Intermodal Logistics Park North Ltd

INTERMODAL LOGISTICS PARK NORTH (ILPN)

Intermodal Logistics Park North (ILPN) Strategic Rail Freight Interchange (SRFI)

Project reference TR510001

Preliminary Environmental Information Report (PEIR)

Appendix 11.6: Great Crested Newt Report

October 2025

Planning Act 2008

The Infrastructure Planning (Environmental Impact Assessment) Regulations 2017

This document forms a part of a Preliminary Environmental Information Report (PEIR) for the Intermodal Logistics Park North (ILPN) project.

A PEIR presents environmental information to assist consultees to form an informed view of the likely significant environmental effects of a proposed development and provide feedback.

This PEIR has been prepared by the project promoter, Intermodal Logistics Park North Ltd. The Proposed Development is described in Chapter 3 of the PEIR and is the subject of a public consultation.

Details of how to respond to the public consultation are provided at the end of Chapter 1 of the PEIR and on the project website:

https://www.tritaxbigbox.co.uk/our-spaces/intermodal-logistics-park-north/

This feedback will be taken into account by Intermodal Logistics Park North Ltd in the preparation of its application for a Development Consent Order for the project.

TECHNICAL NOTE: Great Crested Newt

Intermodal Logistics Park North Limited

Intermodal Logistics Park North

September 2025

1.0 INTRODUCTION

1.1 The following Technical Note for great crested newt *Triturus cristatus* (GCN), has been prepared by FPCR Environment and Design Ltd. on behalf of Intermodal Logistics Park North Limited, to support a Development Consent Order (DCO) application for the development of a Nationally Significant Infrastructure Project (NSIP), Intermodal Logistics Park North, located to the east of Newton-Le-Willows (Central OS Grid Ref: SJ 6129 9507).

Context

1.2 The entire area of the Proposed Development is herein referred to as 'the DCO Site'. The Proposed Development covers a number of areas in the wider DCO Site including; the Main Site (the strategic rail freight terminal and logistics park); the Western Rail Chord (a railway spur to the west of the Main Site); the Northern Mitigation Area (land north of the existing railway line to be used for compensatory habitat creation); the 'Lane Head Relief Road' (located to the north-east of the Main Site); 'Remote Highway Works' including a number of options for highways improvements in the wider locality; and 'Soils Reuse Area' which includes agricultural land to the east of Winwick Lane, to be used for the reuse/spreading of soils onto agricultural land.

1.3 The area assessed by the surveys contained within this report includes the Main Site, and the Western Rail Chord. Herein referred to as 'the Survey Area'.

1.4 Note that the Remote Highway Works have been assessed at a high level in a separate highways mitigation options document. The Soils Reuse Area and the Lane Head Relief Road areas were not accessible for survey at the time of survey.

1.5 This Technical Report supports an Ecological Impact Assessment (EcIA) provided within the ecology chapter (Chapter 11) of the Preliminary Environmental Information Report (PEIR).

Site Location

1.6 The DCO Main Site is a roughly triangular area of approximately 198ha, bound by the Liverpool to Manchester railway line and Highfield Moss SSSI to the north, Winwick Lane (A579) to the east and south-east, the M6 motorway to the south-west, and an area of woodland and scrub and the M6 Motorway to the west. The Main Site also includes a small area of roughly triangular land north of the railway line bound by Parkside Road to the west and railway lines on all other aspects.

1.7 Of note is Highfield Moss SSSI, directly north of the Main Site. This SSSI is designated for raised mire habitats but also includes areas of scrub, woodland and lowland acid grassland, bounded by a ditch to the south which holds water except in periods of extreme dry weather.

1.8 The Western Rail Chord is a thin curved area of land that connects to the Liverpool to Manchester railway line in the north (just west of the M6). The Chord runs in an arc from the north-east to south-east through an area of mixed woodland, scrub and grassland on land which was formerly occupied by Parkside Colliery. At the south-eastern point of the arc the boundary of the area runs directly northwards (for a proposed access road).

1.9 The Main Site is predominantly occupied by land in agricultural cultivation. A small area of woodland is located in the north-east of the area, with two small, isolated stands of woodland located in the central area of the Main Site. An area of modified grassland is located in the north-central area associated with the runway and facility areas of Kenyon Hall Farm Airstrip. There are a number of ponds located across the Main Site, including recently constructed balancing ponds associated with Parkside Link Road East. There are a number of buildings within the Main Site including Highfield Farm and associated barn in the north of the Main Site, Parkside Farm and associated buildings in the central area adjacent to Parkside Road, and a scrap/storage yard in the north-east of the Main Site.

1.10 The Western Rail Chord extends through areas of woodland, scrub and grassland associated with the former Parkside Colliery.

Proposed Development

1.11 The Main Site is proposed to be developed as a Strategic Rail Freight Terminal and logistics park with large commercial/industrial buildings and associated access and landscaping. It is assumed that all habitats within the Main Site will be cleared during the Proposed Development with the exception of boundary hedgerows and trees (where present).

1.12 The Western Rail Chord is proposed to be developed as a railway line spur which, in the future will serve a separate proposed development (Parkside West). It is assumed that the majority of habitats within the footprint of the railway chord will be lost to the development with the exception of a small area of woodland and grassland in the south-western area.

2.0 LEGISLATION

2.1 GCN and the places they use for refuge and breeding are protected under Schedule 5 of the Wildlife and Countryside Act 1981 (as amended) and the Conservation of Habitats & Species Regulations 2017 (as amended).

2.2 They are a European Protected Species (EPS) and protected under Annexes II and IV of the EU Habitats and Species Directive and Appendix II of the Bern Convention.

2.3 In summary, it is an offence to:

- deliberately or recklessly take, injure or kill a great crested newt;
- intentionally or recklessly damage, destroy or obstruct access to any structure or place used for breeding, shelter or protection by the species;
- intentionally or recklessly disturb while it is occupying a structure or place which it uses for such purpose;
- intentionally take or destroy the eggs of a great crested newt.

2.4 This legislation equally protects all life stages, including eggs, efts and adults.

2.5 Proposals which could lead to any of the above would require a derogation licence from Natural England alongside appropriate avoidance, mitigation and compensation measures, or application to a District Level Licensing (DLL) Scheme.

3.0 METHODOLOGY

Desk Study

3.1 In order to compile existing baseline information, relevant ecological information was requested from both statutory and non-statutory nature conservation organisations as well as review of publicly accessible datasets including:

- Greater Manchester Local Records Centre (GMLRC);
- Record (Biological Records Centre for Cheshire and the Wirral); and
- Merseyside Biobank; and
- Multi Agency Geographic Information for the Countryside (MAGIC - https://magic.defra.gov.uk/)

3.2 Further inspection of colour 1:25,000 OS base maps (www.ordnancesurvey.co.uk) and aerial photographs from Google Earth (www.maps.google.co.uk) was also undertaken in order to provide additional context and identify any features of potential importance for nature conservation in the wider countryside.

Habitat Suitability Assessment (HSI)

3.3 The habitats within the survey area were assessed for their potential to support GCNs during both their breeding and terrestrial phases, including an assessment of waterbodies. In addition, access was sought to assess waterbodies within a 250m radius of the survey area which had suitable connective habitat to the site.

3.4 All accessible waterbodies were assessed using a Habitat Suitability Index (HSI)[1]. The HSI incorporates ten suitability indices, all of which are factors known to affect this species:

- Geographic location
- Pond area
- Pond drying
- Water quality
- Shade
- Presence of waterfowl
- Presence of fish
- Number of linked ponds
- Terrestrial habitat
- Macrophytic coverage

3.5 A score is assigned for each attribute, and a total score is calculated between 0 and 1. Pond suitability is then determined according to the scale in Table 1.

[1] Oldham, R.S., Keeble, K., Swan, M.J.S. & Jeffcote, M. (2000) Evaluating the suitability of habitat for the Great Crested Newt (*Triturus cristatus*). *Herpetological Journal*, 10(4), 143-155.

Table 1: HSI Scale

HSI Score	Pond Suitability
<0.5	Poor
0.5-0.59	Below average
0.6-0.69	Average
0.7-0.79	Good
>0.8	Excellent

Environmental DNA (eDNA) Sampling

3.6 eDNA sampling was undertaken on twenty-seven (27) waterbodies in accordance with the recommended protocol[2]. Waterbody locations are shown in Figure 1.

3.7 This methodology has been approved by Natural England for the determination of GCN presence/likely absence. Sampling was undertaken during the recommended survey season (15th April – 30th June inclusive) by appropriately licenced ecologists who collected a sample of water from the pond. Sampling was undertaken using kits obtained from ADAS Biotechnology. This comprised taking samples of agitated water from 20 locations around the pond and mixing thoroughly. Fifteen millilitres of this water was then placed into each of the 6 sterile sample tubes containing preservative, precipitates and a DNA sequence that was used for degradation control. All samples were stored in accordance with the protocols provided by the laboratory.

3.8 Sampling was undertaken by suitably trained and licenced ecologists supervised by Paul Andrews FPRC Associate ecologist holding a GCN survey Class licence (2022-10302-CL08-GCN) and sent to the ADAS laboratory in Beeston, Nottingham for analysis. The possible results are summarised in Table 2.

Table 2: Possible results of eDNA Analysis

Result	Description
Positive	A positive result means GCN eDNA was detected and they have been present within the water in the 20 days preceding sampling. A score is provided indicating the number of positive replicates from a series of twelve.
Negative	GCN eDNA was not detected. Where samples are negative, further testing for PCR inhibitors and degradation of the sample is undertaken.
Inconclusive	Controls indicate degradation or inhibition of the sample. Therefore, the lack of detection of GCN eDNA is not conclusive evidence for determining the absence of this species using the sample provided.

[2] Biggs, J. *et al.* (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.

4.0 RESULTS

Desk Study

4.1 Four records of GCN were identified from within 1km of the Main Site and Western Rail Chord within the previous 20 years as detailed below:

Table 1: Summary of Desktop Study Results

Species	Location	Date of Most Recent Record	Description
GCN	910m N of Main Site	01.06.2007	No further information
GCN	350m SW of Western Rail Chord	25.06.2019	No further information
GCN	620m SE of Main Site	01.06.2017	No further information
GCN	740m SE of Main Site	01.06.2017	No further information

Habitat Suitability Assessment

4.2 Suitable habitats within the Main Site included areas of unmanaged modified grassland south of Highfield Moss SSSI, hedgerows, woodland stands and woodland edge habitats.

4.3 Suitable habitats across the Western Rail Chord included areas of woodland, scrub and grassland, in particular a wet grassland area in the south-west of the area.

4.4 The waterbodies within the survey area were subject to GCN Habitat Suitability Index Assessment. Eight waterbodies were assessed to have above average suitability, five waterbodies to have average suitability, three waterbodies to have below average suitability and four waterbodies to have poor habitat suitability.

4.5 Four waterbodies were found to be dry at the time of survey, and no access was available to three waterbodies.

4.6 A summary of the HSI results is provided in Appendix A.

Environmental DNA Sampling

4.7 27 ponds and ditches were sampled for environmental DNA (eDNA), the locations of which are provided on Figure 1. Results of laboratory analysis are provided appended to this note as Appendix B.

4.8 Two ponds were found to be positive for GCN eDNA, Pond 2 and Pond 3 (highlighted on Figure 1). As such, these ponds are considered to support GCN.

4.9 No access was available to ponds P13, P15, and P19 (locations shown on Figure 1).

4.10 Ponds P6 and P20 were dry at the time of survey and unable to be sampled.

4.11 All other ponds within the DCO Site and the wider area were found to be negative for great crested newt eDNA and as such considered to be likely absent of the species.

Table 2: Summary of Pond Sampling

Pond	Access Y/N	Description	eDNA Result
P1	Y	Pond at centre of Highfield Moss SSSI	Negative
P2	Y	Central norther area of Main Site, isolated pond surrounded by small woodland south of airfield	Positive
P3	Y	Central northern area of Main Site, northernmost pond in 'Moss Pits' pond complex	Positive
P3A	Y	Central northern area of Main Site, pond in 'Moss Pits' pond complex, adjacent to P4 linked in times of high water via a small channel.	Negative
P4	Y	Central northern area of Main Site, pond in 'Moss Pits' pond complex	Negative
P5A	Y	Central northern area of Main Site, pond in 'Moss Pits' pond complex	Negative
P5B	Y	Central northern area of Main Site, pond in 'Moss Pits' pond complex, adjacent to P5A linked in times of high water via a small channel.	Negative
P6	Y	Dry at time of survey. Balancing pond adjacent to Parkside Road (central)	N/A – Dry
P7	Y	Balancing pond adjacent to Parkside Road East (south-east)	Negative
P8	Y	Small pond west of roundabout on Parkside Road/Winwick Lane junction	Negative
P9	Y	Small pond west of roundabout on Parkside Road/Winwick Lane junction	Negative
P10	Y	Pond at Parkside Farm	Negative
P11	Y	Pond in woodland north-west of Main Site, adjacent to east of M6	Negative
P12	Y	Dry at time of survey pond within woodland west of Main Site	Negative
P13	N	Pond to rear of Hollow Dene (residence)	N/A
P14	Y	Long pond, almost dry at time of survey	Negative
P15	N	Small pond near entrance to aggregates quarry.	N/A
P16	Y	Larger pond at Kenyon Hall Farm	Negative
P17	Y	Smaller pond at Kenyon Hall Farm	Negative
P18	Y	Pond in field to east of Winwick Lane and west of Kenyon	Negative
P19	N	Pond in woodland within grounds of Sandfield Hall north of Main Site	N/A
P20	Y	Dry at time of survey. Pond south of Parkside Link Road and south-east of Western Rail Chord	N/A - Dry
P21	Y	Small pond identified to the west of the M6 in the north-east area of the Western Rail Chord (outside the red line of the Order Limits)	Negative
D1	Y	Ditch south-west of Highfield Moss – evidence of some drying out at time of survey	Negative
D2	Y	Ditch south-east of Highfield Moss – evidence of some drying out at time of survey	Negative
D3	Y	Small ditch south of scrap yard in north-east of Main Site, evidence of pollution (oily sheen, orange coloured staining around ditch)	Negative
D4	Y	Ditch within woodland area north-east of western rail chord- dry at time of survey	N/A - Dry

Other Amphibians

4.12 The desk study identified four records of common toad *Bufo bufo* within 1 km of the Main Site and Western Rail Chord within the previous 20 years.

Table 2: Summary of Desktop Study Results for Other Amphibians

Species	Location	Date of Most Recent Record	Description
Common Toad	50m NE of Main Site	03.09.2014	Within Highfield Moss SSSI, western grassland area.
Common Toad	170m NW of Main Site	03.09.2014	Within Highfield Moss SSSI near pond.
Common Toad	110m W of Western Rail Chord proposed access road	June 2007	Adjacent to Newton Park Farm, west of proposed access road and east of Western Rail Chord.
Common Toad	840m SE of Main Site	May 2008	Beyond M6 motorway

4.13 Common toads were encountered incidentally during reptile surveys under artificial refugia (reptile mats). Common toad does not have any additional legal protection but is a Species of Principal Importance (SPI) listed on Schedule 41 of the NERC Act 2006 and as such should be considered within the planning process.

4.14 Three common toads were identified in the south of the Western Rail Chord within an area of scattered scrub and grassland near to woodland.

4.15 A single common toad was identified in the eastern area of the Western Rail Chord (within the area of proposed new access road to Newton Park Farm).

4.16 It is considered that common toad are located within the area of the Western Rail Chord and also are potentially present in Highfield Moss SSSI (due to historical records). Given that common toad are a relatively mobile species their presence in the Main Site cannot be completely discounted, particularly in the areas immediately adjacent to Highfield Moss SSSI.

5.0 DISCUSSION AND RECOMMENDATIONS

Great Crested Newt

5.1 Two ponds within the DCO Main Site were found to be positive for GCN eDNA. All other accessible ponds within the Main Site and Western Rail Chord and within 250m of these areas were found to be negative for GCN eDNA and are considered to be likely absent of GCN.

5.2 Although no population size class surveys were undertaken, given that only two ponds were found to be positive whilst other ponds in proximity and the wider area were negative this implies that the population of GCN in this area is likely to be low.

5.3 The area of the Main Site within which the ponds that tested positive are located is within a Natural England District Level Licencing (DLL) Area with an Amber Level risk rating.

5.4 It is recommended that the client seeks a DLL in relation to GCN compensation and mitigation. Granting of a DLL provides compensation for loss of GCN populations and/or habitats at a district level, with funds being used to provide optimal habitats in areas most suitable for the species and which will contribute to the long term favourable conservation status of the species at the district level. Should a DLL be granted for the DCO Site no traditional GCN mitigation would be required.

5.5 Although no traditional GCN mitigation would be required with a DLL in place, precautionary working methods will be prescribed within a Construction Environmental Management Plan (CEMP) to reduce the potential of harming or killing individual GCN. Should any individual GCN be encountered during construction the appointed Ecological Clerk of Works (ECoW) would provide further advice on how to proceed, including (if required) the translocation of individual GCN into suitable habitats outside of the construction area.

Common Toad

5.6 Common toads are considered likely to be present across the Main Site and Western Rail Chord, in particular around areas of scrub and woodland within the Western Rail Chord and potentially in the area immediately adjacent to the south of Highfield Moss SSSI within the Main Site.

5.7 Precautionary measures to protect common toads will be provided in a CEMP.

N

- Draft Order Limits
- Study Area (Main Site and WRC)
- 250m from Main Site and WRC
- Pond locations

P1 P2 P3 P4 P3A P5A P5B P6 P7 P8 P9 P10 P11 P12 P13 P14 P15 P16 P17 P18 P19 P20 P21

D1 D2 D3 D4

50 100 150 200 250 m

date 24/10/25

drwn/chkd PRA / PRA

client

Intermodal Logistics Park North Limited

project

ILPN

title

Pond Locations

scale 1:12,000 @ A3

number

FIGURE 1

rev -

FPCR | environment & design

L:\13100\13196\ECO\QGIS_BNG\Biodiversity Metric 4.1 - Onsite - Rev C - Sept 2025 Updates\Net Gain Habitat Mapping.qgs

t: 01509 672772 e: mail@fpcr.co.uk w: www.fpcr.co.uk

N

Draft Order Limits
Study Area (Main Site and WRC)
250m from Study Area

eDNA Results
Positive
Negative
Dry
No Access

P19
P1
Negative
D3
Negative
Negative
P18
P21
P11
D2
D1
P2
P3
P4
P3A
P5A
P5B
P12
Dry
D4
P10
P16
P17
P6
P20
P7
P8
P9
P15
P14
P13

50 100 150 200 250 m

date	drwn/chkd
24/10/25	PRA / PRA

client

Intermodal Logistics Park North Limited

project

ILPN

title	scale
eDNA Results	1:12,000 @ A3

number	rev
FIGURE 2	-

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APPENDIX A: HSI Results

Pond	Location	Pond Area m2	Permanence	Water Quality	Shade %	Waterfowl	Fish	Density (ponds in 1km)	Terrestrial habitat	Macrophytes %	HSI	Suitabilty
P1	A	1648	Sometimes Dries	Good	10	Minor	Possible	13	Good	16-20%	0.791973	Above Average
P2	A	288	Rarely Dries	Poor	100	Minor	Absent	12	Poor	<1%	0.552002	Below Average
P3	A	260	Sometimes Dries	Poor	100	Minor	Absent	12	Moderate	16-20%	0.576766	Below Average
P3A	A	526	Sometimes Dries	Poor	100	Minor	Absent	12	Moderate	16-20%	0.612299	Average
P4	A	746	Rarely Dries	Poor	100	Minor	Absent	12	Moderate	<1%	0.623565	Average
P5A	A	393	Sometimes Dries	Poor	50	Minor	Absent	12	Moderate	<1%	0.66832	Average
P5B	A	735	Rarely Dries	Poor	70	Minor	Absent	12	Moderate	<1%	0.716288	Above Average
P6 (DRY)	A	3379	Sometimes Dries	N/A	N/A	N/A	N/A	N/A	Poor	N/A	N/A	DRY
P7	A	2506	Sometimes Dries	Bad	0	Major	Absent	11	Poor	26-30%	0.308158	Poor
P8	A	241	Rarely Dries	Moderate	0	Minor	Absent	11	Poor	26-30%	0.730599	Above Average
P9	A	214	Rarely Dries	Moderate	0	Minor	Absent	11	Poor	36-40%	0.734172	Above Average
P10	A	390	Never Dries	Moderate	90	Minor	Absent	16	Moderate	66-80%	0.783	Above Average
P11	A	356	Sometimes Dries	Bad	80	Minor	Absent	8	Moderate	<1%	0.436604	Poor
P12 (DRY)	A	1055	Sometimes Dries	N/A	N/A	N/A	N/A	8	N/A	N/A	N/A	DRY
P13	A	119	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	No Access
P14	A	202	Never Dries	Bad	0	Absent	Absent	10	Moderate	<1%	0.48275	Poor
P15	A	42	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	No Access
P16	A	965	Rarely Dries	Moderate	60	Minor	Possible	11	Poor	11-15%	0.727605	Above Average
P17	A	228	Rarely Dries	Poor	30	Minor	Possible	11	Poor	26-30%	0.647075	Average
P18	A	228	Rarely Dries	Moderate	45	Minor	Possible	11	Poor	16-20%	0.682013	Average
P19	A	679	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	No Access
P20 (DRY)	A	739	Sometimes Dries	N/A	N/A	N/A	N/A	4	N/A	N/A	N/A	DRY

P21	A	50	Sometimes Dries	Poor		Absent	Absent	4	Moderate	51-55%	0.553652	Below Average
D1	A	1063	Sometimes Dries	Poor	75	Absent	Absent	17	Good	<1%	0.735823	Above Average
D2	A	518	Sometimes Dries	Poor	70	Absent	Absent	17	Good	<1%	0.74039	Above Average
D3	A	29	Dries Annually	Poor	25	Absent	Absent	12	Poor	11-15%	0.435433	Poor
D4 (DRY)	A	213	Sometimes Dries	N/A	N/A	N/A	N/A	5	N/A	N/A	N/A	DRY

APPENDIX B: eDNA Analysis Certificates

Client: Natasha Pickett,
FPCR

ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

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

www.adas.uk

Sample ID: ADAS-7719	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: P1	Description: pond water samples in preservative	
Date of Receipt: 22/04/2025	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	24/04/2025
Degradation Control[§]	Within Limits	Real Time PCR	24/04/2025
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	24/04/2025
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:	24/04/2025	Date of issue:	24/04/2025

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: Natasha Pickett,
FPCR

ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

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

www.adas.uk

Sample ID: ADAS-7720	Condition on Receipt: Medium Sediment	Volume: Passed
Client Identifier: P2	Description: pond water samples in preservative	
Date of Receipt: 22/04/2025	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	24/04/2025
Degradation Control[§]	Within Limits	Real Time PCR	24/04/2025
Great Crested Newt*	1 of 12 (GCN positive)	Real Time PCR	24/04/2025
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:	24/04/2025	Date of issue:	24/04/2025

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ADAS
Spring Lodge
172 Chester Road
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Tel: 01159 229249
Email: Helen.Rees@adas.co.uk

www.adas.uk

Sample ID: ADAS-7721	Condition on Receipt: Medium Sediment	Volume: Passed
Client Identifier: P3	Description: pond water samples in preservative	
Date of Receipt: 22/04/2025	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	24/04/2025
Degradation Control[§]	Within Limits	Real Time PCR	24/04/2025
Great Crested Newt*	10 of 12 (GCN positive)	Real Time PCR	24/04/2025
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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Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	24/04/2025	Date of issue:	24/04/2025

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: ADAS-7722	Condition on Receipt: White Precipitate	Volume: Passed
Client Identifier: P4	Description: pond water samples in preservative	
Date of Receipt: 22/04/2025	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	24/04/2025
Degradation Control[§]	Within Limits	Real Time PCR	24/04/2025
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	24/04/2025
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:	24/04/2025	Date of issue:	24/04/2025

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: ADAS-7723	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: P5A	Description: pond water samples in preservative	
Date of Receipt: 22/04/2025	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	0 of 2	Real Time PCR	24/04/2025
Degradation Control[§]	Within Limits	Real Time PCR	24/04/2025
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	24/04/2025
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:	24/04/2025	Date of issue:	24/04/2025

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: ADAS-7725	Condition on Receipt: Algae Present	Volume: Passed
Client Identifier: P7	Description: pond water samples in preservative	
Date of Receipt: 22/04/2025	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	24/04/2025
Degradation Control[§]	Within Limits	Real Time PCR	24/04/2025
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	24/04/2025
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:	24/04/2025	Date of issue:	24/04/2025

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.

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Sample ID: ADAS-7726	Condition on Receipt: Good	Volume: Passed
Client Identifier: P8	Description: pond water samples in preservative	
Date of Receipt: 22/04/2025	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	23/04/2025
Degradation Control[§]	Within Limits	Real Time PCR	23/04/2025
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	23/04/2025
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:	24/04/2025	Date of issue:	24/04/2025

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: ADAS-7727	Condition on Receipt: Good	Volume: Passed
Client Identifier: P9	Description: pond water samples in preservative	
Date of Receipt: 22/04/2025	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	23/04/2025
Degradation Control[§]	Within Limits	Real Time PCR	23/04/2025
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	23/04/2025
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:	24/04/2025	Date of issue:	24/04/2025

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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-7728	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: P10	Description: pond water samples in preservative	
Date of Receipt: 22/04/2025	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	23/04/2025
Degradation Control[§]	Within Limits	Real Time PCR	23/04/2025
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	23/04/2025
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:	24/04/2025	Date of issue:	24/04/2025

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** 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: ADAS-7729	Condition on Receipt: Good	Volume: Passed
Client Identifier: P11	Description: pond water samples in preservative	
Date of Receipt: 22/04/2025	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	23/04/2025
Degradation Control[§]	Within Limits	Real Time PCR	23/04/2025
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	23/04/2025
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:	24/04/2025	Date of issue:	24/04/2025

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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-7730	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: P12	Description: pond water samples in preservative	
Date of Receipt: 22/04/2025	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	24/04/2025
Degradation Control[§]	Within Limits	Real Time PCR	24/04/2025
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	24/04/2025
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:	24/04/2025	Date of issue:	24/04/2025

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-7732	Condition on Receipt: White Precipitate	Volume: Passed
Client Identifier: P14	Description: pond water samples in preservative	
Date of Receipt: 22/04/2025	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	24/04/2025
Degradation Control[§]	Within Limits	Real Time PCR	24/04/2025
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	24/04/2025
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:	24/04/2025	Date of issue:	24/04/2025

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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.

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Sample ID: ADAS-7734	Condition on Receipt: White Precipitate	Volume: Passed
Client Identifier: P16	Description: pond water samples in preservative	
Date of Receipt: 22/04/2025	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	0 of 2	Real Time PCR	24/04/2025
Degradation Control[§]	Within Limits	Real Time PCR	24/04/2025
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	24/04/2025
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:	24/04/2025	Date of issue:	24/04/2025

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.

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Sample ID: ADAS-7735	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: P3A	Description: pond water samples in preservative	
Date of Receipt: 22/04/2025	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	24/04/2025
Degradation Control[§]	Within Limits	Real Time PCR	24/04/2025
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	24/04/2025
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:	24/04/2025	Date of issue:	24/04/2025

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: ADAS-7737	Condition on Receipt: Algae Present	Volume: Passed
Client Identifier: P18	Description: pond water samples in preservative	
Date of Receipt: 22/04/2025	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	24/04/2025
Degradation Control[§]	Within Limits	Real Time PCR	24/04/2025
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	24/04/2025
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:	24/04/2025	Date of issue:	24/04/2025

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: ADAS-7738	Condition on Receipt: Algae Present	Volume: Passed
Client Identifier: D1	Description: pond water samples in preservative	
Date of Receipt: 22/04/2025	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	0 of 2	Real Time PCR	24/04/2025
Degradation Control[§]	Evidence of degradation or residual inhibition	Real Time PCR	24/04/2025
Great Crested Newt*	Indeterminate	Real Time PCR	24/04/2025
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:	24/04/2025	Date of issue:	24/04/2025

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: ADAS-7739	Condition on Receipt: Medium Sediment	Volume: Passed
Client Identifier: D2	Description: pond water samples in preservative	
Date of Receipt: 22/04/2025	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	0 of 2	Real Time PCR	24/04/2025
Degradation Control[§]	Within Limits	Real Time PCR	24/04/2025
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	24/04/2025
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:	24/04/2025	Date of issue:	24/04/2025

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: ADAS-7740	Condition on Receipt: Algae Present	Volume: Passed
Client Identifier: D3	Description: pond water samples in preservative	
Date of Receipt: 22/04/2025	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	24/04/2025
Degradation Control[§]	Within Limits	Real Time PCR	24/04/2025
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	24/04/2025
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:	24/04/2025	Date of issue:	24/04/2025

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Sample ID: ADAS-7741	Condition on Receipt: Medium Sediment	Volume: Passed
Client Identifier: P21	Description: pond water samples in preservative	
Date of Receipt: 22/04/2025	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	24/04/2025
Degradation Control[§]	Within Limits	Real Time PCR	24/04/2025
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	24/04/2025
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:	24/04/2025	Date of issue:	24/04/2025

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: ADAS-7744	Condition on Receipt: Good	Volume: Passed
Client Identifier: P5B	Description: pond water samples in preservative	
Date of Receipt: 22/04/2025	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control†	2 of 2	Real Time PCR	24/04/2025
Degradation Control§	Within Limits	Real Time PCR	24/04/2025
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	24/04/2025
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:	24/04/2025	Date of issue:	24/04/2025

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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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