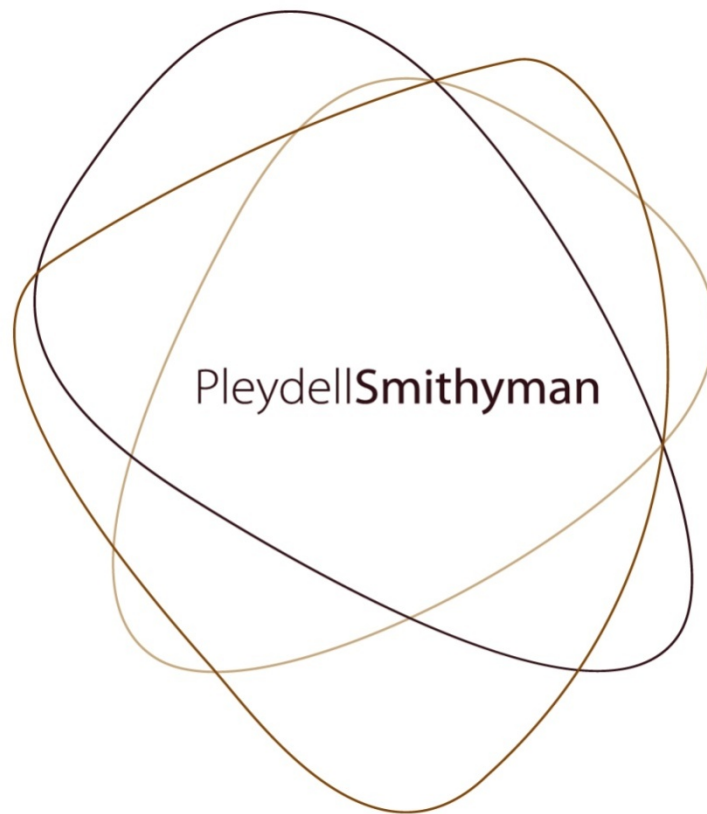




**GREAT CRESTED NEWT SURVEY
RELATING TO LAND AT THE PROPOSED WESTERN EXTENSION AT
DENBIGH QUARRY, DENBIGHSHIRE
APPLICATION FOR PLANNING PERMISSION**

For Breedon Southern Limited

DECEMBER 2019

PSL Report Reference M18.155.R.009

PREPARED BY PLEYDELL SMITHYMAN LIMITED

**20a The Wharfage,
Ironbridge,
Telford,
Shropshire,
TF8 7NH**

T: 01952 433211 F: 01952 433323

psl@pleydellsmithyman.co.uk

**GREAT CRESTED NEWT SURVEY AT THE PROPOSED WESTERN EXTENSION OF
DENBIGH QUARRY, DENBIGHSHIRE**

Report Prepared for:
Breedon Southern Limited,
Breedon Quarry,
Main Street,
Breedon on the Hill,
Derby,
DE73 8AP

Great Crested Newt Survey on land at
Denbigh Quarry,
Plas Chambres Road,
Denbigh,
Denbighshire,
LL16 5US

Main Contributors:

Kelly Hopkins

By:
Pleydell Smithyman Limited

Issued By



December 2019

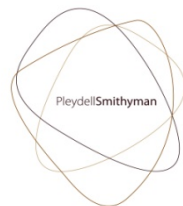
.....
Kelly Hopkins BSc (Hons) ACIEEM

Report Independently Checked By

Dr Sarah Cox CEcol CEnv MCIEEM
.....

Pleydell Smithyman Limited

20a The Wharfage
Ironbridge
Telford
Shropshire
TF8 7NH
Tel: 01952 433211
Fax: 01952 433323
Email: psl@pleydellsmithyman.co.uk



**GREAT CRESTED NEWT SURVEY AT THE PROPOSED WESTERN EXTENSION OF
DENBIGH QUARRY, DENBIGHSHIRE**

Reference	Contents	Page Number
1.0	Introduction	1
2.0	Survey Methodology	4
3.0	Results	8
4.0	Mitigation Measures	11
5.0	Conclusions and Recommendations	12
6.0	References	13

Drawings

M18.155.D.013 GCN Survey

Appendices

Appendix 1 - Additional Pond Survey Information

Appendix 2 - eDNA Survey Results

Appendix 3 - Great crested newt Survey Information

GREAT CRESTED NEWT SURVEY AT THE PROPOSED WESTERN EXTENSION OF DENBIGH QUARRY, DENBIGHSHIRE

1.0 INTRODUCTION

Background and Proposals

- 1.1 Pleydell Smithyman Limited was instructed by Breedon Southern Limited to undertake great crested newt (*Triturus cristatus*) surveys on four ponds within 500m of Denbigh Quarry, Denbighshire.
- 1.2 The surveys were required to inform the preparation and submission of a planning application for the proposed western extension of the existing quarry and to help ensure compliance with national and European legislation. The existing quarry and the proposed western extension combined is hereafter referred to as 'the site'.
- 1.3 Surveys were carried out on four ponds identified as potentially suitable by Pleydell Smithyman Limited (2019). Please see Drawing Number M18.155.D.013 for a plan showing the ponds surveyed and the site boundary. The initial Preliminary Ecological Appraisal completed on the site covered the whole site, however the assessment and further survey work focussed on any areas of change (i.e. the proposed western extension) as the existing quarry will continue to work in the same manner.
- 1.4 An additional pond (Pond 5) as shown on this plan was assessed for its suitability for great crested newt due to it being within 500m of the quarry boundary, however was not subject to further survey due to it being approximately 600m from the proposed extension.

Site Location

- 1.5 The site is located off Fford Y Graig Road, approximately 950m to the north of the centre of Denbigh in Denbighshire. The site is centred on grid reference SJ050671.

Site Description

- 1.6 The site comprised an existing working quarry with bare earth, open water and cliff faces. The working quarry was surrounded by areas of plantation and semi-natural woodland with small areas of scattered scrub. The proposed western extension was largely dominated by improved grassland with a number of scattered trees and a defunct species-poor hedgerow. The north-western boundary of the extension was bordered by Crest Mawr Wood Site of Special Scientific Interest (SSSI).

GREAT CRESTED NEWT SURVEY AT THE PROPOSED WESTERN EXTENSION OF DENBIGH QUARRY, DENBIGHSHIRE

- 1.7 In the wider area, arable and pastoral land dominated the majority of the northern and western landscape, with the town of Denbigh present to the south and east. An industrial park was also present to the east. Additional scattered ponds were present further to the east, on the eastern side of Fford y Graig Road.

Legislation

- 1.8 All British amphibian species receive a degree of protection under the Wildlife & Countryside Act 1981 (as amended). The level of protection varies from protection from sale or trade only, as is the case with species such as common toad (*Bufo bufo*) and smooth newt (*Lissotriton vulgaris*), to full protection afforded to species such as great crested newt.
- 1.9 The great crested newt is a European Protected Species and as such receives full protection under the Conservation of Habitats and Species Regulations 2017 (as amended), making it an offence to:
- Deliberately capture, injure or kill a great crested newt;
 - Deliberately disturb great crested newt, including in particular any disturbance which is likely to:
 - o impair their ability to survive, reproduce or to rear or nurture their young;
 - o impair their ability to hibernate or migrate; or
 - o Significantly affect their local distribution or abundance.
 - Deliberately take or destroy eggs of great crested newt;
 - Damage or destroy a breeding site or resting place of great crested newt;
 - Possess or control any live or dead specimen or anything derived from a great crested newt; and
 - Sell, offer for sale, possess or transport a great crested newt (live or dead, part or derivative) for the purpose of sale or advertise for buying or selling.
- 1.10 The implications of the above for the proposed project are that the works could result in the injury, death or disturbance of individual great crested newt as well as the damage and destruction of their breeding sites and terrestrial habitat if they are present on the site. As suitable waterbodies that could be used as breeding sites, as well as suitable terrestrial habitat, have been identified on the site, it is vital to establish if great crested newt are present and breeding within these waterbodies and

GREAT CRESTED NEWT SURVEY AT THE PROPOSED WESTERN EXTENSION OF DENBIGH QUARRY, DENBIGHSHIRE

other waterbodies within dispersal distance of the site. If this species is found within the site or within proximity of the site (500m), a programme of licensed avoidance and mitigation measures may need to be put in place to provide for the protection of great crested newt and to ensure compliance with the above legislation.

- 1.11 Great crested newt, common toad and Natterjack toad (*Epidalea calamita*) are the amphibians listed on the Natural Environment and Rural Communities Act 2006 Section 42 list of species of principal importance for conservation of biological diversity in Wales.

Aims and Objectives of the Survey

- 1.12 The key objective of the great crested newt survey was to determine the presence or likely absence of great crested newt in suitable waterbodies within the site and within a 500m radius, of which account must be taken prior to and during the planned works in accordance with the Wildlife and Countryside Act 1981 (as amended) and the Conservation of Habitats and Species Regulations, 2017 (as amended).
- 1.13 The surveys would help determine the need for a European Protected Species licence to be obtained for the works and help inform the design and scope of any mitigation measures that might be proposed.

2.0 SURVEY METHODOLOGY

Desk Study

- 2.1 A data search for all protected and notable species and all sites of conservation importance within a 2km radius of SJ049671 was completed to inform the PEA. Please refer to the PEA survey report for full details (Pleydell Smithyman Limited, 2019).

Habitat Suitability Index (HSI) Assessment

- 2.2 HSI Assessments were undertaken on all still waterbodies 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 in line with Oldham *et al.* (2000). The methodology for these assessments is detailed in the PEA report (Pleydell Smithyman Limited, 2019).
- 2.3 The HSI assessment was carried out on 11th April 2019 by Kelly Hopkins of Pleydell Smithyman Limited. The full HSI calculations are included within the appendices of the PEA report. Drawing No. M18.155.D.013 – GCN Survey shows the locations of the five assessed waterbodies. One additional pond is shown on some OS maps and aerial imagery towards the south-eastern boundary of the site approximately 115m to the south of Pond 2. This pond was not present at the time of the assessment.

Great Crested Newt environmental DNA (eDNA) Surveys

- 2.4 Water samples were taken from Ponds 1, 2, 3 and 4 in order to identify any environmental DNA of great crested newt within these ponds. This method of identifying the presence of great crested newt was approved by Natural England in March 2014. This method involves taking a total of 20 water samples from different places around the pond and mixing these samples with ethanol in order to preserve them. These samples then get sent to a laboratory to be tested for any presence of mitochondrial DNA of great crested newt within the ponds. The institution that was used to test these water samples was Surescreen Scientifics Ltd.

Great Crested Newt Population Surveys

- 2.5 Population surveys comprising six survey visits were completed on all ponds that were returned as positive following the eDNA surveys. The surveys were completed in line with best practice guidelines (English Nature, 2001).
- 2.6 The great crested newt surveys were carried out using three standard survey methods at each pond on every occasion. The methods used were as follows:

GREAT CRESTED NEWT SURVEY AT THE PROPOSED WESTERN EXTENSION OF DENBIGH QUARRY, DENBIGHSHIRE

- Egg searching was carried out by searching the leaves of suitable aquatic and marginal plants for eggs. Newts lay their eggs individually on the leaves of water plants, which are then folded around the egg. The larger size and yellowish coloration readily distinguishes the eggs of great crested newt from those of the smaller (non-EPS protected) smooth newt and palmate newts (*Lissotriton helveticus*). When great crested newt eggs are found, egg searching at that pond is terminated to avoid unnecessary damage to eggs and developing larvae.
 - Torch surveys were carried out using a high-powered torch (Clulite, 1,000,000 candle power). Torch surveys were carried out after dark and in predominantly suitable weather conditions (>5°C, no/little wind, no rain). They involve walking slowly around accessible margins of each pond and scanning the water with the torch to search for newts or other amphibians. The presence or absence of fish and any other relevant information is also recorded during torch surveys. Torch surveys are most effective in ponds with relatively clear water, with easy access to the banks and which are not excessively choked with vegetation.
 - Bottle trap surveys were carried out using traps made from 2 litre plastic bottles attached to canes. Bottle traps were inserted around accessible margins of each pond, taking care to retain an air reservoir in the top of each bottle. The traps were left overnight and checked early the following morning, in accordance with best practice guidelines. Ideally, bottle traps are set at approximately 2m intervals around the pond margin. However, in practice the density and distribution of traps is dictated by safety and habitat considerations, with bottle trapping effort being focussed on parts of a pond where access can safely be gained and where the habitat is most suitable for great crested newt. The bottle traps were set in the evening before the torch survey was carried out and were retrieved the following morning.
- 2.7 The surveys were carried out by a number of experienced and great crested newt licensed surveyors. These included Kelly Hopkins BSc (Hons) ACIEEM - Natural Resources Wales Licence Number: S086523/1, Lucy Elliott, Nick Lishman, Matt Kirby, George Hicks, Sam Morris and Veronica Cantero.
- 2.8 Table 1 presents the dates and prevailing weather conditions during each of the survey visits. Details of the water turbidity and vegetation cover of each of the ponds during each survey can be found in Appendix 1.

GREAT CRESTED NEWT SURVEY AT THE PROPOSED WESTERN EXTENSION OF DENBIGH QUARRY, DENBIGHSHIRE

Table 1. Dates and weather conditions during the survey visits

Date	Weather Conditions
13/5/2019 Evening	Dry, light breeze, air temp 11°C.
14/5/2019 Morning	Dry, light breeze, air temp 15°C.
15/5/2019 Evening	Dry, light breeze, air temp 13°C.
16/5/2019 Morning	Light rain, light breeze, air temp 15°C.
20/5/2019 Evening	Dry, light breeze, air temp 11°C.
21/5/2019 Morning	Dry, light breeze, air temp 5°C.
6/6/2019 Evening	Dry, light breeze, air temp 11.5°C.
7/6/2019 Morning	Dry, light breeze, air temp 12.5°C.
10/6/2019 Evening	Dry, light breeze, air temp 9.8°C.
11/6/2019 Morning	Dry, light breeze, air temp 10°C.
18/6/2019 Evening	Moderate rain, light breeze, air temp 12.2°C.
19/6/2019 Morning	Dry, light breeze, air temp 13.1°C.

2.9 Where great crested newt populations were recorded, these population figures were assessed using Natural England population assessment guidelines which can be classed as:

- ‘**Small**’ for maximum counts up to 10.
- ‘**Medium**’ for maximum counts between 11 and 100.
- ‘**Large**’ for maximum counts over 100.

Constraints and Limitations

- 2.10 Due to the timing of the return of the environmental DNA survey results, all population surveys were completed from 13th May onwards. Therefore the beginning half of the survey season was missed and thus peak counts of great crested newts may have been different should the surveys have started sooner. The late commencement of the surveys also meant that some surveys were completed close together which may have affected the survey results. However, it is considered unlikely that earlier surveys would have provided an assessment of large populations of great crested newt.
- 2.11 Vegetation levels in Pond 3 were particularly high (between 80 and 95%) during the surveys which made torching difficult on occasions. Netting was not used as this is not a favoured method of survey by Natural Resources Wales and the use of bottle traps was considered to be effective.
- 2.12 Turbidity levels in Pond 2 were quite high during most surveys which meant that only newts present towards the surface were able to be seen. Netting was not used as this is not a favoured method of survey by Natural Resources Wales and the use of bottle traps was considered to be effective.

**GREAT CRESTED NEWT SURVEY AT THE PROPOSED WESTERN EXTENSION OF
DENBIGH QUARRY, DENBIGHSHIRE**

- 2.13 It was not possible to access the entire perimeter of the ponds due to dense vegetation or steep sides. The majority of the ponds were surveyed effectively and the lack of access around the whole perimeter is considered unlikely to have significantly altered the results of the survey.

**GREAT CRESTED NEWT SURVEY AT THE PROPOSED WESTERN EXTENSION OF
DENBIGH QUARRY, DENBIGHSHIRE**

3.0 RESULTS

Desk Study

- 3.1 The data search returned two records of great crested newt from a 2km radius surrounding the central point of the site. These were both located in a pond (Pond 3) approximately 400m to the south-west of the site. These records were dated between 1993 and 2004. No more recent great crested newt records were returned from the data search.

eDNA Survey Results

- 3.2 Environmental DNA surveys were completed on Ponds 1, 2, 3 and 4. The results were returned as negative for presence of great crested newt eDNA in Pond 1 and positive for presence of great crested newt eDNA in Ponds 2, 3 and 4. For the full eDNA survey results please see Appendix 2.

Population Survey Results

- 3.3 The table below show the peak count of great crested newts recorded in each pond during the surveys. For full survey results please see Appendix 3. Details relating to other amphibians recorded during the surveys can also be found in Appendix 3.

Table 2. Peak counts of adult great crested newt and breeding evidence recorded in each pond during the surveys

	Pond 2	Pond 3	Pond 4
Great crested newt Adults	3	2	6
Great crested newt breeding evidence	None	1 juvenile newt, no eggs	4 juvenile newts, no eggs
Population size	Small	Small	Small

- 3.4 A small sized population of great crested newt was recorded in Ponds 2, 3 and 4 during the surveys. Despite extensive searches of aquatic vegetation no great crested newt eggs were recorded. Juvenile great crested newt were recorded in Pond 3 and Pond 4 and adult male and females were recorded in all three ponds. It is therefore assumed that all three ponds are used as breeding ponds.

GREAT CRESTED NEWT SURVEY AT THE PROPOSED WESTERN EXTENSION OF DENBIGH QUARRY, DENBIGHSHIRE

- 3.5 It is considered that the great crested newts in these three ponds form two metapopulations: Ponds 3 and 4 together and Pond 2.

Potential Impacts

- 3.6 There was one great crested newt breeding pond (Pond 2) present within the site; however this was approximately 330m from the proposed western extension boundary. This pond is present towards the entrance of the quarry and is surrounded by access roads. Suitable terrestrial habitat is present in the woodland to the north and south of the pond. The woodland to the south connects to suitable habitat present within the proposed western extension.
- 3.7 A further two breeding great crested newt ponds were present approximately 350m to the south-west of the proposed extension. Suitable terrestrial habitat is present surrounding these ponds which is connected to suitable habitat present within the proposed western extension. Mitigation measures are therefore required to be implemented to avoid any negative impacts upon the local metapopulations of great crested newt.
- 3.8 None of the proposed extension falls within 250m of great crested newt ponds.
- 3.9 The proposed extension covers an area of 4.9 hectares, of which approximately 2.9 hectares of the proposed extension falls within 500m of great crested newt ponds. The habitat present within this area includes 2.1 hectares of improved grassland, 0.7 hectares of broad-leaved plantation woodland, 0.1 hectare of quarry; scattered scrub and scattered trees and a defunct species-poor hedgerow (total length 88m).
- 3.10 The quarry will continue to function as it does currently, and no suitable terrestrial habitat will be removed within the quarry with the exception of that contained within the proposed western extension area.
- 3.11 It is considered that without mitigation measures, following a rapid risk assessment tool shown within the table below, there is an amber risk and it is considered that an offence is likely. This has been calculated due to the proposals involving the damage/loss of 4.9 hectares of land further than 250m from a great crested newt breeding pond. This table has been calculated as an overall effect upon the great crested newt metapopulations within the three ponds that were found to support breeding great crested newt during the 2019 surveys (Ponds 2, 3 and 4).

**GREAT CRESTED NEWT SURVEY AT THE PROPOSED WESTERN EXTENSION OF
DENBIGH QUARRY, DENBIGHSHIRE**

Table 3. Likely effect of the proposals upon great crested newt without mitigation

Component	Likely effect (select one for each component; select the most harmful option if more than one is likely; lists are in order of harm, top to bottom)	Notional offence probability score
Great crested newt breeding pond(s)	No effect	0
Land within 100m of any breeding pond(s)	No effect	0
Land 100-250m from any breeding pond(s)	No effect	0
Land >250m from any breeding pond(s)	1 - 5 ha lost or damaged	0.04
Individual great crested newts	Minor disturbance of newts	0.5
Maximum:		0.5
Rapid risk assessment result:	AMBER: OFFENCE LIKELY	

3.12 This rapid risk assessment tool does not take into account population size, terrestrial habitat quality, presence of dispersal barriers, timing and duration of works, detailed layout of the development in relation to newt resting and dispersal. It is therefore considered that due to the majority of the habitat being removed being largely unsuitable for great crested newt (improved grassland) and the presence of suitable habitat between the ponds and the proposed extension, the mitigation measures detailed within this document are considered sufficient and therefore it is deemed unlikely that a European Protected Species Licence (EPSL) will be necessary in order to protect any individual great crested newt and their associated habitat.

GREAT CRESTED NEWT SURVEY AT THE PROPOSED WESTERN EXTENSION OF DENBIGH QUARRY, DENBIGHSHIRE

4.0 **MITIGATION MEASURES**

- 4.1 Within a previous study it has been determined that 95% of summer newt refuges are located within 63m of a breeding pond and it is generally agreed that very few great crested newt disperse more than 250m from a breeding pond (Jehle, 2000). A report undertaken by Cresswell and Whitworth, states that at distances greater than 200-250m, capture operations will hardly ever be appropriate (English Nature, 2004).
- 4.2 The proposals do not involve the loss or damage of any breeding great crested newt ponds. No habitat within 250m of a great crested newt pond will be impacted. The closest habitat removal works will be approximately 325m from Pond 2 and 390m from Ponds 3 and 4.
- 4.3 The measures below detail the steps that will be taken to ensure that the risk of any offences being committed with regards to great crested newt is minimised.
- 4.4 It is considered that following the process of a non-licensed method statement with reasonable avoidance measures will be appropriate to minimise any impacts to great crested newts due to the low likelihood of encountering them within the proposed western extension.
- 4.5 The non-licensed method statement will need to include details such as toolbox talks; supervised removal of hedgerows and woodland and phased clearance of the woodland. The full details of this should be discussed with the county ecologist and Natural Resources Wales.
- 4.6 Should any great crested newt be identified during the works, all works must stop and advice should be sought from the consultant ecologist. Development may need to be suspended until a development licence from Natural Resources Wales is obtained.
- 4.7 The restoration plan for the quarry should incorporate features that provide benefits to great crested newt. Prior to the commencement of the restoration works, update surveys will be required on all ponds to inform the proposals and to understand the current metapopulations in the local area.

**GREAT CRESTED NEWT SURVEY AT THE PROPOSED WESTERN EXTENSION OF
DENBIGH QUARRY, DENBIGHSHIRE**

5.0 CONCLUSIONS AND RECOMMENDATIONS

- 5.1 Three ponds, each supporting a small population of great crested newt were found to be present within 500m of the site. Great crested newt is protected by European law and is granted the status of a European Protected Species (EPS).
- 5.2 Land within 500m of breeding ponds is likely to be impacted by the works and as a result mitigation measures will be required. Land within 250m of breeding ponds (the most likely dispersal distances for great crested newt) will not be impacted by the proposals.
- 5.3 It is considered that the mitigation measures described above will ensure that there is negligible risk of any offences being committed with regards to great crested newt. The use of a non-licensed method statement will provide protection to the local great crested newt metapopulations in the long-term. The restoration of the site should aim to provide more suitable habitat for great crested newt and result in an overall positive impact upon the local metapopulations.

GREAT CRESTED NEWT SURVEY AT THE PROPOSED WESTERN EXTENSION OF DENBIGH QUARRY, DENBIGHSHIRE

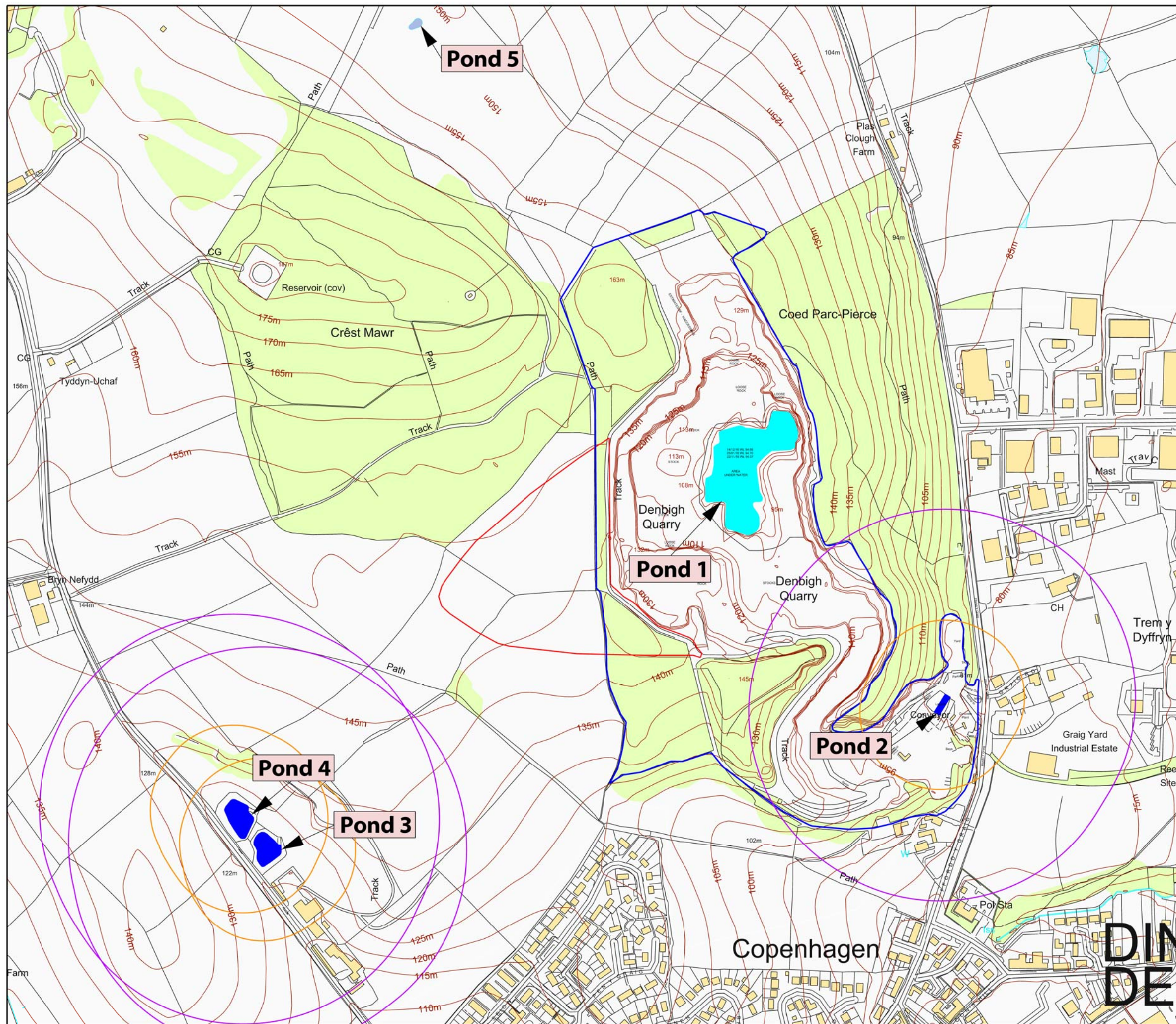
6.0 REFERENCES

- 1) English Nature, 2001. *Great crested newt mitigation guidelines*. Version: August 2001
- 2) English Nature, 2004. *An assessment of the efficiency of capture techniques and the value of different habitats for the Great Crested Newt (Triturus cristatus)*. Peterborough, English Nature.
- 3) Jehle, R., 2000. *The terrestrial habitat of radio-tracked Great Crested Newts (Triturus cristatus) and Marbled Newts (T. Marmoratus)*. Herpetological Journal, 10, pp.137-142.
- 4) Natural England, 2013. *European Protected Species: Mitigation Licensing – How to get a licence. WML – G12. Version December 2013*. Online at: <http://publications.naturalengland.org.uk/publication/4727870517673984> [Last accessed 20/11/2014]
- 5) Pleydell Smithyman Limited, 2019. *Preliminary Ecological Appraisal relating to land at the proposed western extension at Denbigh Quarry, Denbighshire*. Ironbridge, Pleydell Smithyman Limited.

DRAWINGS

DRAWING M18.155.D.013

GCN SURVEY



Legend

Land under control of the applicant

Limit of extension

Survey Pond with GCN Present

Survey Pond with no GCN Present

Pond HSI assessed not surveyed

100m Buffer from GCN Pond

250m Buffer from GCN Pond

Reproduced from Ordnance Survey digital map data © Crown Copyright. All rights reserved.
Licence number 0100031673

REV	AM'D	NOTES	DATE

DRAWING STATUS PLANNING	
PROJECT Denbigh Quarry	
CLIENT Breedon Southern Ltd	
TITLE GCN Survey	
DATE Oct 2019	SCALE 1:5,000 @A3
DRAWN RSB	CHECKED KH
DRAW NO. M18.155.D.013	

PleydellSmithyman

THIS DRAWING MAY NOT BE USED WITHOUT CONSENT OF:
PLEYDELL SMITHYMAN LIMITED
20A THE WHARFAGE, IRONBRIDGE
SHROPSHIRE TF8 7NH
T. 01952 433211 F. 01952 433323
E. psl@pleydellsmithyman.co.uk
www.pleydellsmithyman.co.uk

DIN
DEN

APPENDICES

APPENDIX 1

Additional Pond Survey Information

**GREAT CRESTED NEWT SURVEY AT THE PROPOSED WESTERN EXTENSION OF
DENBIGH QUARRY, DENBIGHSHIRE**

Survey	Turbidity ¹			Veg Cover (%)		
	Pond			Pond		
	2	3	4	2	3	4
1	4	0	0	0	80	30
2	4	1	1	0	80	30
3	4	1	1	0	90	35
4	4	1	1	0	95	50
5	4	1	1	0	95	50
6	4	1	1	0	95	50

¹ Turbidity is measured on a scale from 1 -5 with 1 showing that the water is very clear and 5 showing that the water is opaque.

APPENDIX 2

eDNA Survey Information

Folio No: E4887
Report No: 1
Order No: ONO.117.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
2092	Denbigh Quarry, Pond 2	SJ052 668	Pass	Pass	Pass	Negative	0
2134	Denbigh Quarry, Pond 3	SJ043 666	Pass	Pass	Pass	Positive	6
2135	Denbigh Quarry, Pond 4	SJ0434 6668	Pass	Pass	Pass	Positive	6
2137	Denbigh Quarry, Pond 1	SJ0504 6711	Pass	Pass	Pass	Positive	2

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: Jennifer Higginbottom

Approved by: Sarah Evans

End Of Report

APPENDIX 3

Great Crested Newt Survey Information

Table 1. Pond 2 Survey results

Method	Species	Survey 1	Survey 2	Survey 3	Survey 4	Survey 5	Survey 6
Torch Count	Great Crested Newt	2m	1m	0	0	1f	1m, 1f
	Smooth Newt	2m	9m	29m, 1 juv	6m	5m	3m
	Palmate Newt	0	0	0	0	0	0
	*Smooth/palmate female	66	43	28	50	61	45
	Common Frog	0	0	0	0	0	0
	Common Toad	0	0	0	0	0	0
Bottle Trapping	Great Crested Newt	1m, 1f	0	1m	1f	0	0
	Smooth Newt	1m, 2f	2f	2m, 2f	2m, 2f	1f	3m, 1f
	Palmate Newt	1f	0	0	1f	0	0
	Common Frog	0	0	0	0	0	0
	Common Toad	0	0	0	0	0	0
Egg Searching	Great Crested Newt	0	0	0	0	0	0
	Smooth Newt	0	0	0	0	0	0
	Palmate Newt	0	0	0	0	0	0
	Common Frog	0	0	0	0	0	0
	Common Toad	0	0	0	0	0	0

* It can be difficult to distinguish between smooth and palmate females unless they are in the hand. Palmate females can be identified by the presence of two small, pale tubercles on the underside of the hind feet. During torch surveys these tubercles may not be visible depending on the attitude of the animal in the water and so any records of such unidentified females are recorded in this row.

m=Male, f = Female, im = immature newt, - = survey method not conducted

Table 2. Pond 3 Survey results

Method	Species	Survey 1	Survey 2	Survey 3	Survey 4	Survey 5	Survey 6
Torch Count	Great Crested Newt	1m, 1f	1m	0	0	1f	0
	Smooth Newt	4m, 13f	1f	0	1m	0	0
	Palmate Newt	0	0	0	0	0	0
	*Smooth/palmate female	0	0	0	0	0	0
	Common Frog	0	0	0	0	0	0
	Common Toad	0	0	0	0	0	0
Bottle Trapping	Great Crested Newt	0	0	0	0	1m, 1f, 1 juv	0
	Smooth Newt	0	0	0	1m	3m	3m, 1f
	Palmate Newt	0	0	0	0	0	0
	Common Frog	0	0	0	0	0	0
	Common Toad	0	0	0	0	0	0
Egg Searching	Great Crested Newt	0	0	0	0	0	0
	Smooth Newt	0	0	0	0	0	1 eft
	Palmate Newt	0	0	0	0	0	0
	Common Frog	0	0	0	0	0	0
	Common Toad	0	0	0	0	0	0

* It can be difficult to distinguish between smooth and palmate females unless they are in the hand. Palmate females can be identified by the presence of two small, pale tubercles on the underside of the hind feet. During torch surveys these tubercles may not be visible depending on the attitude of the animal in the water and so any records of such unidentified females are recorded in this row.

m=Male, f = Female, im = immature newt, - = survey method not conducted

Table 3 - Pond 4 Survey results

Method	Species	Survey 1	Survey 2	Survey 3	Survey 4	Survey 5	Survey 6
Torch Count	Great Crested Newt	1m	0	0	0	1f	0
	Smooth Newt	7f	2m, 2f	2m, 5f	0	1m, 6f	2m, 1f
	Palmate Newt	0	0	0	0	0	0
	*Smooth/palmate female	0	0	0	0	0	0
	Common Frog	0	0	0	0	0	0
	Common Toad	0	2	0	0	0	0
Bottle Trapping	Great Crested Newt	1f	0	1m	0	1 juv	2m, 4f, 4 juv
	Smooth Newt	0	1m, 1f	1m, 2f	1f	0	0
	Palmate Newt	0	0	0	0	0	0
	Common Frog	0	0	0	0	0	0
	Common Toad	0	0	0	0	0	0
Egg Searching	Great Crested Newt	0	0	0	0	0	0
	Smooth Newt	0	0	0	0	0	0
	Palmate Newt	0	0	0	0	0	0
	Common Frog	0	0	0	0	0	0
	Common Toad	0	0	0	0	0	0

* It can be difficult to distinguish between smooth and palmate females unless they are in the hand. Palmate females can be identified by the presence of two small, pale tubercles on the underside of the hind feet. During torch surveys these tubercles may not be visible depending on the attitude of the animal in the water and so any records of such unidentified females are recorded in this row.

m=Male, f = Female, im = immature newt, - = survey method not conducted