

Great Crested Newt Survey

**Land off Rockingham Roundabout,
Hoyland, near Barnsley**

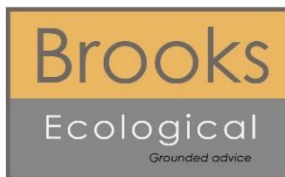
Gregory Property Group

Report Reference: R-3806-03

May 2019

Report Title:	Great Crested Newt Assessment & Report Land off Rockingham Roundabout, Hoyland
Report Reference:	R-3806-03
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Date:	02.05.19

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Introduction

1. Brooks Ecological Ltd was commissioned by Gregory Property Group to carry out surveys for Great Crested Newt at a pond located off Rockingham Roundabout, Hoyland (grid reference SE 3533 0081).
2. Survey is required to establish the presence or likely absence of Great Crested Newt (GCN) in the on-site pond and, if applicable, to collect a sufficient level of information on the GCN population resident within the pond - to support an application for a Natural England (NE) European Protected Species Mitigation Licence (EPSML).

Baseline Information

3. A Preliminary Ecological Appraisal was initially undertaken by Brooks Ecological Ltd in February 2019. This identified a single pond (Pond 1) on the eastern boundary of the Site. A further two ponds are located within a 500m radius of the Site (Ponds 2 and 3 on Figure 2), to the north, but both are separated from it by major infrastructure (Dearne Valley Parkway) which would form a major barrier to amphibian dispersal from these ponds into the Site. As such, the potential presence of amphibians in these ponds is not considered further.
4. The Desk Study identified a single historic record of GCN larvae within Pond 1, dating back to 2013; however, eDNA sampling carried out in June 2018 as part of a nearby development returned a negative result. Given the mixed results of previous surveys, it was thought possible that the pond supports a very small GCN population that only visits this pond sporadically.
5. Survey was required, as if GCN are present in Pond 1, they would be highly likely to be present on the wider Site; at which point they would represent a potential constraint to development.



Figure 1

Pond 1 located on eastern boundary of the Site.

Figure 2 Pond location plan



Methodology

6. Survey work was directed by Kate Wright BSc (Hons) MSc GradCIEEM, Natural England Class Licence CL09 (2015-17624-CLS-CLS).

Surveying

7. The pond was surveyed twice in April 2019 using traditional techniques. A summary of survey conditions is shown in the table below.

Table 1 Survey Conditions.

Visit	Date	Temp.	Weather
1	10 th April 2019	9°C	Dry. Light wind (B2). Clear skies. Cool.
2	24 th April 2019	11°C	Dry (rain earlier in day). Light wind (B1). Overcast with 95% cloud. Humid.

8. Each visit used a range of methods including egg search, netting, funnel trapping and torch counts in accordance with English Nature GCN Mitigation Guidelines 2001.
- Egg search involved an examination of submerged marginal vegetation to determine the presence of newt eggs.
 - A hand held net was used along the margins of the pond to collect any adult newts or larvae.
 - Torch counting involved 'sweeping' the whole area of the ponds using a powerful torch to determine the presence and relative abundance of any adult amphibians and to note the presence of larvae.
 - Funnel traps were positioned around the pond margins and within the ditch and left overnight, with any captured newts being released early the following morning.

eDNA Sampling

9. eDNA testing involves taking water samples from 20 different locations around the pond, focusing on areas where newts are more likely to gather. The samples are combined and redistributed into six sample pots before being sent off for analysis. Sterile kits, supplied by SureScreen Scientifics, were used and the methodology as advised in the Natural England Technical Advice Note (WC1067) was followed, taking all necessary measures to avoid contamination.
10. Water samples were collected on 15th April 2019 and sent off for laboratory analysis (kit number 0467; lab reference E4489).

Results

Surveying

11. Pond 1 was surveyed twice in April 2019 using traditional techniques. No evidence of GCN was found during these visits, but other amphibians were detected, including common frog (adults and spawn) and smooth newt (adults and juvenile).
12. A copy of the survey conditions and results are shown in Appendix 2.

eDNA Sampling

13. eDNA results were returned on 1st May 2019 and the pond (Pond 1) tested negative for GCN eDNA.
14. A copy of the laboratory report can be seen in Appendix 1.
15. Following the return of a negative eDNA test, traditional survey was curtailed.

Conclusion

16. A combination of traditional survey and eDNA sampling has been able to demonstrate the likely absence of GCN from Pond 1, and therefore also from the proposed development Site.
17. Proposals to develop the Site therefore present a negligible risk of impacting on GCN and no further survey or specific mitigation is required.

Appendices

Appendix 1 Full Survey Results

Appendix 2 SureScreen eDNA Results

References

English Nature (2001) Great Crested Newt Mitigation Guidelines.

http://www.naturalengland.org.uk/Images/GreatCrestedNewts_tcm6-21705.pdf

Natural England (2013) Standing Advice Species Sheet: Great crested newts

<http://publications.naturalengland.org.uk/publication/810429?category=30014>

Appendix 1 - Full Survey Results

The below tables contain all the data collected for each pond during the survey. During each visit the condition of each pond, in terms of Vegetation Cover (VC) and Turbidity (TRB), was assessed.

Table A1.1: Amphibian Survey Summary: Pond 1

Visit	Date	Weather Conditions	Amphib's	Survey Methods				Notes
				Torch	Bottle Traps	Netting	Egg Search	
1	10.4.19	Dry. Light wind (B2). Clear skies. Cool. 9°C.	GCN	0	0	0	0	VC = 1 TRB = 3
			Other	1 FS		0	0	
2	24.4.19	Dry (rain earlier in day). Light wind (B1). Overcast with 95% cloud. Humid. 11 °C.	GCN	0	0	0	0	VC = 1 TRB = 1-3 (Clear in shallows, becoming quite turbid in deeper water)
			Other	3 F, FTP 3 SN	1 F 1 SN (J)	0	0	

GCN=great crested newt SN=smooth newt PN= palmate newt F=frog T=toad FS=Frog spawn
TS= Toad spawn FTP=Frog Tadpole TTP=Toad Tadpole

♀= female ♂= male J=Juvenile

VC is scored from 0 = no vegetation to 5 = fully covered
TRB is scored from 0 = clear to 5 = very turbid.

Appendix 2 - SureScreen eDNA Results

Folio No: E4489
Report No: 1
Order No: 3806
Client: BROOKS ECOLOGICAL
Contact: Kate Wright
Contact Details: kw@brooks-ecological.co.uk
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: 17/04/2019
Date Reported: 01/05/2019
Matters Affecting Results: None

RESULTS

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
0467	Rockingham	-	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: Troy Whyte

Approved by: Sarah Evans

End Of Report
