



Ballswood
Protected Species Surveys: eDNA Surveys

in respect of

***Section 73 Application For 3No. Changes to the Limits of
Extraction within the Existing Permitted Planning Boundary of
Ballswood Quarry***

for

Cheshire and North Wales Sand and Gravel Ltd

Prepared By

Heatons

August 2020

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1.0 INTRODUCTION

1.1 Heatons have been commissioned by Cheshire and North Wales Sand and Gravel Limited to undertake surveys for great crested newts (GCN), at Ballswood Quarry (hereafter referred to as the site).

1.2 These surveys have been undertaken to fulfil the protected species survey requirements identified in the Preliminary Ecological Appraisal (PEA) completed in March 2020.

Survey Scope

1.3 The scope of these surveys is to identify:

- the presence or likely absence of GCN within the Site;
- the presence or likely absence of GCN within a 500m radius of the Site; and
- the GCN population size, in areas where they found to be present.

1.4 GCN are afforded full protection under European and British law which makes it an offence to deliberately kill or injure individuals, damage their breeding or resting places, and/or obstruct access to their breeding or resting places.

1.5 Sufficient ecological information is required to fully inform the site design and the proposed works. Reports will enable the project to satisfy all current UK and European legal wildlife requirements, as well as national and local planning regulations. All public bodies have statutory obligations under the Natural Environment and Rural Communities Act 2006 to conserve and enhance biodiversity.

Site location and description

1.6 Ballswood Quarry is located at Gegin Lane, Wrexham, LL12 0NU, and is one of three active sand and gravel quarries in the County Borough of Wrexham (the others being Borrass Quarry and Hafod Quarry). Ballswood Quarry is operated by Cheshire and North Wales Sand and Gravel Ltd.

1.7 The majority of the site is comprised of existing quarry, with areas of scattered scrub and tall ruderal vegetation located around existing soil bunds. The boundaries of the existing quarry are comprised of broad-leaved semi natural

woodland and hedgerows. There are a number of waterbodies located within the existing quarry. The eastern section of the site is comprised of arable fields and heavily grazed improved grassland.

Proposed Development

- 1.8 The proposed development is to enable 3 proposed changes to the extraction boundary of Ballswood Quarry utilising existing quarry infrastructure and access. The proposed development will release currently sterilised mineral within the permitted planning boundary of the site. Access to the site for the purpose of extending mineral extraction from the existing quarry to the west would best be supplied through the existing quarry site.

2.0 LEGISLATION

2.1 GCN are a European protected species and are safeguarded by both the Wildlife and Countryside Act 1981 (as amended) and the Habitat Directive. Legislation extends protection to the animals, their eggs, their breeding sites and resting places and is a means by which sites can be protected from undesirable change.

- Actions that would cause an offence under UK law include:
- The deliberate capture, killing, disturbance or injury of GCN
- Damaging or destroying a breeding or resting place
- Obstructing access to their resting or sheltering places (deliberately or by not taking enough care)
- Possessing, selling, controlling or transporting live or dead newts, or parts of them
- Taking GCN eggs

2.2 The legislation covers each of the life stages including eggs, tadpoles and adult equally.

2.3 A European Protected Species (EPS) licence may be required from Natural Resources Wales (NRW) if the disturbance or damaging of their habitats (ponds and the land around ponds) cannot be avoided. An application can only be made once planning permission for the scheme has been approved. In order to obtain a protected species licence, the client must demonstrate that the work cannot be avoided and that there are no alternatives.

2.4 The protected species licence will also require a suitable protection and mitigation method to be adopted.

3.0 ASSESSMENT METHODOLOGY

Desktop Study

- 3.1 In order to inform the extended Phase 1 habitat survey and to compile background information on the site and its immediate surroundings, information on statutory and non-statutory designated sites within a 2km radius of the site was obtained from the COFNOD North Wales Environmental Information Service.
- 3.2 The COFNOD North Wales Environmental Information Service was also commissioned to undertake a data search for information on protected and notable species within the area. The National Biodiversity Network (NBN) database was also searched for protected species records within 2km of the site boundary.
- 3.3 Reference was also made to Ordnance Survey maps and aerial photography, which were used to determine the presence of open water and ponds in the area and to provide information on land use and habitat connectivity throughout the area.

Habitat Suitability Index

- 3.4 HSI Assessments were undertaken on all still water bodies within the site as well as those on the near side of major dispersal barriers (major roads, rivers and railways) within 500m of the site. This assessment was undertaken in order to establish the likelihood of great crested newts either breeding on the site or dispersing to the site in their terrestrial phase.
- 3.5 The HSI is a standard assessment method developed specifically to evaluate the habitat suitability for great crested newts. A series of factors must be considered. Each factor is assessed along suitability guidelines and allocated a value of between 0.1 (highly unsuitable) to 1.0 (highly suitable). The geometric mean of these values provides an overall suitability score for each pond. Although this is no substitute for a dedicated survey it does give an indication of whether such a survey is needed.
- 3.6 The HSI assessment was carried out on the waterbodies within the site during the extended Phase 1 habitat survey undertaken in March 2020. Previous HSI assessments had been undertaken on the site prior to the eDNA surveys being conducted in 2019. These assessments were then updated as part of the PEA Survey undertaken in March 2020.

Great Crested Newt eDNA Surveys

- 3.7 Environmental DNA analysis is a method of monitoring specific fauna within waterbodies. It is used to generate fast results for GCN presence or absence by collecting water samples from the waterbody margins. The analysis is used as it picks up fragments of DNA displaced in the water i.e. skin, faeces or animal fatalities. This method is most effective on waterbodies that hold a depth of 10cm or greater.
- 3.8 GCN eDNA surveys were undertaken on four waterbodies in total, these were Ponds 1,2,3 and 4.
- 3.9 The methodology used for surveying GCN eDNA is approved by Natural Resources Wales (NRW) following Defra funded research into the suitability of using this protocol to determine presence/likely absence of GCN. eDNA kits were supplied and analysed by SureScreen Scientifics.
- 3.10 The eDNA survey included taking 20 water samples from each of the four ponds spread out evenly around the pond edge. The samples were taken from both open water and vegetated areas, avoiding water less than 10cm deep. The water column was mixed gently avoiding stirring up sediment from the bottom.
- 3.11 All water samples were collected in a Whirl-Pak. bag and shaken for 10 seconds to mix evenly. Using a clear plastic pipette, 15ml of water was taken from the Whirl-Pak. bag, and transferred into each of six conical tubes containing 35ml preserving fluid (i.e. filling the tube to the 50ml mark). The samples were then transported to the laboratory for analysis.
- 3.12 GCN Surveys were undertaken in April 2019 by suitably licenced ecologist commissioned by Heatons.
- 3.13 These surveys were undertaken during the optimum survey season time between April and June following the methodology that of Froglife: Great Crested Newt Conservation Handbook.

Constraints

- 3.14 There were no constraints identified whilst carrying out the GCN eDNA Surveys.

4.0 RESULTS

Desk Study

Statutory Designated Sites

- 4.1 There are three Statutory Designated Sites located within 2km of the site boundary. The closest of these is the Chwarel Singret Special Site of Scientific Interest (SSSI). This is located approximately immediately adjacent to the quarry plant site and is designated for geological interest. There were no records for GCN for this site, however there was one record returned for smooth newt, (*Lissotriton vulgaris*).
- 4.2 The Marford Quarry SSSI is located 0.6km to the south-east of the site boundary. This site is designated for its large diversity of lepidopteran species which include a variety of nationally rare species. In addition to this there is a number of floral species indicative of a calcareous sand and gravel substrate, with a range of seral succession stages from bare ground to scrub/woodland. There were records for GCN returned within this designated site.
- 4.3 The Alyn waters Local Nature Reserve (LNR) is located approximately 1.9km to the south-west of the site boundary. The site is designated for its rich meadow habitat, variety of grassland, woodland. There were no records returned for GCN within this designated site.

Non-Statutory Designated Sites

- 4.4 The COFNOD search returned records for seven non-statutory designated sites within 2km of the site boundary. The closest of these is the Ballswood Quarry and Cockpit Wood Local Wildlife Site (LWS) which covers the existing quarry and the wider extending broad-leaved semi natural woodlands. There were no records for GCN returned for this (LWS).
- 4.5 The Marford Quarry SSSI site is also designated as a North Wales Wildlife Trust Reserve. This site returned records for GCN.
- 4.6 There were no records for GCN returned within any other non-statutory designated sites.

Species Records

- 4.7 COFNOD returned three counts of great crested newts (GCN), all located 800m from the site boundary in the Marford Quarry SSSI and LWS. These records were dated from 2003 to 2005.
- 4.8 The NBN search returned over 700 records GCN located approximately 1.2km to the south of the site. A large number of these records were dated from 2019.

Habitat Suitability Index

- 4.9 The PEA survey identified seven waterbodies within the site. In addition, there was one additional waterbody located approximately 200m to the west of the site boundary. All of the waterbodies located within the existing quarry were active quarry lagoons. Of the quarry lagoons, ponds 1,2 and 3 had been established for a period of time and therefore vegetation had begun to establish within the pond. Ponds 5,6 and 7 were assessed as silt lagoons. In addition, pond 8 was a newly created pond that had been recently excavated. The pond located 200m to the west of the site boundary is a large pond, surrounded by willow and alder tree species.
- 4.10 As all these ponds are present within 500m of the site (maximum dispersal distance for great crested newts), Habitat Suitability Index (HSI) assessments were carried out for each of the ponds. Please see Table 1 below for the full HSI assessment details.
- 4.11 Table 1 HSI calculations for each waterbody assessed and their suitability for GCN

Waterbody	HSI Score	Suitability
Pond 1 (Grid Reference SJ348565)	0.43	Poor
Pond 2 (Grid Reference SJ349564)	0.43	Poor
Pond 3 (Grid Reference SJ350562)	0.7	Average
Pond 4 (Grid Reference SJ344559)	0.42	Poor
Pond 5 (Silt Lagoon) (Grid Reference SJ348563)	0.43	Poor
Pond 6 (Silt Lagoon) (Grid Reference SJ348563)	0.43	Poor
Pond 7 (Silt Lagoon) (Grid Reference SJ348563)	0.43	Poor
Pond 8 (Grid Reference SJ346560)	0.43	Poor

4.12 The above table indicates that Pond 3 has an average suitability for GCN. This was assessed as this pond is closer to the southern boundary broad-leaved semi natural woodland providing higher quality terrestrial habitat for GCN. In addition, this pond contained a higher percentage of macrophyte cover providing suitable egg laying habitat for GCN.

4.13 Ponds 1, 2,4,5,6, 7 and 8 were all considered to provide a poor suitability for GCN. These ponds were all within the centre of the quarry workings and therefore the surrounding terrestrial habitat was poor. In addition, the macrophyte cover and water quality of these waterbodies was also assessed as poor.

eDNA Survey Results

4.14 GCN eDNA samples were undertaken on four waterbodies. Ponds 1,2 and 3 were located within the existing quarry and Pond 4 is located approximately 200m to the west of the site boundary.

Table 2 Results Summary of the eDNA Samples

GCN Pond Number	Distance from Site	Description of Waterbody	HSI Score	eDNA Result
1	Within the site boundary	Existing quarry lagoon, aquatic vegetation beginning to form	0.43	Negative
2	Within the site boundary	Existing quarry lagoon, aquatic vegetation beginning to form	0.43	Negative
3	Within the site boundary	Existing quarry lagoon, aquatic vegetation more established	0.7	Negative
4	250m to the South East of site boundary	Waterbody surrounded by woodland and fence. Limited aquatic vegetation suggesting the presence of fish	0.42	Negative

- 
- 4.15 All waterbodies tested for GCN eDNA returned negative results. Please see Appendix A for the result certificate provided by Surescreen Scientifics.

5.0 CONCLUSIONS

- 5.1 The results of the GCN eDNA test were negative for all four ponds testing within the site and within 500m of the site boundary. It is not anticipated that GCN would be present within any of the waterbodies scoped out as part of the HSI assessment.
- 5.2 In the unlikely case that any GCN are discovered during the works, then all works must be stopped immediately, and a suitably qualified ecologist must be informed so the issue can be dealt with accordingly.

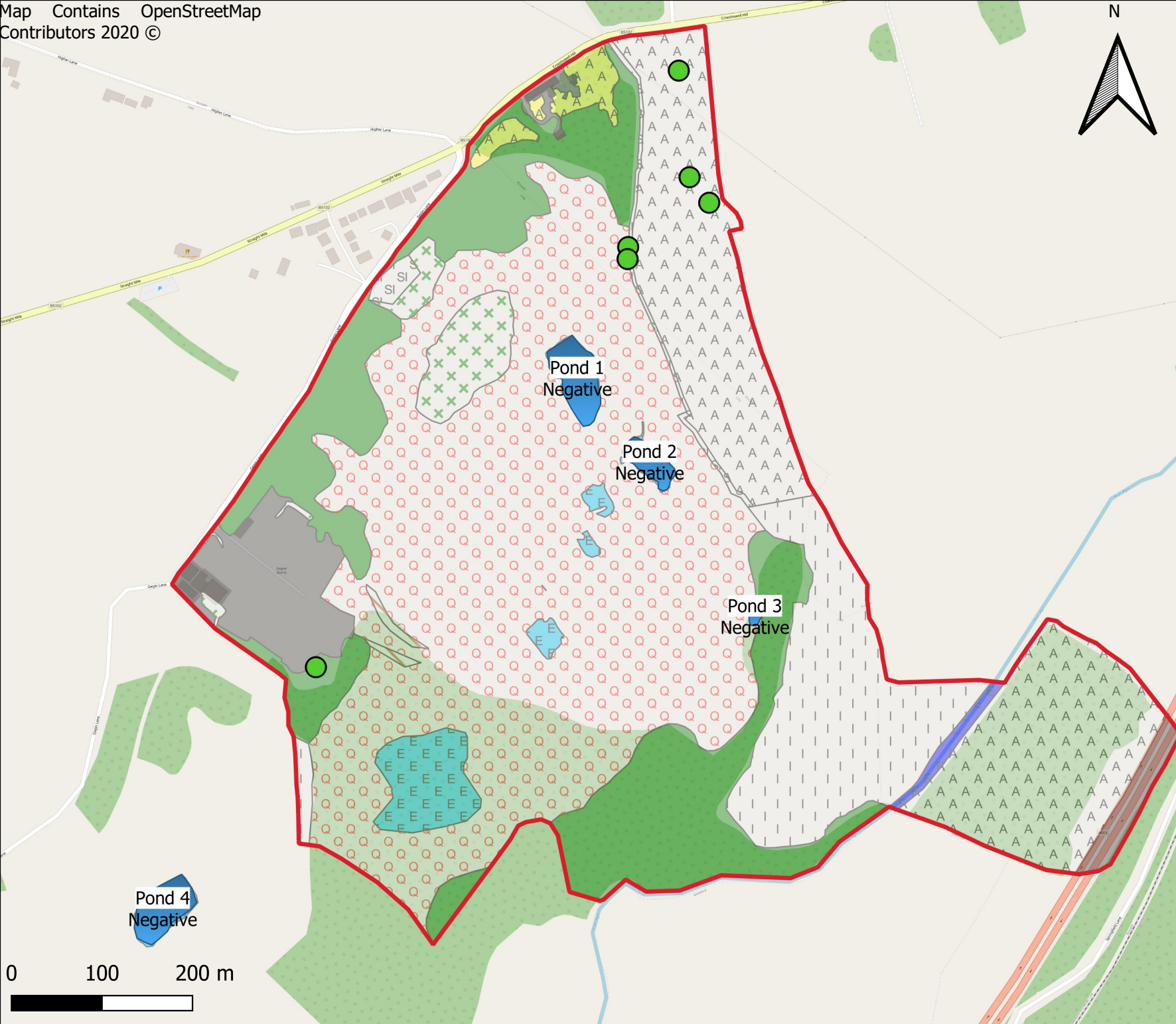
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Appendix A

eDNA Ponds Map



LEGEND

- eDNA Tested Ponds
- Site Boundary
- Scattered Tree

Phase 1 Habitats

- A1.1.1 - BL Woodland/SN
- A2.2 - Scrub - scattered
- B4 - Improved grassland
- Poor SI Grassland
- C3.1 - Tall ruderal
- G1.1 - Standing water - EU
- G2.1 - Running water - EU
- I2.1 - Quarry
- J1.1 - Arable
- J1.2 - Amenity grassland
- J3.6 - Buildings
- Hardstanding

OpenStreetMap

Heatons
Planning Environment Design

PROJECT
Ballswood Quarry

DRAWING TITLE
eDNA Tested Ponds Locations

DATE REFERENCE
Aug 2020 KEDL-003-M (ED.005)

SCALE
See Map
STATUS
FINAL



Appendix B

eDNA Test Results

Folio No: E4886
Report No: 1
Order No: ONO.124.M18
Client: PLEYDELL SMITHYMAN
Contact: Kelly Hopkins
Contact Details: kellyh@pleydellsmithyman.co.uk
Date: 07/05/2019

TECHNICAL REPORT

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

Date sample received at Laboratory: 02/05/2019
Date Reported: 07/05/2019
Matters Affecting Results: None

RESULTS

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
2090	Ballswood Quarry, pond 4	SJ3441 5594	Pass	Pass	Pass	Negative	0
2091	Ballswood Quarry, pond 1	SJ3487 5653	Pass	Pass	Pass	Negative	0
2094	Ballswood Quarry, pond 2	SJ3495 5643	Pass	Pass	Pass	Negative	0
2093	Ballswood Quarry, pond 3	SJ3507 5626	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
