# Barn at Shornhill Farm, Withington, GL54 4BJ

# Great Crested Newt eDNA Analysis

May 2022

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## Document Control

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## Notice to Readers:

The results of the survey and assessment work undertaken by All Ecology are representative at the time of surveying.

Every endeavour has been made to identify the presence of protected species on site, where this falls within the agreed scope of works.

The flora and fauna detailed within this report are those noted during the field survey and from anecdotal evidence. It should not be viewed as a complete list of flora and fauna species that may frequent or exist on site at other times of the year.

Up to date standard methodologies have been used, which are accepted by Natural England and other statutory conservation bodies. No responsibility will be accepted where these methodologies fail to identify all species on-site.

All Ecology cannot take responsibility where Government, national bodies or industry subsequently modify standards.

All Ecology cannot accept responsibility for data collected from third parties.

Reference to sections or particular paragraphs of this document taken out of context may lead to misrepresentation.

# Contents

# 1.0 Introduction

## Background

1.1 In April 2022, All Ecology was commissioned to facilitate Great Crested Newt environmental DNA (eDNA) analysis of a pond in relation to a site known as Barn at Shornhill Farm, Withington, GL54 4BJ. Land at Springfields, Shuthonger. The site consists of two connected barn buildings with attached lean-tos surrounded by areas of bare ground, grass, scrub and hard standing. The site is bound by fencing, hedges and overhanging trees. The local area consists of woodland, arable and grassland fields.

1.2 The proposals for the site are to convert the barn to a dwelling with the immediate surrounding areas to be re-landscaped as new garden.

1.3 A previous Ecological Appraisal included a Habitat Suitability Index (HSI) Assessment of a pond adjacent to the west boundary of the site which found this pond to score 0.52 and rated as 'below average' for this species. This is above the 0.5 threshold at which further surveys are usually required when impacts to Great Crested Newts as a result of any development of a site cannot be ruled out. There was a second pond within 100 m of the site however, this scored below 0.5 so does not require further survey.

1.4 The aim of the survey was to identify presence or absence of Great Crested Newts from the pond using eDNA extracted from pond water samples and determine whether a district licence application would be required.

## Site Location

Figure 1: Site location plan.

## Pond Location


Figure 2: Pond location plan.

# 2.0 Legislation and Status

2.1 As Great Crested Newts are listed on Schedule 5 of The Wildlife and Countryside Act (1981), they receive protection under Section 9 of this Act. The Act has been amended several times, most recently by the Countryside and Rights of Way Act 2000 which added 'or recklessly' to Section 9(4)(a) and (b). Thus, it is an offence to:

- intentionally kill, injure or take a Great Crested Newt
- possess or control any live or dead specimen or anything derived from a Great Crested Newt
- intentionally or recklessly damage, destroy or obstruct access to any structure or place used for shelter or protection by a Great Crested Newt
- intentionally or recklessly disturb a Great Crested Newt while it is occupying a structure which it uses for that purpose
- transport for sale or exchange, or offer for sale or exchange a live or dead Great Crested Newt or any part of a Great Crested Newt.

2.2 The Conservation of Habitats and Species Regulations 2010 make it an offence to:

- deliberately capture or kill a Great Crested Newt
- deliberately disturb a Great Crested Newt
- deliberately take or destroy the eggs of a Great Crested Newt
- damage or destroy a breeding site or resting place of a Great Crested Newt
- keep, transport, sell or exchange or offer for sale any Great Crested Newts or anything derived from this species.

2.3 The Great Crested Newt is a NERC Priority Species (JNCC, 2017).

2.4 Smooth or Common Newts, Palmate Newts, Common Toad and Common Frog are listed under Schedule 5 of The Wildlife and Countryside Act (1981). However, only part of Section 9(5) applies to these species. As such it is an offence to transport for sale or exchange, or offer for sale or exchange alive or dead individual or any part of an individual of these species

# 3.0 Methodology

## Presence/Absence eDNA Survey

3.1 Great Crested Newt eDNA water samples of the ponds were collected on the 27th April 2022.

3.2 The eDNA water sample collection was undertaken in accordance with guidance set out in Analytical and methodological development for improved surveillance of the Great Crested Newt, WC1067, Appendix 5. Technical advice note for field and laboratory sampling of Great Crested Newt environmental DNA (Biggs et al, 2014). The sampling kit was provided by SureScreen Scientifics, who also performed the qPCR eDNA testing, which is also in accordance to the Technical advice note (Biggs et al, 2014).

3.3 In summary, 20 water samples were taken from the pond on a day with suitable weather conditions between mid-April and the end of June. Samples were taken as deep as possible without disturbing the silt. Care was taken to prevent cross contamination of the ditch and gloves and sterile equipment was used. The 20 samples were pooled into six 15 ml sub-samples and stored in sterile tubes containing ethanol to preserve the eDNA. These samples were then kept refrigerated at 2-4°C until delivery of the samples to the laboratory.

3.4 In summary, the laboratory testing is conducted in two parts. Firstly, all six samples are pooled together for the extraction process. This pooled sample is tested with real time PCR (qPCR) which amplifies the selected part of the DNA to allow it to be detected and measured, giving a positive or negative result. Each pooled sample is replicated eight times to ensure accurate results.

3.5 If results are returned as positive for Great Crested Newt eDNA, it is concluded that this species is present although the results are not a reliable indicator of population size and detailed population assessment using traditional survey methods may be required depending on the nature, scale and potential impacts of the proposals. If the results are negative, it is concluded that Great Crested Newts are absent from the pond and no further surveys are required.

## Limitations

3.6 Pond 1 was fully accessible allowing the 20 water samples to be taken at well-spaced, representative sampling points around the edges. There were no other limitations to carrying out the survey of this waterbody in accordance with the current guidelines (Biggs et al, 2014).

# 4.0 Results

## Laboratory results

4.1 A full copy of the laboratory results can be found in Appendix 1.

4.2 The replicates for the pond were all confirmed as negative therefore giving a negative result for Great Crested Newt eDNA being present in the pond at the time of survey, or up to 7-21 days prior. This indicates that Great Crested Newts are likely to be absent from the pond.

# 5.0 Conclusion

5.1 Great Crested Newts are likely to be absent from the pond and no restrictions on the proposed works with respect to Great Crested Newts, are required; a district licence is not required to permit works.

5.2 In the unlikely event a Great Crested Newt is discovered during works, work should cease immediately and a licensed ecologist be contacted for advice.

5.3 There is one other pond within 100 m and although this did not score well for Great Crested Newts, the following precautionary methods of working should be followed on site:

- Species of amphibians, including Great Crested Newts could be found on site throughout the construction phase although any potential is minimal. Any contractors on site should be advised to carry out all work with care and vigilance for this species. Should any Great Crested Newts be found during works, then works should cease immediately and a licensed Ecologist consulted before works continue.
- Works should be restricted to the designated development area and the impact of works on adjacent habitats avoided by the clear demarcation of the works area.
- Ground clearance works should ideally be undertaken during the period of March to June when newts are most likely to be in ponds and away from the site.
- The duration of any groundworks should be kept as short as possible and any trenches left overnight should be covered or provided with ramps to prevent amphibians (and other animals) from becoming trapped.
- Any debris, spoil collected during site clearance should be removed from the site immediately to avoid it becoming used as refugia by amphibians and reptiles. Any building materials should be kept off site or where not possible stored on pallets to prevent them being used as cover.

# 6.0 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*. Freshwater Habitats Trust, Oxford [online] Available at: http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&Completed=0&ProjectID=18650 [accessed 11th May 2022].

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*. Defra Project WC1067. Freshwater Habitats Trust: Oxford [online] Available at: http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&Completed=0&ProjectID=18650 [accessed 11th May 2022].

Natural England (2014) *EPS Mitigation Licensing: Latest developments - April 2014* [online] Available at: http://webarchive.nationalarchives.gov.uk/20140910073652/http://www.naturalengland.org.uk/ourwork/regulation/wildlife/species/epsnewsletters.aspx [accessed 11th May 2022].

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, p143-155.

# 7.0 Appendices

## Appendix 1 – SureScreen Scientifics eDNA Analysis Report

SureScreen Scientifics

Folio No: E13086
Report No: 1
Purchase Order: 2259
Client: ALL ECOLOGY LTD
Contact: Laura Cuming

### TECHNICAL REPORT

**ANALYSIS OF ENVIRONMENTAL DNA IN POND WATER FOR THE DETECTION OF GREAT CRESTED NEWTS (TRITURUS CRISTATUS)**

**SUMMARY**

When great crested newts (GCN), *Triturus cristatus*, inhabit a pond, they continuously release small amounts of their DNA into the environment. By collecting and analysing water samples, we can detect these small traces of environmental DNA (eDNA) to confirm GCN habitation or establish GCN absence.

**RESULTS**

**Date sample received at Laboratory:** 28/04/2022
**Date Reported:** 08/05/2022
**Matters Affecting Results:** None

| Lab Sample No. | Site Name | O/S Reference | SIC | DC | IC | Result | Positive Replicates |
|---|---|---|---|---|---|---|---|
| 3003 | Pond 1 Shornhill Farm | SO 00576 16251 | Pass | Pass | Pass | Negative | 0 |

If you have any questions regarding results, please contact us: ForensicEcology@surescreen.com

**Reported by:** Chris Troth **Approved by:** Gabriela Danickova

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## METHODOLOGY

The samples detailed above have been analysed for the presence of GCN eDNA following the protocol stated in DEFRA WC1067 'Analytical and methodological development for improved surveillance of the Great Crested Newt, Appendix 5.' (Biggs et al. 2014). Each of the 6 sub-sample tubes are first centrifuged and pooled together into a single sample which then undergoes DNA extraction. The extracted sample is then analysed using real time PCR (qPCR), which uses species-specific molecular markers to amplify GCN DNA within a sample. These markers are unique to GCN DNA, meaning that there should be no detection of closely related species.

If GCN DNA is present, the DNA is amplified up to a detectable level, resulting in positive species detection. If GCN DNA is not present then amplification does not occur, and a negative result is recorded.

Analysis of eDNA requires scrupulous attention to detail to prevent risk of contamination. True positive controls, negative controls and spiked synthetic DNA are included in every analysis and these have to be correct before any result is declared and reported. Stages of the DNA analysis are also conducted in different buildings at our premises for added security.

SureScreen Scientifics Ltd is ISO9001 accredited and participate in Natural England's proficiency testing scheme for GCN eDNA testing. We also carry out regular inter-laboratory checks on accuracy of results as part of our quality control procedures.

## INTERPRETATION OF RESULTS

**SIC:** **Sample Integrity Check** [Pass/Fail]
When samples are received in the laboratory, they are inspected for any tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to inconclusive results.

**DC:** **Degradation Check** [Pass/Fail]
Analysis of the spiked DNA marker to see if there has been degradation of the kit or sample between the date it was made to the date of analysis. Degradation of the spiked DNA marker may lead indicate a risk of false negative results.

**IC:** **Inhibition Check** [Pass/Fail]
The presence of inhibitors within a sample are assessed using a DNA marker. If inhibition is detected, samples are purified and re-analysed. Inhibitors cannot always be removed, if the inhibition check fails, the sample should be re-collected.

**Result:** **Presence of GCN eDNA** [Positive/Negative/Inconclusive]
**Positive:** GCN DNA was identified within the sample, indicative of GCN presence within the sampling location at the time the sample was taken or within the recent past at the sampling location.
**Positive Replicates:** Number of positive qPCR replicates out of a series of 12. If one or more of these are found to be positive the pond is declared positive for GCN presence. It may be assumed that small fractions of positive analyses suggest low level presence, but this cannot currently be used for population studies. In accordance with Natural England protocol, even a score of 1/12 is declared positive. 0/12 indicates negative GCN presence.
**Negative:** GCN eDNA was not detected or is below the threshold detection level and the test result should be considered as evidence of GCN absence, however, does not exclude the potential for GCN presence below the limit of detection.