.uk

www.adas.uk

Sample ID: 2020-1371 Condition on Receipt: White Precipitate Volume: Passed
Client Identifier: F, Carlton Main, 403.03563.00037 Description: pond water samples in preservative
Date of Receipt: 12/06/2020 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	0 of 2	Real Time PCR	15/06/2020
Degradation Control [§]	Evidence of degradation or residual inhibition	Real Time PCR	15/06/2020
Great Crested Newt [*]	Indeterminate	Real Time PCR	15/06/2020
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:	16/06/2020	Date of issue:	16/06/2020

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.

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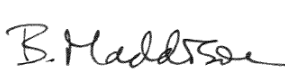
Tel: 01159 516747
Email: Helen.Rees@adas.co.uk

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Sample ID: 2020-1380 Condition on Receipt: White Precipitate Volume: Passed
Client Identifier: E, Carlton Main, 403.03563.00037 Description: pond water samples in preservative
Date of Receipt: 12/06/2020 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	0 of 2	Real Time PCR	15/06/2020
Degradation Control [§]	Evidence of degradation or residual inhibition	Real Time PCR	15/06/2020
Great Crested Newt*	Indeterminate	Real Time PCR	15/06/2020
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: 16/06/2020 Date of issue: 16/06/2020

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.

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Sample ID: 2020-1381 Condition on Receipt: Good Volume: Passed
Client Identifier: H Offsite,
Carlton Main, 403.03563.00037 Description: pond water samples in preservative
Date of Receipt: 12/06/2020 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	15/06/2020
Degradation Control [§]	Within Limits	Real Time PCR	15/06/2020
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	15/06/2020
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: 16/06/2020 Date of issue: 16/06/2020

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.

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[#] 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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Sample ID: ADAS-Filter 1 Condition on Receipt: Low Sediment Volume: 290ml

Client Identifier: Pond 1, Carlton
Main Brickworks. SE41105 08072 Description: pond water samples in filters
403.03653.00037

Date of Receipt: 03/05/2022 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	09/05/2022
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	09/05/2022
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: 11/05/2022 Date of issue: 11/05/2022

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.

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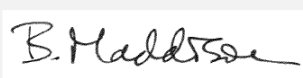
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Sample ID: ADAS-Filter 2 Condition on Receipt: Low Sediment Volume: 440ml

Client Identifier: Pond 2, Carlton
Main Brickworks. SE41372 08084
403.03653.00037 Description: pond water samples in filters

Date of Receipt: 03/05/2022 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	09/05/2022
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	09/05/2022
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:	11/05/2022	Date of issue:	11/05/2022

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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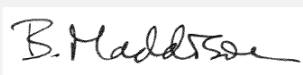
Tel: 01159 229249
Email: Helen.Rees@adas.co.uk

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Sample ID: ADAS-Filter 3 Condition on Receipt: Low Sediment Volume: 480ml

Client Identifier: Pond 3, Carlton
Main Brickworks. SE41343 08086 Description: pond water samples in filters
403.03653.00037

Date of Receipt: 03/05/2022 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	09/05/2022
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	09/05/2022
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:	11/05/2022	Date of issue:	11/05/2022

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