masterplanning
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graphic design

Lockington Hall
Lockington
Derby
DE74 2RH

Tel: 01509 672772
Fax: 01509 674565
mail@fpcr.co.uk
www.fpcr.co.uk

Our ref: 11119/ MJ

Beth Evans
Freeths Ltd
Cumberland Court
80 Mount Street,
Nottingham
NG1 6HH

6th July 2023

Dear Beath,

**Re: Land off Coupals Way, Woodlands Hotel, Haverhill: Great Crested Newt eDNA Survey**

This letter provides the results of the environmental DNA (eDNA) survey to determine GCN presence/absence undertaken on four off-site ponds (P1, P2, P4 and P5) which are located within the confines of the adjacent golf club (Haverhill Golf Club) access was not permitted for the offsite pond P3. Works are required at the above site affecting both potential terrestrial and aquatic GCN habitat.

Water sampling/analysis at P1, P2, P4 and P5 was undertaken in accordance with the guidance as set out in the *Analytical and Methodological Development for Improved Surveillance of Great Crested Newt; WC1067; Appendix 5; Technical advice note for field and laboratory sampling of great crested newt (Triturus cristatus) environmental DNA*[1]. This methodology has been approved by Natural England for the determination of GCN presence/ absence.

Sampling was undertaken on 14th June 2023 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. This comprised taking samples of agitated water from 20 locations around each of the ponds 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. The samples were then transported under suitable conditions to ADAS's laboratory for analysis. Following analysis, results provided by the laboratory could have one of three outcomes which are described in Table 1 below.

[1] http://randd.defra.gov.uk/Document.aspx?Document=11976_WC1067_Appendix_5_TechnicalAdviceNote.pdf

**Table 1: Description of Possible Results of eDNA Analysis**

| Result | Description |
|---|---|
| Positive | A positive result means that eDNA from GCN was detected and they have been present within the water in the 20 days preceding sampling. An eDNA score would be provided indicating the number of positive replicates from a series of twelve. |
| Negative | DNA from GCN was not detected; in the case of negative samples the DNA extract is further tested for PCR inhibitors and degradation of the sample. |
| Inconclusive | Controls indicate degradation or inhibition of the sample, therefore the lack of detection of GCN DNA is not conclusive evidence for determining the absence of the species in the sample provided. Degradation can occur through poor storage of the samples or kits and inhibition can occur through unexpected chemicals in the sample. |

Results returned were negative for GCN DNA and GCN are considered to be absent from P1, P2, P4 and P5. Full results of the analysis are provided in the attached laboratory report.

Pond P3 was located c.280m to the southwest of the site within an area of grassland surrounded by woodland, while some suitable habitat connectivity was present in the form of grassland and linear trees/scrub, it was considered unlikely that any GCN potentially present in this waterbody would be utilising the small area of suitable habitat on site. Research suggests that the maximum routine migratory range for great crested newt has been estimated as approximately 250m from a breeding pond[2,3,4]. In addition, Jehle3 determined a terrestrial zone of 63m, within which 95% of summer refuges were located, and in a separate analysis Jehle and Arntzen5 recorded 64% of non-GCN within 20m of the pond edge.

Given the nature of the application site and the small scale of the proposed works it is considered unlikely that GCN would be present and hence the species is not considered to pose a statutory constraint to the proposals.

Please do not hesitate to contact me should you wish to discuss or have any queries.

Yours sincerely,

[redacted]

**Mark Jackson**
Senior Ecologist
FPCR Environment and Design Ltd

mark.jackson@fpcr.co.uk

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[2] *The migratory ecology and terrestrial habitat preferences of the crested newt Triturus cristatus, at Little Wittenham Nature Reserve*. Franklin, P. M. Phil Thesis, De Montford University, Dept of Applied Biology & Biotechnology. 1993.
[3] *Status of the warty newt Triturus cristatus.* Nature Conservancy Council, CSD Report no. 703. Oldman, R.S. and Nicholson, M., 1986.
[4] *The terrestrial summer habitat of radio-tracked great crested newts (Triturus cristatus) and marbled newts (Triturus marmoratus).* Herpetological Journal: 10(4): 137-142. Jehle, R., 2000.
[5] *Post-breeding migrations of newts with contrasting ecological requirements.* Journal of Zoology, London: 251: 297-306. Jehle, R. and Arntzen, J. W., 2000.

Client: Nick Grant,
FPCR Environment and Design Ltd.

ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

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

www.adas.uk

| | | |
|---|---|---|
| Sample ID: ADAS-678 | Condition on Receipt: Good | Volume: Passed |
| Client Identifier: P4 | Description: pond water samples in preservative | |
| Date of Receipt: 16/06/2023 | Material Tested: eDNA from pond water samples | |

| **Determinant** | **Result** | **Method** | **Date of Analysis** |
|---|---|---|---|
| Inhibition Control[†] | 2 of 2 | Real Time PCR | 20/06/2023 |
| Degradation Control[§] | Within Limits | Real Time PCR | 20/06/2023 |
| Great Crested Newt* | 0 of 12 (GCN negative) | Real Time PCR | 20/06/2023 |
| 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: | 20/06/2023 | Date of issue: | 20/06/2023 |

*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-687 | Condition on Receipt: Good | Volume: Passed |
| Client Identifier: P2 | Description: pond water samples in preservative | |
| Date of Receipt: 16/06/2023 | Material Tested: eDNA from pond water samples | |

| **Determinant** | **Result** | **Method** | **Date of Analysis** |
|---|---|---|---|
| Inhibition Control[†] | 2 of 2 | Real Time PCR | 20/06/2023 |
| Degradation Control[§] | Within Limits | Real Time PCR | 20/06/2023 |
| Great Crested Newt* | 0 of 12 (GCN negative) | Real Time PCR | 20/06/2023 |
| 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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*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-693 | Condition on Receipt: Good | Volume: Passed |
| Client Identifier: P5 | Description: pond water samples in preservative | |
| Date of Receipt: 16/06/2023 | Material Tested: eDNA from pond water samples | |

| **Determinant** | **Result** | **Method** | **Date of Analysis** |
|---|---|---|---|
| Inhibition Control[†] | 2 of 2 | Real Time PCR | 20/06/2023 |
| Degradation Control[§] | Within Limits | Real Time PCR | 20/06/2023 |
| Great Crested Newt* | 0 of 12 (GCN negative) | Real Time PCR | 20/06/2023 |
| 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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| Date of preparation: | 20/06/2023 | Date of issue: | 20/06/2023 |

*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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FPCR Environment and Design Ltd.

ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

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

www.adas.uk

| | | |
|---|---|---|
| Sample ID: ADAS-694 | Condition on Receipt: Good | Volume: Passed |
| Client Identifier: P1 | Description: pond water samples in preservative | |
| Date of Receipt: 16/06/2023 | Material Tested: eDNA from pond water samples | |

| **Determinant** | **Result** | **Method** | **Date of Analysis** |
|---|---|---|---|
| Inhibition Control[†] | 2 of 2 | Real Time PCR | 20/06/2023 |
| Degradation Control[§] | Within Limits | Real Time PCR | 20/06/2023 |
| Great Crested Newt* | 0 of 12 (GCN negative) | Real Time PCR | 20/06/2023 |
| 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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*[†] 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.*

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# Appendix 1: Interpretation of results

## Sample Condition

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

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

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

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

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

## What do my results mean?

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

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

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

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

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