Supplementary Ecology Report
Great Crested Newt

November 2024

Land North-East of Humber Doucy Lane, Ipswich

Prepared by
CSA Environmental

On behalf of
Barratt David Wilson Homes & Hopkins Homes

Report No: CSA/6675/13

CSA environmental

masterplanning | ecology | landscape | heritage

This report may contain sensitive ecological information. It is the responsibility of the Local Authority to determine if this should be made publicly available.

Report Reference	Revision	Date	Prepared by	Approved by	Comments
CSA/6675/13	-	05/11/2024	CH	JW	

1.0 Legislation

1.1 Great crested newts *Triturus cristatus* are legally protected as European Protected Species (EPS) under Regulation 43 of the Conservation of Habitats and Species Regulations 2017. These Regulations make it an offence to:

- Deliberately capture, injure or kill a great crested newt
- Deliberately disturb great crested newts, impairing their ability to survive, breed, reproduce or rear/nurture their young
- Damage or destroy a breeding site or resting place used by a great crested newt

1.2 Great crested newts are also fully protected under the Wildlife & Countryside Act 1981 (as amended), making it an offence to:

- Intentionally or recklessly disturb a great crested newt while it is occupying a structure or place of shelter or protection
- Intentionally or recklessly obstruct access to any structure or place of shelter or protection

1.3 Disturbance of great crested newts is covered by both the 2017 Regulations and the 1981 Act. Disturbance that impairs survival or successful reproduction would be covered by the Regulations, while less significant acts of disturbance may only be covered by the Act.

1.4 It is important to note that great crested newts and their habitats (such as breeding ponds) are protected throughout the year, regardless of whether or not newts are present at the time.

1.5 Great crested newts are also listed as a species of principal importance for the conservation of biodiversity in England, under Section 41 (S41) of the Natural Environment and Rural Communities (NERC) Act 2006. The S41 species list is used to guide decision-makers, including planning authorities, in implementing their duty under Section 40 of the NERC Act to have regard to the conservation of biodiversity in England, when carrying out their normal functions.

<u>Licensing</u>

1.6 Where development is proposed that would result in an offence under the Habitats and Species Regulations, a statutory derogation licence may be granted by Natural England to permit an act that would otherwise be unlawful. To obtain an EPS licence for development, it must be demonstrated that the purpose of the act to be licensed is for:

- "preserving public health or public safety or other imperative reasons of overriding public interest including those of social or economic nature and beneficial consequences of primary importance for the environment" (Regulation 55(2)(e))

1.7 In addition, Natural England will not grant an EPS licence unless they are satisfied that:

- "There is no satisfactory alternative" (Regulation 55(9)(a))
- "The action authorised will not be detrimental to the maintenance of the population of the species concerned at a favourable conservation status in their natural range" (Regulation 55(9)(b))

2.0 Methods

Desk Study

2.1 In accordance with Natural England's Great Crested Newt Mitigation Guidelines (2001), a desktop search was undertaken in August 2023 to identify ponds within 500m of the Site which may have potential to support breeding great crested newts, using Ordnance Survey (OS) mapping, the MAGIC database and aerial photography. 500m is the generally accepted typical maximum dispersal range of this species, with great crested newt most likely to use terrestrial habitat within 250m of breeding ponds.

Habitat Suitability Index (HSI) Assessment

2.2 Where ponds were situated within a 250m radius and connected to the Site by traversable terrestrial habitats, access permission was requested to undertake a Habitat Suitability Index (HSI) assessment, using the standard approach set out by Oldham *et al*. (2000).

2.3 The data search found records of great crested newt that were associated with and in close proximity to P19 dating from 2008 to 2017. Given that these are recent records, despite being greater than 250m from the Site boundary, P19 was included within the assessment in addition to P11 and P12 which are located close to P19.

2023

2.4 HSI assessments were undertaken on 15 September 2023 by Laura Farrar ACIEEM (Class Survey Licence CL08 – Registration Number 2019-41162-CLS-CLS) of ponds P1, P3, P4, P5, P6, P7, P11, P12 and P19.

2024

2.5 Update HSI assessments were undertaken on 06 June 2024 by Laura Farrar and Matthew Dale (Class Survey Licence CL08 – Registration Number 2022-10646-CLS-CLS) of ponds P3, P4, P5, P6, P7, P11, P12 and P19.

Environmental DNA (eDNA) Sampling

2.6 Environmental DNA (eDNA) sampling was used to determine the presence/likely absence of great crested newts from ponds. This method has been shown to be a highly effective in detecting the presence of great crested newts (Biggs *et al*., 2014).

2023

2.7 Water samples were collected from ponds P1, P3, P5 and P11 on 28 September 2023 by Carly Howes ACIEEM (Class Survey Licence CL08 – Registration Number 2017-32238-CLS-CLS and Matthew Dale following the recommended procedure. Appropriate biosecurity measures were taken to avoid cross contamination of great crested newt eDNA. Subsequently the samples were sent to ADAS for DNA analysis.

2024

2.8 Water samples were collected from ponds P3, P5, P6, P11, P12 and P19 on 06 June 2024 by Laura Farrar and Matthew Dale.

Limitations

HSI Assessment

2023

2.9 No access was granted for assessments of ponds P8, P9 or P10. Pond P2 was also not viewed but the homeowner stated it was currently dry. There were no limitations to the HSI surveys which were undertaken, the surveys were conducted at suitable times of year and in good conditions.

2024

2.10 No access was granted for assessments of ponds P1, P2, P8, P9 or P10. There were no limitations to the HSI surveys which were undertaken, the surveys were conducted at suitable times of year and in good conditions.

eDNA Sampling

2023

2.11 The eDNA surveys were undertaken outside of the standard practice period for sampling, which is between 15 April and 30 June. A negative eDNA result therefore cannot confirm likely absence (however a positive eDNA result can confirm presence). eDNA surveys undertaken in 2024 were undertaken between 15 April and 30 June and therefore, supersede the 2023 surveys.

2.12 A water sample could not be collected for pond P19 as it had dried up between the HSI and eDNA sampling surveys.

3.0 Results

Desk Study

3.1 The desktop search for ponds and subsequent site visits identified 19 water bodies occurring within 500m of the Site. These ponds are identified on the Pond Plan (CSA/6675/102).

3.2 Access for further survey work was not sought for ponds P13, P14, P15, P16, P17 or P18 due to the distance/separation from the Site and lack of recent records of GCN from these ponds.

Habitat Suitability Index (HSI) Assessment

2023

3.3 A summary of the HSI assessment for surveyed ponds is provided in Table 1 below.

3.4 Ponds P4 and P7 were found to be dry at the time of survey and are likely dry for most of the year, only filling with water during periods of abundant rainfall.

Table 1. Habitat suitability index (HSI) results for surveyed ponds (2023)

Pond Reference	Suitability Score	Suitability Rating	Comments
1	0.66	Average	
2	N/A	N/A	Informed pond is dry by homeowner.
3	0.38	Poor	
4	N/A	N/A	Pond dry.
5	0.35	Poor	
6	N/A	N/A	Pond dry.
7	N/A	N/A	Pond dry.
11	0.66	Average	
12	N/A	N/A	Pond dry.
19	0.71	Good	

2024

3.5 A summary of the HSI assessment for surveyed ponds is provided in Table 2 below.

Table 2. Habitat suitability index (HSI) results for surveyed ponds (2024)

Pond Reference	Suitability Score	Suitability Rating	Comments
3	0.25	Poor	
4	N/A	N/A	Pond dry.
5	0.23	Poor	
6	0.24	Poor	
7	N/A	N/A	Pond dry.
11	0.77	Good	
12	0.46	Poor	
19	0.79	Good	

3.6 Full HSI results for ponds surveyed in 2024 are provided below.

Environmental DNA (eDNA) Sampling

2023

3.7 Environmental DNA sampling was undertaken of ponds P1, P3, P5 and P11 in September 2023.

3.8 All ponds returned a negative result for great crested newts.

2024

3.9 Environmental DNA sampling was undertaken of ponds P3, P5, P6, P11, P12 and P19 in June 2024.

3.10 Ponds P3, P5 and P6 returned a negative result for great crested newts, indicating a likely absence of GCN in the ponds at this time.

3.11 Water samples for P11, P12 and P19 returned a positive result for great crested newts, indicating great crested newt presence in the ponds at this time.

3.12 The full eDNA lab results from ADAS are included at the end of this report.

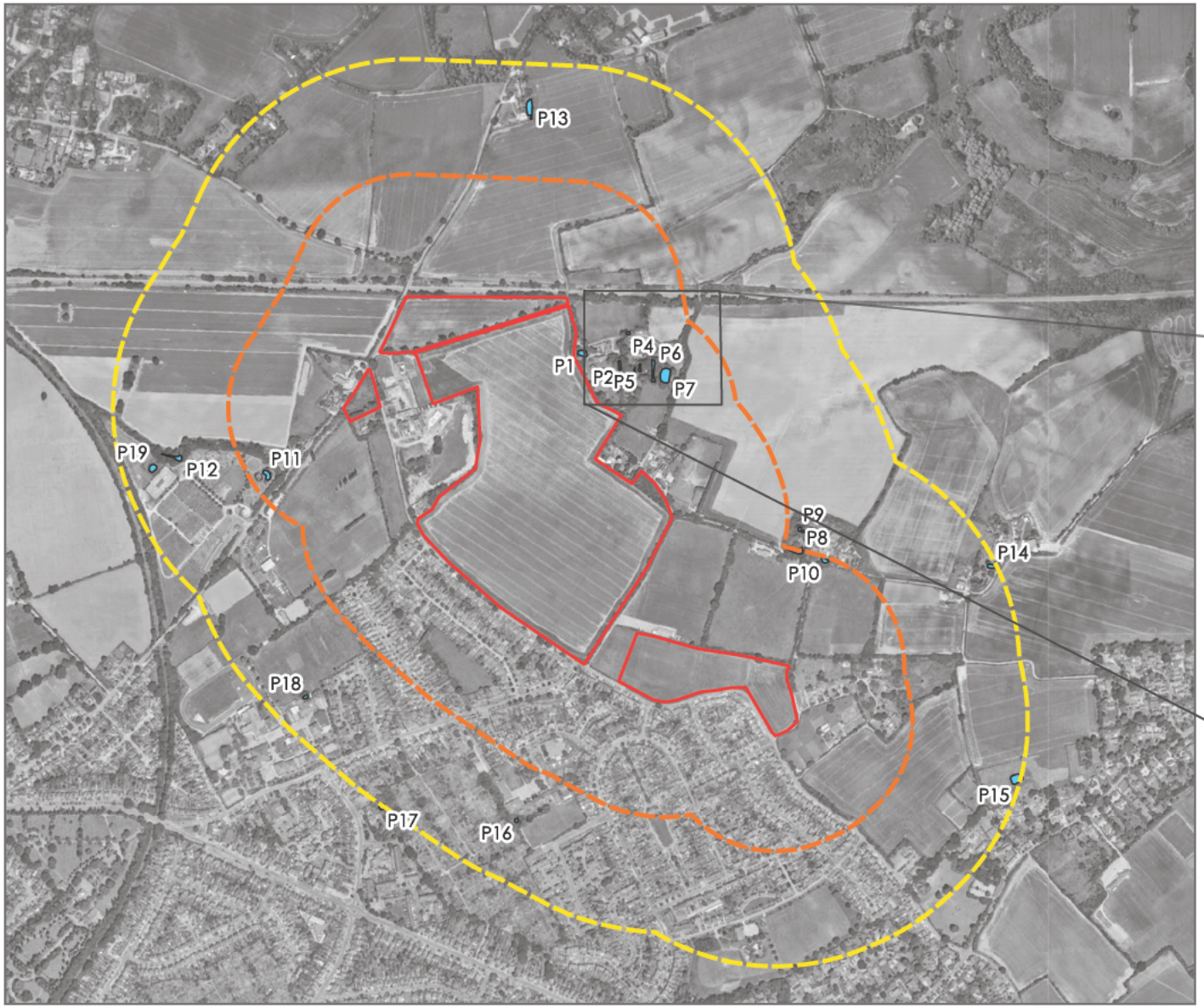
3.13 Based on the survey effort it can be concluded that GCN are likely absent from ponds P3, P5 and P6, and are confirmed to be present within ponds P11, P12 and P19.

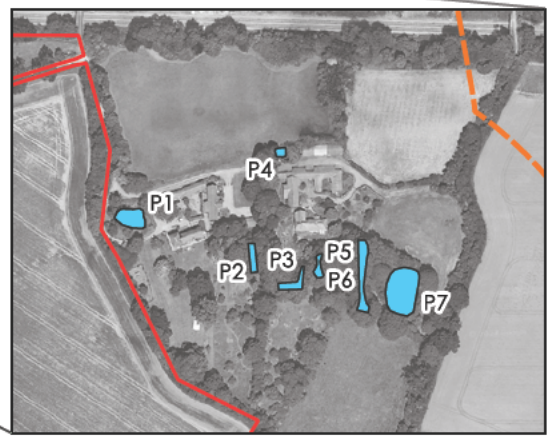
4.0 References

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. Oxford: Freshwater Habitats Trust.

English Nature, 2001. *Great Crested Newt Mitigation Guidelines.* Peterborough: English Nature.

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.





Site boundary
250m Buffer
500m Buffer
Ponds (with number)

N

0 0.5 1 km

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Project	Land North-East of Humber Doucy Lane, Ipswich	Date	February 2024	Drawing No.	CSA/6675/102
Drawing Title	Waterbodies Plan	Scale	Refer to scale	Rev	-
Client	Barratt David Wilson Homes & Hopkins Homes	Drawn	MD	Checked	CH

Habitat Suitability Factors:		Pond Number and Grid Reference																		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
		TM 18709 47140	TM 18771 47084	TM 18812 47095	TM 18811 47184	TM 18833 47113	TM 18846 47100	TM 18884 47092	TM 19163 46724	TM 19181 46764	TM 19239 46692	TM 18026 46878	TM 17827 46918	TM 18596 47677	TM 19604 46684	TM 19652 46221	TM 18566 46134	TM 18270 46172	TM 18106 46399	TM 17777 46893
Map location	Category			Zone A		Zone A	Zone A					Zone A	Zone A							Zone A
	SI Value			1		1	1					1	1							1
Pond area in m^2	Category			125m2		50-100m2	225m2					200m2	150m2							250m2
	SI Value			0.25		0.1	0.45					0.4	0.3							0.5
Permanence / Desiccation	Category			Sometimes Dries		Sometimes Dries	Dries Annually					Rarely Dries	Dries Annually							Sometimes Dries
	SI Value			0.5		0.5	0.1					1	0.1							0.5
Water quality	Category			Bad		Bad	Bad					Good	Poor							Good
	SI Value			0.01		0.01	0.01					1	0.33							1
Percentage perimeter shade to at least 1m from shore	Category			91-95%		91-95%	86-90%					66-70%	96-100%							0-60%
	SI Value			0.3		0.3	0.4					0.8	0.2							1
Waterfowl impact (excluding moorhen)	Category			Major		Major	Major					Minor	Absent							Minor
	SI Value		0	0.01		0.01	0.01					0.67	1							0.67
Fish presence	Category			Absent		Absent	Absent					Possible	Absent							Possible
	SI Value			1		1	1					0.67	1							0.67
Number of ponds within 1km not separated by barriers	Category			>12		>12	>12					>12	>12							>12
	SI Value			1		1	1					1	1							1
Terrestrial habitat	Category			Good		Good	Good					Moderate	Moderate							Good
	SI Value			1		1	1					0.67	0.67							1
Percentage of pond surface occupied by aquatic vegetation (March – May)	Category			<1%		<1%	<1%					41-45%	<1%							46-50%
	SI Value			0.3		0.3	0.3					0.75	0.3							0.8
Product				0.000001125		0.1	0.00000054					0.07218312	0.00039798							0.08978
HSI Score				0.254164711		0.23191048	0.236177965					0.768853446	0.457073586							0.78581074
HSI Suitability		N/A	N/A	**Poor**	N/A	**Poor**	**Poor**	N/A	N/A	N/A	N/A	**Good**	**Poor**	N/A	N/A	N/A	N/A	N/A	N/A	**Good**

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Sample ID: ADAS-2016	Condition on Receipt: Good	Volume: Passed
Client Identifier: P1	Description: pond water samples in preservative	
Date of Receipt: 03/10/2023	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	06/10/2023
Degradation Control[§]	Within Limits	Real Time PCR	06/10/2023
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	06/10/2023
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:	[redacted]	Signed:	[redacted]
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	10/10/2023	Date of issue:	10/10/2023

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-954	Condition on Receipt: Medium Sediment	Volume: Passed
Client Identifier: (B) P3	Description: pond water samples in preservative	
Date of Receipt: 03/10/2023	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	06/10/2023
Degradation Control[§]	Within Limits	Real Time PCR	06/10/2023
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	06/10/2023
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)[#]	4 of 4	Real Time PCR	As above for GCN

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Sample ID: ADAS-955	Condition on Receipt: Medium Sediment	Volume: Passed
Client Identifier: (C) P5	Description: pond water samples in preservative	
Date of Receipt: 03/10/2023	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	06/10/2023
Degradation Control[§]	Within Limits	Real Time PCR	06/10/2023
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	06/10/2023
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)[#]	4 of 4	Real Time PCR	As above for GCN

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Sample ID: ADAS-2686	Condition on Receipt: Good	Volume: Passed
Client Identifier: P11	Description: pond water samples in preservative	
Date of Receipt: 03/10/2023	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	06/10/2023
Degradation Control[§]	Within Limits	Real Time PCR	06/10/2023
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	06/10/2023
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)[#]	4 of 4	Real Time PCR	As above for GCN

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Sample ID: ADAS-5726	Condition on Receipt: Medium Sediment	Volume: Passed
Client Identifier: P3, 6675	Description: pond water samples in preservative	
Date of Receipt: 10/06/2024	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	12/06/2024
Degradation Control[§]	Within Limits	Real Time PCR	12/06/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	12/06/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)[#]	4 of 4	Real Time PCR	As above for GCN

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Sample ID: ADAS-5725	Condition on Receipt: Medium Sediment	Volume: Passed
Client Identifier: P5, 6675	Description: pond water samples in preservative	
Date of Receipt: 10/06/2024	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	12/06/2024
Degradation Control[§]	Within Limits	Real Time PCR	12/06/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	12/06/2024
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:	12/06/2024	Date of issue:	12/06/2024

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Sample ID: ADAS-5727	Condition on Receipt: Medium Sediment	Volume: Passed
Client Identifier: P6, 6675	Description: pond water samples in preservative	
Date of Receipt: 10/06/2024	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control†	2 of 2	Real Time PCR	12/06/2024
Degradation Control§	Within Limits	Real Time PCR	12/06/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	12/06/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)#	4 of 4	Real Time PCR	As above for GCN

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Sample ID: ADAS-5728	Condition on Receipt: Good	Volume: Passed
Client Identifier: P11, 6675	Description: pond water samples in preservative	
Date of Receipt: 10/06/2024	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	12/06/2024
Degradation Control[§]	Within Limits	Real Time PCR	12/06/2024
Great Crested Newt*	1 of 12 (GCN positive)	Real Time PCR	12/06/2024
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:	[redacted]	Signed:	[redacted]
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	12/06/2024	Date of issue:	12/06/2024

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-5729	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: P12, 6675	Description: pond water samples in preservative	
Date of Receipt: 10/06/2024	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	12/06/2024
Degradation Control[§]	Within Limits	Real Time PCR	12/06/2024
Great Crested Newt*	12 of 12 (GCN positive)	Real Time PCR	12/06/2024
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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** 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-5205	Condition on Receipt: Good	Volume: Passed
Client Identifier: P19, 6675	Description: pond water samples in preservative	
Date of Receipt: 10/06/2024	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	12/06/2024
Degradation Control[§]	Within Limits	Real Time PCR	12/06/2024
Great Crested Newt*	12 of 12 (GCN positive)	Real Time PCR	12/06/2024
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
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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.

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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