

Appendix 2 – eDNA Survey Results

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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: 29/04/2021
Date Reported: 02/05/2021
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
1770	HBL-001	SJ 40883 99372	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



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.



Appendix 3: Pond Descriptions and Photographs

Photograph 1. Pond 1 - Northwood fishing pond: situated within Northwood Local Wildlife Site (LWS), small lake with multiple fishing platforms, extensive common reed is present around the margins with occasional yellow flag iris and bulrush. Lily pads and broad-leaved pond were present sporadically within the channel.



Photograph 2. Pond 2 – Northwood pond: situated south of the fishing lake. The pond is dominated by marginal vegetation with limited open water present.



Photograph 3. Pond 3 within Northwood Hills: Seasonally wet ephemeral pond with presence of yellow flag iris and soft rush. Shallow pond within a flooded area, likely to dry annually.



Photograph 4. Pond 4 within Mill Brook Park: margins dominated by common reed throughout with small areas of open water within the centre. Aquatic mint, yellow flag iris and soft rush present. Tadpoles were present during the HSI survey.



Photograph 5. Pond 5, large fishing lake north of Mill Lane within recreational area: Man-made with a wooded island present in the centre. No aquatic or marginal vegetation present. Heavily fish stocked with large numbers of wildfowl present.



Photograph 6. Pond 6, man-made pond on Kirkby golf course: Some recent management and extension to the area of the pond, water was silty and very turbid with occasional marginal vegetation of soft rush and yellow flag iris. A common frog and toad were observed under a piece of wood sheeting next to the pond.



Photograph 7. Pond 7, seasonally wet ephemeral pond in north east corner of golf course: soft rush was present within the waterbody with creeping bent and floating sweet grass noted within the channel, likely to dry annually.



Photograph 8. Pond 8, on-network waterbody: Pond within willow carr woodland which had dried in April 2021. No signs of aquatic vegetation, however water was present in March 2021 and the channel sediment was damp, likely to be seasonally wet.



Photograph 9. Pond 9, off-network waterbody approximately 10m north of pond 8: Pond is present on OS mapping, however was dry at the time of survey in April 2021, depression in ground was damp, although no signs of aquatic vegetation present. Likely to be seasonally wet.



Appendix 4 - Physical description of great crested newt

Should a newt be found, a description and photographs of great crested newt are given below to aid identification. In the UK, great crested newts may grow up to 17cm long (body and tail). They are the largest species of newt found in Britain. Great crested newts have dark grey-brown backs and flanks, and are covered with darker coloured spots so that they appear almost black in colour. They also have fine white spots on their lower flanks, which are more obvious in breeding males. Their undersides are either yellow or orange-coloured and are covered in large black blotches. Males can be distinguished from females by the presence of a jagged crest that runs along their backs, dipping at the rear of the abdomen to a smoother-edged crest above and below the tail. They also have a silver-grey stripe that runs along the tail. The male's crest is more pronounced during the breeding season, and lies flat to the body when the newt is out of water. Females lack a crest, but have a yellow-orange stripe along the lower edge of their tails.



Male and female adult great crested newt



Underside of female great crested newt



Smooth newt (at top, smaller and brown) and great crested newt (large and black)

Description of other newt species

Smooth newts are Britain's most common and widespread newt species. Adults are up to 10 cm in length. Skin is varying shades of grey or brown. Males develop a wavy crest along the back during the breeding season. Belly is yellow or orange, usually with black spots and/or blotches. Throat is spotted. Females and non-breeding males have smooth brown skin as visible in the photograph above.

Palmate Newts look very similar to smooth newts but they have more of a preference for shallow ponds on acidic soils. Adults up to 9 cm in length. Smooth skin that is brown, green or grey. Yellow belly, often with dark spots. Unspotted pink or yellow throat (unlike Smooth Newts, which have spotted throats). During the breeding season males develop a filament at the tip of their tail and black webbing on their back feet.