



Great Crested Newt Environmental DNA Survey Report

Briefing Note Report

Old Well Cottage, Powney Street, Milden, Ipswich, Suffolk IP7 7AL



Ms J. Baker, Old Well Cottage, Powney Street, Milden, Ipswich, Suffolk IP7 7AL

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Report prepared by Dr J. Huckle for Huckle Ecology Ltd

Declaration: The information and advice which we have prepared and presented it true and has been developed in accordance with the Chartered Institute of Ecology and Environmental Management Code of Professional Conduct. We confirm that any opinions expressed are our own and my true and bona fide opinions.

Digital Signature:



Dr Jon Huckle CEnv MCIEEM MSc BSc (Hons)

Executive Summary

- Huckle Ecology was commissioned in June 2023 by Ms J. Baker to undertake Environmental DNA surveys to determine the presence or likely absence of great crested newts within water bodies located within the grounds of Old Well Cottage, Powney Street, Milden, Suffolk. A Preliminary Ecological Appraisal undertaken in June 2023 had identified a small garden pond and a section of wet ditch that were considered to provide "...potential opportunities for GCN and other amphibians". The PEA report stated that "Environmental DNA (eDNA) surveys will be required of the onsite pond and vegetated ditch to determine the presence or absence of great crested newts".
- An eDNA survey was undertaken on June 23rd 2023, following a walkover survey on 20th June 2023 to confirm the aquatic habitats present and the suitability of the water bodies for eDNA sampling.
- The section of wet ditch held only shallow water for a short section of ditch and was considered to be generally unsuitable as great crested newt aquatic habitat.
- The on site pond was small and artificial in character with a butyl line present around a small area of aquatic and marginal vegetation. eDNA samples were collected and analysed by the Surescreen Scientifics Laboratory.
- The eDNA survey of the on site pond detected no DNA of great crested newt and this is considered sufficient to conclude that great crested newts are absent from the pond. Based on the small size of the site, the sub-optimal aquatic and terrestrial habitats present within the site, and the relative isolation of the site from other suitable ponds, it is considered unlikely that great crested newts will be present.
- Mitigation and enhancement measures have been recommended that demonstrate good practice and will deliver a net gain for biodiversity in proportion to the scale and character of the proposed development.

1 Introduction

Terms of Reference

- 1.1 Huckle Ecology was commissioned in June 2023 by Ms J. Baker to undertake Environmental DNA surveys to determine the presence or likely absence of great crested newts within water bodies located within the grounds of Old Well Cottage, Powney Street, Milden, Suffolk. A Preliminary Ecological Appraisal undertaken in June 2023 had identified a small garden pond and a section of wet ditch that were considered to provide "...potential opportunities for GCN and other amphibians". The PEA report stated that "Environmental DNA (eDNA) surveys will be required of the onsite pond and vegetated ditch to determine the presence or absence of great crested newts".

Aim of Report

- 1.2 This report provides the findings of eDNA surveys undertaken by Huckle Ecology in June 2023. The eDNA surveys were specified as requirements in a Preliminary Ecological Appraisal report (Arbtech, June 2023), as noted above.
- 1.3 This report does not constitute a comprehensive Ecological Impact Assessment (EIA) but is considered an addendum to, and should be considered in combination with, the PEA report prepared for the Site.

2 Methodology

Walkover Survey of Site

- 2.1 An initial walkover survey was undertaken on June 20th 2023 to determine the suitability of the onsite pond and ditch for great crested newt eDNA surveys taking into account the sampling limitations and requirements inherent within the eDNA survey methodology.

Great crested newt eDNA Survey

- 2.2 Both water bodies were very small in size and held reduced levels of water during both the walkover survey on June 20th and the eDNA sampling survey on June 23rd 2023.
- 2.3 The onsite pond held water with a maximum depth of approx. 20cm and was vegetated within a central area with a combination of marginal and aquatic plants. The pond was an artificial garden pond, with a butyl liner, reported to be damaged and leaking, resulting in the pond often being dry and being re-filled through rain water.
- 2.4 The ditch was dry at the time of the walkover survey (20th June) but following rain, held a shallow level of water (<5cm deep) for a short length of the ditch (ca. 2-3m) at the time of eDNA survey (23rd); this ditch is understood to receive surface drainage water from the garden and also outflow from a domestic treatment plant set within the garden. Due to the low levels of water present within the ditch, eDNA sampling was not possible, and was considered to not meet survey protocols recommended for the eDNA sampling, due to the shallow depth being less than 10cm, and the ditch containing an inflow and outflow of water.
- 2.5 Following the walkover survey, it was considered highly unlikely that great crested newt would be present, based on the size, condition and relative isolation of the pond and ditch from other suitable water bodies. However, eDNA sampling of the on site pond was undertaken, as described below.

Environmental DNA Survey Methodology

- 2.6 The eDNA sampling was undertaken by Dr Jon Huckle, who holds a Level 1 Class Licence to survey great crested newts (Class Licence No. 2016-20200-CLS-CLS) and who has been trained in the sampling protocol for great crested newt eDNA surveys.
- 2.7 The onsite pond was surveyed using the prescribed methodology (Biggs J E. N., 2014) with one eDNA kit per pond as the pond was less than 1 ha in area.
- 2.8 eDNA kits were received from the testing laboratory, Surescreen Scientifics Division Ltd, on June 21st, with samples collected on June 23rd 2023. Collected samples were returned promptly, received by the laboratory on 27th June 2023.
- 2.9 All laboratory work was conducted in accordance with guidelines set out in WC1067 Analytical and Methodological Development for Improved Surveillance of The Great Crested Newt (Biggs J E. N., 2014).

Limitations

- 2.10 The samples were collected in dry, sunny conditions with a temperature of 20-21 C, which are optimal conditions for collecting samples.

- 2.11 The samples were collected from the pond edge from water greater than 10cm deep. The small size of the pond, and the dense cover of floating vegetation meant that sampling locations were limited to areas where small areas of open water were present; consequently multiple samples were taken from a small number of locations. The pond had no active inflows at the time of survey but appeared to have received recent rain fall.
- 2.12 There were no limitations to areas considered likely to support egg laying and display areas; the pond supported marginal and aquatic vegetation, but due to the small size and artificial character was considered to provide sub-optimal habitat for great crested newts. Table 1 Guidelines for assessing the potential suitability of proposed development sites for bats, taken from Collins 2016.

Environmental DNA Survey Results

- 2.13 The results of the eDNA analysis are summarised in Table 1 below, with the full lab report included at Appendix B below.

Table 1 Results of Lab eDNA analysis

Water body	OS Grid Ref	Lab Sample No.	No. of positive replicates ¹	Detection of great crested newt
Old Well Cottage – Pond 1	TL 95209 46059	6441	0	Negative
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.				

Conclusions

- 2.14 The eDNA lab results confirmed that the onsite pond tested negative for great crested newt DNA and this result is sufficient evidence to assume that great crested newts are absent.
- 2.15 No specific mitigation measures for great crested newts are considered necessary.
- 2.16 It is recommended that to offset the loss of the small pond and to provide enhancements for biodiversity, including other amphibians species and aquatic invertebrates, that a pond of at least 20m² is created as an integral part of the design of the proposed development; a new pond would ideally be located at the end of the proposed development site, to provide habitat connectivity to adjacent hedgerows and areas of mature trees and scrub.

3 References

- Arbtech. (June 2023). *Preliminary Ecological Appraisal: Old Well Cottage, Powney Street, Milden, Ipswich, Suffolk, IP7 7AL.*
- Biggs J, E. N. (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.

Appendix A – Surescreen Scientifics Technical Report

Analysis Of Environmental DNA in pond water for the detection of great crested newts (*Triturus cristatus*)

Folio No: E18352
Report No: 1
Purchase Order: 20279
Client: HUCKLE ECOLOGY LTD
Contact: Jon Huckle

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: 27/06/2023
Date Reported: 04/07/2023
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
6441	Oldwell Cottage - Pond 1	TL 95209 46059	Pass	Pass	Pass	Negative	0

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

Reported by: Chris Troth

Approved by: Jackson Young



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.

