



13a North Common Road, Uxbridge, UB8 1PD.

Great Crested Newt eDNA Analysis



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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.

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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.

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1.0 Introduction

Background

- 1.1 In March 2018, All Ecology was commissioned to facilitate Great Crested Newt environmental DNA (eDNA) analysis of a pond on a site known as 13a North Common Road, Uxbridge, UB8 1PD. The site comprises a detached house with driveway and small lawn to the front (south), with access around the east side to a rear garden where lawns, sheds and a greenhouse are present. Next to this is a large pond surrounded by recently cleared trees and vegetation. The site is enclosed by a mix of fencing and hedges and there are a number of mature trees on the boundary. The site is bordered by residential gardens and houses on all aspects. The driveway connects to a narrow unsealed access road that connects to North Common Road to the south beyond which is a large open space which is Uxbridge Common.
- 1.2 The site is the subject of a planning application to permit the demolition of the existing house followed by the construction of a new residential development of 6 units. The majority of the pond would be filled in but a section would be retained and enhanced.
- 1.3 A previous Ecological Appraisal of the site carried out in February 2019 recorded the pond on site and one in the surrounding area. The pond on site (Pond 1) and Pond 2 on Uxbridge Common provide potential breeding habitat for amphibians, including Great Crested Newts and these were therefore subject to the Great Crested Newt HSI Assessment. Pond 1 scored 0.69 and a rating of 'average'. Pond 2 scored 0.82 and a rating of 'excellent'. These are above the 0.5 threshold at which further consideration is usually necessary. Great Crested Newt surveys of these ponds were carried out by All Ecology in 2015. These recorded a maximum count of one in the Uxbridge Pond; they were found to be absent from the pond on site although a maximum count of 31 Smooth Newts was recorded.
- 1.4 Although Pond 2 was previously found to support Great Crested Newts it is relatively isolated from the site with newts having to cross roads, driveways, and gardens in order to access the site. Nevertheless, newts present in this pond could potentially access the site including the pond. Great Crested Newts were previously found to be absent from Pond 1; however, this survey took place nearly four years ago and it is possible that they have colonised the pond although this was considered unlikely.
- 1.5 Due to the length of time that has passed since the previous survey in 2015, it was considered necessary to determine Great Crested Newt presence/absence from the pond on site. Due to the previous recorded absence of Great Crested Newts from this pond it was recommended that the eDNA method be used.

Objectives and Aim

- 1.6 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 population assessment was required to inform a mitigation strategy should they be present

Site Location



Figure 1: Site location plan.

Aerial Photograph



Figure 2: Aerial photograph of the site.

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 were collected on the 15th April 2019.
- 3.2 The surveys were undertaken by a Licensed Consultant Ecologist (Class Licence Registration 2016-20363-CLS-CLS).
- 3.3 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 kits were 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).
- 3.4 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 pond 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 2-4oC until delivery of the samples to the laboratory.
- 3.5 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.
- 3.6 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

- 3.7 The pond was fully accessible allowing the 20 water samples to be taken at well spaced, representative sampling points around the pond edges. There were no limitations to carrying out the survey 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 each pond were all confirmed as negative therefore giving a negative result for Great Crested Newt eDNA being present in this 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 on site and therefore the site. To take into account the known presence of Great Crested Newts in Pond 2, all works should be carried out in accordance with precautionary methods of working and reasonable avoidance measures detailed in a method statement secured by a suitably worded planning condition.

6.0 References

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7.0 Appendices

Appendix 1 – SureScreen Scientifics eDNA Analysis Report

Folio No: E4622
Report No: 1
Order No: 1917
Client: ALL ECOLOGY LTD
Contact: James Godbeer
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Date: 01/05/2019

TECHNICAL REPORT

ANALYSIS OF ENVIRONMENTAL DNA IN POND WATER FOR THE DETECTION OF GREAT CRESTED NEWTS

Date sample received at Laboratory: 23/04/2019
Date Reported: 01/05/2019
Matters Affecting Results: None

RESULTS

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
1028	13a North Common Rd	TQ 05973 85192	Pass	Pass	Pass	Negative	0

SUMMARY

When Great Crested Newts (GCN); *Triturus cristatus* inhabit a pond, they deposit traces of their DNA in the water as evidence of their presence. By sampling the water, we can analyse these small environmental DNA (eDNA) traces to confirm GCN habitation, or establish GCN absence.

The water samples detailed below were submitted for eDNA analysis to the protocol stated in DEFRA WC1067 (Latest Amendments). Details on the sample submission form were used as the unique sample identity.

RESULTS INTERPRETATION

Lab Sample No.- When a kit is made it is given a unique sample number. When the pond samples have been taken and the kit has been received back in to the laboratory, this sample number is tracked throughout the laboratory.

Site Name- Information on the pond.

O/S Reference – Location/co-ordinates of pond.

SIC- Sample Integrity Check. Refers to quality of packaging, absence of tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to results errors. Inspection upon receipt of sample at the laboratory. To check if the Sample is of adequate integrity when received. Pass or Fail.

DC- Degradation Check. Analysis of the spiked DNA marker to see if there has been degradation of the kit since made in the laboratory to sampling to analysis. Pass or Fail.

IC- Inhibition Check- PCR inhibitors can cause false results. Inhibitors are analysed to check the quality of the result. Every effort is made to clean the sample pre-analysis however some inhibitors cannot be extracted. An unacceptable inhibition check will cause an indeterminate sample and must be sampled again.

Result- NEGATIVE means that GCN eDNA was not detected or is below the threshold detection level and the test result should be considered as no evidence of GCN presence. POSITIVE means that GCN eDNA was found at or above the threshold level and the presence of GCN at this location at the time of sampling or in the recent past is confirmed. Positive or Negative.

Positive Replicates- To generate the results all of the tubes from each pond are combined to produce one eDNA extract. Then twelve separate analyses are undertaken. If one or more of these analyses are positive the pond is declared positive for the presence of GCN. 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.

METHODOLOGY

The laboratory testing adheres to strict guidelines laid down in WC1067 Analytical and Methodological Development for Improved Surveillance of The Great Crested Newt, Version 1.1

The analysis is conducted in two phases. The sample first goes through an extraction process where all six tubes are pooled together to acquire as much eDNA as possible. The pooled sample is then tested via real time PCR (also called q-PCR). This process amplifies select part of DNA allowing it to be detected and measured in 'real time' as the analytical process develops. qPCR combines PCR amplification and detection into a single step. This eliminates the need to detect products using gel electrophoresis. With qPCR, fluorescent dyes specific to the target sequence are used to label PCR products during thermal cycling. The accumulation of fluorescent signals during the exponential phase of the reaction is measured for fast and objective data analysis. The point at which amplification begins (the Ct value) is an indicator of the quality of the sample. True positive controls, negatives and blanks as well as spiked synthetic DNA are included in every analysis and these have to be correct before any result is declared so they act as additional quality control measures.

The primers used in this process are specific to a part of mitochondrial DNA only found in GCN ensuring no DNA from other species present in the water is amplified. The unique sequence appropriate for GCN analysis is quoted in DEFRA WC 1067 and means there should be no detection of closely related species. We have tested our system exhaustively to ensure this is the case in our laboratory. We can offer eDNA analysis for most other species including other newts.

Analysis of eDNA requires scrupulous attention to detail to prevent risk of contamination. Kits are manufactured by SureScreen Scientifics to strict quality procedures in a separate building and with separate staff, adopting best practice from WC1067 and WC1067 Appendix 5. Kits contain a 'spiked' DNA marker used as a quality control tracer (SureScreen patent pending) to ensure any DNA contained in the sampled water has not deteriorated in transit. Stages of the DNA analysis are also conducted in different buildings at our premises for added security.

SureScreen Scientifics Ltd also participate in Natural England's proficiency testing scheme and we also carry out inter-laboratory

checks on accuracy of results as part of our quality procedures.

Reported by: Derry Hickman

Approved by: Chris Troth

End Of Report
