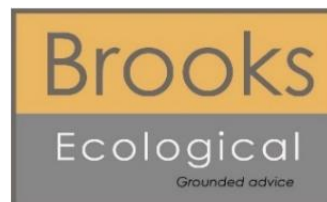


Site Inspection Report

Land off Barugh Green Road, Barnsley



Project:	Land off Barugh Green Road, Barnsley	Site Inspection reference:	SI-4661-01
Date of inspection:	21.04.2021	No. of Pages:	8

Inspection carried out by: Victoria Baker BSc (Hons) MSc ACIEEM Ecologist

Task description eDNA survey

Two ponds (Pond 1 and 2) were identified on mapping within a 500m radius of the Site in Brooks Ecological's Preliminary Ecological Appraisal Report (report reference ER-4661-01).

Wildscapes, on behalf of Barnsley Council also identified the presence of an additional group of 3 waterbodies (Ponds 3-5) within a 500m radius (report reference P1310).

Three drainage ditches are also located within or immediately adjacent to the Site.

Figure 1

The 5 ponds within a 500m radius and 3 drainage ditches on or adjacent to the Site.



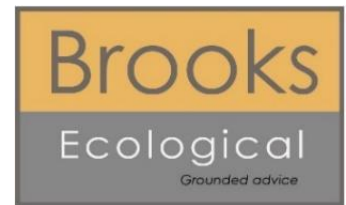
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Subsequently Wildscapes requested further information on the status of great crested newt (GCN) within the eight aforementioned waterbodies, and these were subject to eDNA survey to confirm the presence or likely absence of GCN.

The eDNA survey involved taking water samples from 20 different locations around the pond's margin, focusing on areas where newts are most likely to gather. This involved using sterile kits supplied by Surescreen Scientifics laboratory and followed methodology as advised in the Natural England Technical Advice Note (WC1067).

Survey

The waterbodies were inspected on the 21st April 2021 and are described below:

Pond 1- vertical concrete sided and supported a large population of large carp.

Pond 2- located along a minor watercourse, with some pooled standing water surrounding it (although less standing water than observed during the 2020 visit).

Pond 3- part of the same watercourse as pond 2 and 4. In this area the watercourse diverges into multiple tributaries before reforming.

Pond 4- narrow, open culverted section of fast flowing water.

Pond 5- seemingly a small drainage tank (stone wall surround), fed by the steeply sloped gardens before the water exits into the adjacent off-site drainage ditch. Held c.5cm of water and was infilled significantly with leaf litter.

Ditch 1- Held c.20cm of water with a slow flow.

Ditch 2- Dry at the time of survey.

Ditch 3- Dry at the time of survey.

Whilst the ecologist's professional judgement of Pond 1 on both the 21st April and as concluded in the PEA report was that it would be highly unlikely to support GCN, following the request by Wildscapes, an eDNA test of this waterbody was still undertaken.

In addition, Pond 2 and Ditch 1 (titled 'Northern' on the SureScreen eDNA report) were also subject to eDNA analysis.

Ponds 3 - 5 and Ditch's 2 - 3 were not subject to eDNA testing due to Ponds 3 and 4 being sections of the same watercourses with no standing water available, Pond 5 holding only a negligible amount of water and which was part of a moving water system and Ditch's 2 - 3 being dry.



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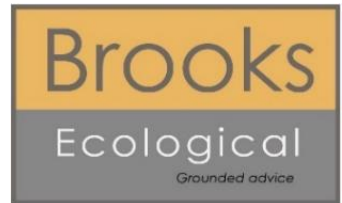


Figure 2

View of Pond 1



Figure 3

Large population of carp within Pond 1



Figure 4

View of Pond 2 (watercourse in foreground and some pooled water in background)



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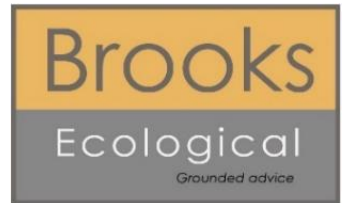


Figure 5

View of 'Pond' 3



Figure 6

View of 'Pond' 4



Figure 7

View of 'Pond' 5



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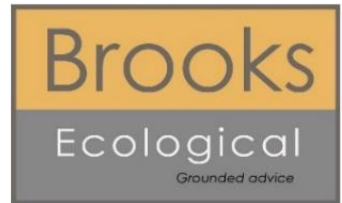


Figure 8

View of Ditch 1
(Northern)



Figure 9

View of Ditch 2



Figure 10

View of Ditch 3



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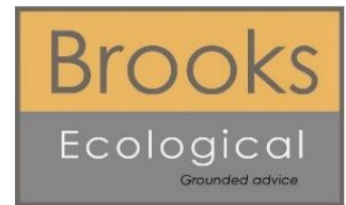
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Results

Water samples were collected on the 21st April 2021 and the results returned on the 7th May 2021 - see the following pages for Surescreen's full report.

A negative result was returned for all three samples, meaning that GCN DNA was not detected within the water samples collected. This result can be used to demonstrate the likely absence of GCN from Pond 1, Pond 3 and Drainage Ditch 1 (Northern).

Conclusions and Recommendations

Based on this information, it is concluded that the risk of GCN being present on Site is negligible and no further survey or specific mitigation is considered necessary.

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.



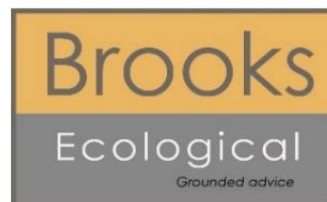
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Site Inspection Report

Land off Barugh Green Road, Barnsley



Folio No: E9599
Report No: 1
Purchase Order: 4661
Client: BROOKS ECOLOGICAL
Contact: Vickie Baker

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: 23/04/2021
Date Reported: 07/05/2021
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
2331	POND 1 BARUGH GREEN		Pass	Pass	Pass	Negative	0
2332	NORTHERN DITCH		Pass	Pass	Pass	Negative	0
2334	POND 2		Pass	Pass	Pass	Negative	0

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

Reported by: Chris Troth

Approved by: Chris Troth



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Page 1 of 2



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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.



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Page 2 of 2



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