JOANNA GRAHAM
ECOLOGY LTD

31a Charnham Street

Hungerford

Berkshire

RG17 0EJ

13th June 2025

Dear Lexi,

Background

Joanna Graham Ecology Ltd was commissioned to undertake Habitat Suitability Index (HSI) assessments and Great Crested Newt (*Triturus cristatus*) environmental DNA (eDNA) surveys of ponds within 250m of a proposed water main. The site is a linear route from Egham Water Treatment Works (WTW) to the south (grid reference TQ 02256 71730) and Iver Water Treatment Works to the north (grid reference TQ 02275 71754). A total of 36* waterbodies were located using an OS base map, see Appendix 1.

Methodology

Prior to the HSI and eDNA surveys, unsuitable water bodies such as fishing lakes and large reservoirs associated with high numbers of waterfowl were removed from the survey scope. Furthermore, within parts of the route the water pipe would be installed by Horizontal Directional Drilling (HDD). The use of HDD avoids the impact to terrestrial and aquatic habitats and therefore any ponds within 250m of the HDD areas were also removed from the scope as no habitat would be affected. A total of 20 ponds were removed from the survey scope, with the locations of the remaining 16 ponds recorded using DEFRA Magic Map within 250m of the open cut sections of the route.

Where access was granted, the ponds were subject to a HSI assessment and eDNA survey. Photographs of each pond were taken, see Appendix 2.

HSI assessment

HSI assessments were carried out following the Amphibian and Reptile Group UK Advice Note 5 following a HSI scoring system (Oldham *et al*, 2000)[1]. A HSI assessment is a measure of habitat suitability and is not a substitute for newt surveys, in general ponds with high HSI are more likely

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

* Note, when numbering the ponds, ponds 5 and 33 were omitted and therefore the map shows ponds 37 and 38.

to support Great Crested Newts. Ten factors are considered to score the ponds which when the geometric mean is calculated gives a suitability score. Pond suitability is as follows: <0.5 is poor, 0.5-0.59 is below average, 0.6-0.69 is average, 0.7-0.79 is good and >0.8 is excellent. The following ten factors are considered:

1. Geographic location;
2. Pond area;
3. Permanence;
4. Water quality;
5. Shade;
6. Water fowl;
7. Fish;
8. Pond count;
9. Terrestrial habitat; and,
10. Macrophytes.

eDNA

eDNA surveys were undertaken on 24th, 25th and 30th April and 9th May 2025 on ponds 1, 6, 9, 11, 12, 16, 17, 18, 20 23, 24, 26, 29, 30, 31 and 34 by Joanna Graham BSc(Hons) MCIEEM (Great Crested Newt licence reference: 2021-52255-CLS-CLS), Beth England (Great Crested Newt licence reference: 2022-10885-CL08-GCN) and Lauren Jones-Mullins.

The survey involved taking water samples from each of the accessible ponds. The surveys were carried out following the standard protocol from the Technical Advice Note for field and laboratory sampling for Great Crested Newt environmental DNA (Biggs *et al*, 2014)[2]. A total of 20 water samples were collected from each pond, evenly spread out around the pond to include open and vegetated areas. The 20 water samples were mixed and 15ml was pipetted into preprepared 35ml test tubes of preservative fluid. The test tubes were sent to ADAS for analysis using a Polymerase Chain Reaction (PCR).

Results

Pond 12 was a balancing pond located to the east of the M25 and it was dry during the survey. No aquatic vegetation was present and therefore it was not subject to a HSI assessment. Pond 6 could not be accessed to undertake an eDNA survey as it was located down a steep slope surrounded by chain link fencing and dense scrub, however the pond was subject to a HSI assessment. It was not possible to access Pond 11 due to health and safety concerns with the unknown use of the land and uncertainty of ownership. Pond 20 was visible from surrounding land but dense scrub prevented access to undertake an eDNA survey. Upon ground truthing pond 18 was part of pond 19, a very large waterbody with steep sides used by the quarry.

[2] Biggs *et al* (2014) Technical Advice Note for field and laboratory sampling for Great Crested Newts (*Triturus cristatus*) environmental DNA.

HSI

Ponds 9 and 29 were assessed as 'poor' suitability, ponds 24 and 31 were 'below average' suitability, ponds 1, 17 and 30 were 'average' suitability and ponds 16, 20, 23, 26 and 34 scored 'good' suitability for Great Crested Newts. Full HSI results can be found in Appendix 3.

eDNA

The eDNA results for ponds 1, 9, 24, 26, 29, 30 and 34 were all negative for the presence of Great Crested Newt DNA. The results from ponds 16, 17, 23 and 31 were indeterminate due to evidence of degradation. The sample for pond 23 and pond 17 contained white precipitate which can form as a result of a chemical reaction between the water and the preservative and interfere with the DNA extraction and PCR processes. The sample for pond 31 contained low sediment which can also interfere with the DNA extraction and PCR processes. There was evidence of degradation for pond 16, the reason was unknown as there was no sediment present and the condition of the sample was good. eDNA samples can be re-tested where the reason for an indeterminate result was high sediment content, however the sample for pond 31 was returned as only low sediment so the re-test may still return an inconclusive result. Where water chemistry was the cause of the indeterminate result, then a re-test would most likely also return an inconclusive result. Given the location of the ponds within and adjacent to the quarry it is likely the ponds are subject to direct/indirect pollution from the quarry site. Full laboratory results can be found in Appendix 4.

Table 1: Survey results

Pond number	Location	HSI result	eDNA result
Pond 1	TQ 04644 80243	Average	Negative
Pond 6	TQ 03758 78205	N/A	N/A
Pond 9	TQ 03546 77087	Poor	Negative
Pond 11	TQ 04476 76739	N/A	N/A
Pond 12	TQ 04071 75961	N/A - dry	N/A - dry
Pond 16	TQ 03710 75118	Good	Indeterminate
Pond 17	TQ 03276 74617	Average	Indeterminate
Pond 20	TQ 03612 74301	Good	N/A
Pond 23	TQ 02980 73607	Good	Indeterminate
Pond 24	TQ 03028 73563	Below average	Negative
Pond 26	TQ 02696 72706	Good	Negative
Pond 29	TQ 02466 72693	Poor	Negative
Pond 30	TQ 02476 72869	Average	Negative
Pond 31	TQ 02517 72965	Below average	Indeterminate
Pond 34	TQ 02359 72368	Good	Negative

Discussion and Recommendations

Biological records from four records centres (Buckinghamshire and Milton Keynes Environmental Records Centre (BMERC), Thames Valley Environmental Records Centre (TVERC), Greenspace Information for Greater London (GiGL) and Surrey Biodiversity Information Centre (SBIC)) were requested as part of the Preliminary Ecological Appraisal process. Only GiGL provided one record of Great Crested Newt from 2018 within a 1km^2 area, however no location was specified. Magic Map showed no licence returns or data from the 2017-2019 pond surveys map layer.

Due to the lack of access and indeterminate results, the presence of Great Crested Newts cannot be ruled out. However, the combination of negative eDNA results and lack of records further reduces the risk of impacting Great Crested Newts. With regard to approaches to mitigation requirements for Great Crested Newts, a maximum routine migratory range of 250m from breeding ponds has been estimated for Great Crested Newts during terrestrial phases, and studies suggest that 95% of newt summer refuges are within 63m of breeding ponds (Jehle, 2000)[3].

Given the close proximity of the route to pond 6, it is recommended that access is gained through cutting a route through the scrub to undertake surveys. Further recommendations include a 63m buffer to be maintained from all other ponds that were either not accessible or returned an indeterminate result. This will protect the core 63m of terrestrial habitat surrounding the ponds. Furthermore, all works that are located within vegetated habitat which is within 250m of either an inaccessible pond or a pond which returned an indeterminate result, should proceed following a Non-licensed Method Statement/Precautionary Working Method Statement (NLMS/PWMS) under the supervision of an Ecological Clerk of Works. The NLMS/PWMS will detail the working methodology to ensure that the risk of encountering Great Crested Newts is further reduced. Where works are within the core 63m of a pond, a fingertip search should be carried out ahead of vegetation removal and log piles or other hibernacula should be avoided during any winter works. If in the very unlikely circumstances a Great Crested Newt is found, works should cease and Natural England contacted.

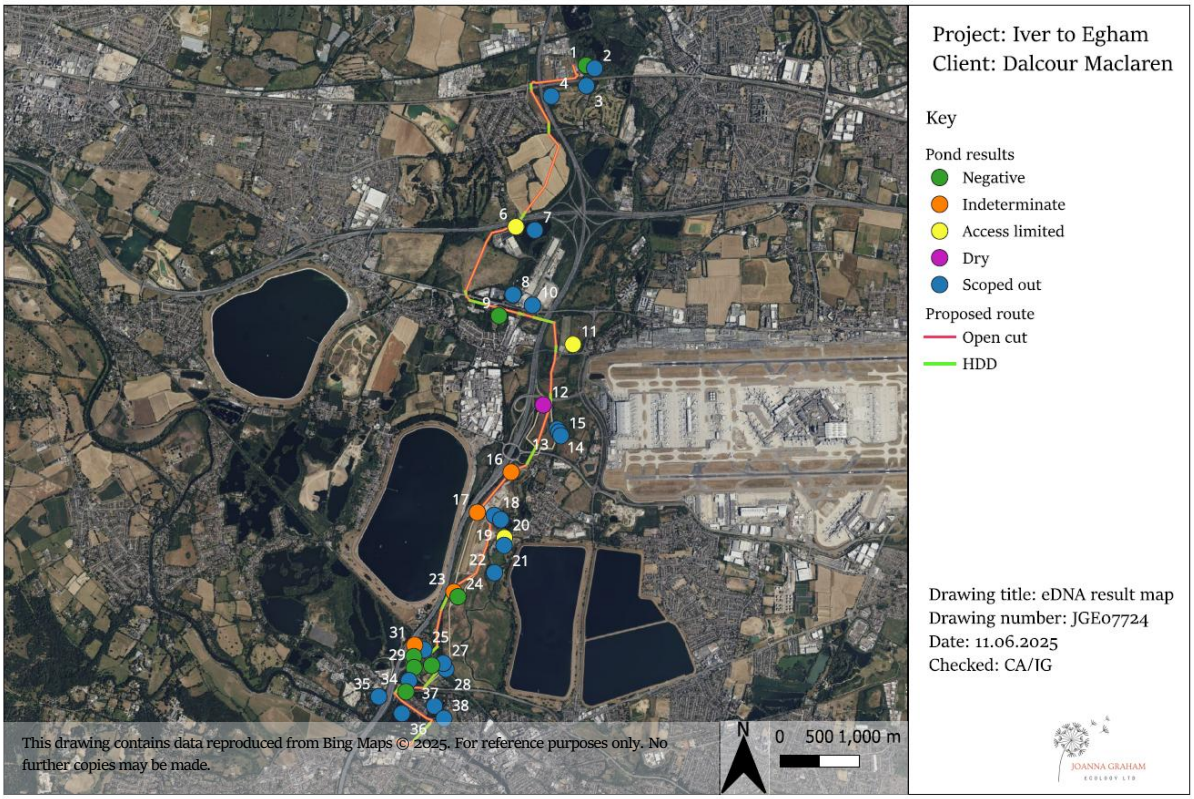
Yours sincerely,

Joanna Graham

Director

[3] Jehle. R (2000) *The terrestrial summer habitat of radio tracked great crested newts (Triturus cristatus) and marbled newts (Triturus marmoratus)* Erpetological Journal, Vol. 1 0, pp. 137-142.

Appendix 1 Pond locations



Appendix 2 Photographs

Photograph 1: Pond 1

Photograph 2: Pond 6 – not accessible

Photograph 3: Pond 9

Photograph 4: Pond 12 - dry

Photograph 5: Pond 16

Photograph 6: Pond 17

Photograph 7: Pond 20

Photograph 8: Pond 23

	
Photograph 9: Pond 24	Photograph 10: Pond 26
	
Photograph 11: Pond 29	Photograph 12: Pond 30
	
Photograph 13: Pond 31	

Appendix 3 HSI results

Factor	Pond 1	Pond 6	Pond 9	Pond 16	Pond 17	Pond 20	Pond 23
Geographic location	Zone A	Zone A	Zone A	Zone A	Zone A	Zone A	Zone A
Pond area	>2000m²	500m²	>2000m²	400m²	100m²	450m²	450m²
Pond permanence	Never dries	Never dries	Never dries	Never dries	Sometimes dries	Never dries	Never dries
Water quality	Moderate	Moderate	Moderate	Moderate	Moderate	Good	Moderate
Shade	0-60%	70%	95%	0-60%	0-60%	0-60%	0-60%
Waterfowl effect	Minor	Minor	Major	Minor	Absent	Minor	Minor
Fish presence	Possible	Possible	Minor	Possible	Possible	Possible	Possible
Pond density	Score: 0.1	Score: 0.1	Score: 0.67	Score: 0.67	Score: 0.7	Score: 0.7	Score: 0.7
Terrestrial habitat	Moderate	Moderate	Moderate	Moderate	Poor	Good	Moderate
Macrophyte cover	20%	20%	95%	40%	60%	10%	50%
Score	0.65	0.61	0.39	0.76	0.63	0.79	0.78
Suitability	Average	Average	Poor	Good	Average	Good	Good

Factor	Pond 24	Pond 26	Pond 29	Pond 30	Pond 31	Pond 34
Geographic location	Zone A	Zone A	Zone A	Zone A	Zone A	Zone A
Pond area	100m²	300m²	>2000m²	>2000m²	>2000m²	450m²
Pond permanence	Dries annually	Rarely dries	Never dries	Never dries	Never dries	Never dries
Water quality	Poor	Moderate	Moderate	Moderate	Poor	Moderate
Shade	0-60%	80%	0-60%	90%	95%	0-60%
Waterfowl effect	Absent	Absent	Minor	Minor	Minor	Minor
Fish presence	Absent	Possible	Major	Minor	Minor	Absent
Pond density	Score: 0.7	Score: 0.82	Score: 0.82	Score: 0.82	Score: 0.82	Score: 0.38
Terrestrial habitat	Moderate	Moderate	Moderate	Good	Moderate	Moderate
Macrophyte cover	0-10%	20%	20%	10%	20%	5%
Score	0.51	0.73	0.47	0.64	0.56	0.78
Suitability	Below average	Good	Poor	Average	Below average	Good

Appendix 4 eDNA results

Client:
IVER, Joanna Graham, Joanna Graham Ecology
1040079-1262, IVER, version 1

RSK ADAS Ltd
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

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

www.adas.uk

Sample ID: ADAS-9041	
Client Identifier: Pond31	Grid references/coordinates: TQ0251772965
Description: pond water samples in preservative	Condition on Receipt: Low Sediment
Date of Receipt : 14/05/2025	Volume: Passed

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	22/05/2027
Degradation Control[§]	Evidence of degradation	Real Time PCR	22/05/2027
Great Crested Newt*	Indeterminate	Real Time PCR	22/05/2027
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:	23/05/2025	Date of issue:	23/05/2025

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 GCN if all of the replicates are negative; positive for GCN 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

[§] 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:
IVER, Joanna Graham, Joanna Graham Ecology
1040079-1262, IVER, version 1

RSK ADAS Ltd
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

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

www.adas.uk

Sample ID: ADAS-9042	
Client Identifier: Pond26	Grid references/coordinates: TQ0269672706
Description: pond water samples in preservative	Condition on Receipt: Low Sediment
Date of Receipt : 14/05/2025	Volume: Passed

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	22/05/2027
Degradation Control[§]	Within limits	Real Time PCR	22/05/2027
Great Crested Newt*	0 of 12 (negative)	Real Time PCR	22/05/2027
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:	23/05/2025	Date of issue:	23/05/2025

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1040079-1262, IVER, version 1

RSK ADAS Ltd
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

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

www.adas.uk

Sample ID: ADAS-9043	
Client Identifier: Pond29	Grid references/coordinates: TQ0246672693
Description: pond water samples in preservative	Condition on Receipt: Good
Date of Receipt : 14/05/2025	Volume: Passed

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	22/05/2027
Degradation Control[§]	Within limits	Real Time PCR	22/05/2027
Great Crested Newt*	0 of 12 (negative)	Real Time PCR	22/05/2027
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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Signed:		Signed:	
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1040079-1262, IVER, version 1

RSK ADAS Ltd
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

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

www.adas.uk

Sample ID: ADAS-9044	
Client Identifier: Pond30	Grid references/coordinates: TQ0247672869
Description: pond water samples in preservative	Condition on Receipt: Good
Date of Receipt : 14/05/2025	Volume: Passed

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	22/05/2025
Degradation Control[§]	Within limits	Real Time PCR	22/05/2025
Great Crested Newt*	0 of 12 (negative)	Real Time PCR	22/05/2025
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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RSK ADAS Ltd
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

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

www.adas.uk

Sample ID: ADAS-9045	
Client Identifier: Pond16	Grid references/coordinates: TQ0371075118
Description: pond water samples in preservative	Condition on Receipt: Good
Date of Receipt : 14/05/2025	Volume: Passed

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	22/05/2025
Degradation Control[§]	Evidence of degradation	Real Time PCR	22/05/2025
Great Crested Newt*	Indeterminate	Real Time PCR	22/05/2025
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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RSK ADAS Ltd
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

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

www.adas.uk

Sample ID: ADAS-9046			
Client Identifier: Pond17		Grid references/coordinates: TQ0327674617	
Description: pond water samples in preservative		Condition on Receipt: Medium White Precipitate	
Date of Receipt : 14/05/2025		Volume: Passed	
Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	22/05/2025
Degradation Control[§]	Evidence of degradation	Real Time PCR	22/05/2025
Great Crested Newt*	Indeterminate	Real Time PCR	22/05/2025
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RSK ADAS Ltd
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

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

www.adas.uk

Sample ID: ADAS-9047			
Client Identifier: Pond23		Grid references/coordinates: TQ0298073607	
Description: pond water samples in preservative		Condition on Receipt: Medium White Precipitate	
Date of Receipt : 14/05/2025		Volume: Passed	
Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	22/05/2025
Degradation Control[§]	Evidence of degradation	Real Time PCR	22/05/2025
Great Crested Newt*	Indeterminate	Real Time PCR	22/05/2025
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RSK ADAS Ltd
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

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

www.adas.uk

Sample ID: ADAS-9048	
Client Identifier: Pond24	Grid references/coordinates: TQ0302873563
Description: pond water samples in preservative	Condition on Receipt: Low Sediment
Date of Receipt : 14/05/2025	Volume: Passed

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	22/05/2025
Degradation Control[§]	Within limits	Real Time PCR	22/05/2025
Great Crested Newt*	0 of 12 (negative)	Real Time PCR	22/05/2025
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RSK ADAS Ltd
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

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

www.adas.uk

Sample ID: ADAS-9049	
Client Identifier: P34	Grid references/coordinates: TQ0232072367
Description: pond water samples in preservative	Condition on Receipt: Good
Date of Receipt : 14/05/2025	Volume: Passed

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	22/05/2027
Degradation Control[§]	Within limits	Real Time PCR	22/05/2027
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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:	23/05/2025	Date of issue:	23/05/2025

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 GCN if all of the replicates are negative; positive for GCN 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

[§] 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:
IVER, Joanna Graham, Joanna Graham Ecology
1040079-1262, IVER, version 1

RSK ADAS Ltd
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

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

www.adas.uk

Sample ID: ADAS-9050	
Client Identifier: Pond1	Grid references/coordinates: TQ0464480243
Description: pond water samples in preservative	Condition on Receipt: Good
Date of Receipt : 14/05/2025	Volume: Passed

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	22/05/2027
Degradation Control[§]	Within limits	Real Time PCR	22/05/2027
Great Crested Newt*	0 of 12 (negative)	Real Time PCR	22/05/2027
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:	23/05/2025	Date of issue:	23/05/2025

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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[§] 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:
IVER, Joanna Graham, Joanna Graham Ecology
1040079-1262, IVER, version 1

RSK ADAS Ltd
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

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

www.adas.uk

Sample ID: ADAS-9052	
Client Identifier: P9	Grid references/coordinates: TQ0354677087
Description: pond water samples in preservative	Condition on Receipt: Low Sediment
Date of Receipt : 14/05/2025	Volume: Passed

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	22/05/2027
Degradation Control[§]	Within limits	Real Time PCR	22/05/2027
Great Crested Newt*	0 of 12 (negative)	Real Time PCR	22/05/2027
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:	23/05/2025	Date of issue:	23/05/2025

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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

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

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

Appendix 1: Interpretation of results

Sample Condition

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

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

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

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

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

What do my results mean?

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

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

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

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

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