

Visual Impact Provision (VIP)

Peak District (East) Project

Appendix 7C Great Crested Newt Survey Report

May 2019 Rev04

Quality information

Prepared by	Checked by	Verified by	Approved by
JW Graduate Ecologist GradCIEEM	HP Principal Ecologist MCIEEM PIEMA	CM Principal Ecologist MCIEEM CEnv	LK Regional Director MCIEEM CEnv MRSB

Revision History

Revision	Revision date	Details	Prepared by	Reviewed by	Approved by
1	01/11/2017	Draft	CW	CM	LK
2	01/06/2017	Updated to include Pond 10 and terminology changes	HM	CM	CM
3	August 2018	Minor wording edits/additions	JW	CM	LK
4	May 2019	Updated following 2019 GCN surveys	JW	HP	CM

Distribution List

# Hard Copies	PDF Required	Association / Company Name

Prepared for:

National Grid

Prepared by:

AECOM Limited
5th Floor, 2 City Walk
Leeds LS11 9AR
United Kingdom

T: +44 (0)113 391 6800
aecom.com

© AECOM Limited. All Rights Reserved.

This document has been prepared by AECOM Limited ("AECOM") for sole use of our client (the "Client") in accordance with generally accepted consultancy principles, the budget for fees and the terms of reference agreed between AECOM and the Client. Any information provided by third parties and referred to herein has not been checked or verified by AECOM, unless otherwise expressly stated in the document.

The conclusions and recommendations contained in this Report take into account information provided by the Client about the Proposed Project and upon the assumption that all relevant information has been provided by those parties from whom it has been requested and that such information is accurate. Information obtained by AECOM has not been independently verified by AECOM, unless otherwise stated in the Report.

The methods adopted, and the sources of information used by AECOM in providing its services are outlined in this Report. The work described in this Report was undertaken in June 2016, August 2018 and May 2019, and is based on the conditions encountered and the information available during those periods. The scope of this Report and the services are accordingly factual and therefore be subject to minor limitations as detailed in Section 3.4.

Where assessments of works or costs identified in this Report are made, such assessments are based upon the information available at the time and where appropriate are subject to further investigations or information which may become available.

AECOM disclaim any undertaking or obligation to advise any person of any change in any matter affecting the Report, which may come or be brought to the attention of AECOM after the date of the Report.

Certain statements made in the Report that are not historical facts may constitute estimates, projections or other forward-looking statements and even though they are based on reasonable assumptions as of the date of the Report, such forward-looking statements by their nature involve risks and uncertainties that could cause actual results to differ materially from the results predicted. AECOM specifically does not guarantee or warrant any estimate or projections contained in this Report.

Where field investigations are carried out, these have been restricted to a level of detail required to meet the stated objectives of the services. The results of any measurements taken may vary spatially or with time and further confirmatory measurements should be made after any significant delay in issuing this Report.

Table of Contents

1. Introduction 1
1.1 The Proposed Project 1
1.2 Defining the Study and Survey Areas 1
1.3 Survey Aims and Objectives 1
2. Legislation and Planning Policy 3
2.1 Relevant Legislation 3
2.2 Planning Policy 3
3. Methodology 5
3.1 Desk Study 5
3.2 Field Survey 5
3.3 Evaluation Methodology 7
3.4 Survey Limitations 8
4. Results 9
4.1 Desk Study 9
4.2 Field Survey 9
5. Evaluation 16
6. References 17
Appendix A : Great Crested Newt Ecology and Survey Method Descriptions 19
Appendix B : Habitat Suitability Index Assessment 21
Appendix C : Pond Photographs 23
Appendix D : Presence / Absence Survey Details 30
Appendix E : Supplier Environmental DNA analysis reports 33

Figures

Figure 1 Great Crested Newt Survey 18

Tables

Table 4.1: Details of the waterbodies identified within the Survey Area 10
Table 4.2: Environmental DNA Survey Results 13
Table 4.3: Great Crested Newt Survey 14

1. Introduction

1.1 The Proposed Project

AECOM was instructed by National Grid to conduct great crested newt (*Triturus cristatus*) surveys at Dunford Bridge, South Yorkshire (centred approximate Ordnance Survey grid reference 41654 40244). The great crested newt surveys were undertaken as part of the Phase 2 protected species surveys to inform the Visual Impact Provision (VIP), Peak District East Project (hereafter referred to as the 'Proposed Project'). The Proposed Project is described in detail in Chapter 2 of the Environmental Assessment Report (EAR).

Ten waterbodies and suitable terrestrial habitat to support great crested newt were identified during the initial Preliminary Ecological Appraisal (PEA) and during subsequent ecology surveys conducted between November 2015 and May 2017. These were subject to a full assessment using the Habitat Suitability Index (Oldham *et al.*, 2000) and all were deemed suitable for the species.

Based upon the findings of the initial PEA, great crested newt surveys were undertaken in 2016/2017 to determine the status of this legally protected species within the Survey Area (as defined below).Given the 2016/17 great crested newt survey data was approaching >2 years old (i.e. >3 great crested newt breeding seasons), a desk study review was carried out in 2019 to identify any potential new ponds within 250m of the Proposed Project. No new ponds were identified, and ponds previously surveyed in 2016/2017 were subjected to great crested newt surveys in 2019 (where applicable). This was to ensure that up to date baseline survey information was available for great crested newts to support the ecological assessment and potential licence applications (where applicable). The results of the 2016/2017 and 2019 surveys are provided in this report. Background information on great crested newt ecology is provided in Appendix A.

1.2 Defining the Study and Survey Areas

Within this report the following terminology is used when referring to the geographic areas within which the great crested newt work has been conducted:

- Ecological Study Area – the area which was subject to collection of background information e.g. desk study records for amphibians to supplement the findings of the survey work; this comprises the Great Crested Newt Survey Area plus a 2km radius; and
- Great Crested Newt Survey Area (hereafter referred to as the Survey Area) – this is the area within which the great crested newt survey work has been conducted; and is shown on Figure 7C.1. This area includes all land within which permanent and temporary works associated with the Proposed Project are located. A 250m radius of the Survey Area has also been shown on Figure 7C.1 to reflect the area within which the presence of additional waterbodies was assessed during the desk study.

1.3 Survey Aims and Objectives

The aim and objectives of the survey work and the subsequent report presented here were to:

1.3.1 Aim

- To determine the status and distribution of great crested newt within the Survey Area in order to inform the Ecological Impact Assessment (EcIA) for the Proposed Project.

1.3.2 Objectives

- Review existing ecological data to identify any records of great crested newt within the Ecological Study Area;
- Assess the presence of potentially suitable waterbodies located within at least a 250m radius of the Proposed Project using aerial mapping and online sources;
- Conduct habitat suitability assessments on identified waterbodies, followed by detailed surveys of suitable (to potentially support amphibians) waterbodies to confirm presence/likely absence of great crested newts;

- If presence of great crested newt is confirmed, conduct a population estimate survey to categorise the size of the population present;
- Record other species of amphibian or aquatic mammal; and
- Evaluate the survey results to determine the nature conservation value of the Survey Area for great crested newt.

2. Legislation and Planning Policy

2.1 Relevant Legislation

The decline in great crested newt conservation status across Europe has led to its listing on Annex IV ('European Protected Species') of the Council Directive 92/43/EEC on the Conservation of Natural Habitats and of Wild Fauna and Flora, adopted in 1992. The Habitats Directive is transposed into English Law through the Conservation of Habitats and Species Regulations 2010 (as amended) (henceforth referred to as the 'Habitats Regulations'). Schedule 2 of the Habitats Regulations lists the European Protected Species of animals, including great crested newt.

Under the Habitat Regulations a person who:

(a) deliberately captures, injures or kills any wild animal of a European protected species;

(b) deliberately disturbs wild animals of any such species;

(c) deliberately takes or destroys the eggs of such an animal; or

(d) damages or destroys a breeding site or resting place of such an animal, is guilty of an offence.

For all species listed on Schedule 2, it is also illegal to possess, control, transport, offer for sale or exchange, or sell or exchange any live or dead individual, or anything derived from or any part of such a species.

The Wildlife and Countryside Act 1981 (as amended) provides protection to certain wild animals – listed on Schedule 5 – including great crested newt. Section 9 (4) & (5) detail the offences concerning great crested newt:

(4) a person is guilty of an offence if intentionally or recklessly:

(b) disturbs any such animal while it is occupying a structure or place which it uses for shelter or protection; or

(c) obstructs access to any structure or place which any such animal uses for shelter or protection.

(5) subject to the provisions of this Part, if any person:

(a) sells, offers or exposes for sale, or has in his possession or transports for the purpose of sale, any live or dead wild animal included in Schedule 5, or any part of, or anything derived from, such an animal; or

(b) publishes or causes to be published any advertisement likely to be understood as conveying that he buys or sells, or intends to buy or sell, any of those things, he shall be guilty of an offence.

2.1.1 NERC Act, 2006

The Natural Environment and Rural Communities (NERC) Act (2006), as amended, put an obligation on public bodies to have regard, so far as is consistent with the proper exercise of their functions, to the purpose of conserving biodiversity. Under the terms of the Act, conserving biodiversity includes restoring or enhancing populations and/or habitats. The local planning authority (LPA) or other determining authority must therefore consider the effects of planning applications upon biodiversity and how it can be mitigated for or enhanced.

A list of species and habitats 'of principal importance for the purpose of conserving biodiversity' is published under Section 41 of the NERC Act (2006). The list which includes 56 habitats and 943 species has been drawn up in consultation with Natural England and draws upon the previous UK Biodiversity Action Plan (BAP) List of Priority Species and Habitats which is now obsolete. Great crested newt is listed as priority species on the NERC Act, 2006.

2.2 Planning Policy

The revised National Planning Policy Framework (NPPF) published on 24th July 2018 sets out the government's planning policies for nature conservation in England and how these are expected to be applied. This revised Framework replaces the previous NPPF published in March 2012.

The NPPF states the commitment of the UK Government to minimising impacts on biodiversity and providing net gains in biodiversity where possible, contributing to the Government's commitment to halt the overall decline in biodiversity. It specifies the obligations that the Local Authorities and the UK Government have regarding statutory designated sites and protected species under UK and international legislation and how this it to be delivered in the planning system. Protected or notable habitats and species can be a material consideration in planning decisions and may therefore make some sites unsuitable for particular types of development, or if development is permitted, mitigation measures may be required to avoid or minimise impacts on certain habitats and species, or where impact is unavoidable, compensation may be required.

2.2.1 Local Biodiversity Action Plan (LBAP)

Barnsley Local Biodiversity Action Plan (LBAP) is being revised, with the revised version previously anticipated to be completed and adopted in 2018. This document is still awaiting full completion at the time of writing. Some specific habitat and species BAPS have been updated and published. Great crested newt are currently listed as a local priority species on the revised section of the LBAP as detailed on the Barnsley Biodiversity Trust web site (Barnsley Biodiversity Trust, 2019).

The Peak District Local Biodiversity Action Plan 2011 – 2020 lists Great crested newt as a priority species (Peak District National Park, 2016).

3. Methodology

3.1 Desk Study

An initial data search was conducted in 2016 to support the initial Scoping stage of the Proposed Project. This search requested records of great crested newt or other protected amphibian species within the Ecological Study Area. The following consultees were contacted.

- Barnsley Biological Records Centre/Barnsley Metropolitan Borough Council (BMBC); and
- Peak District National Park Authority.

The desk study was restricted to data within the last 10 years (post 2005), so that the data collated would be more likely to reflect the current (rather than historic) baseline conditions associated with the Ecological Study Area. An updated request for relevant amphibian records was submitted to Barnsley Biological Records Centre in August 2018, and records returned for amphibian species within the Study Area.

The 2017 and 2019 desk study also involved an online search of aerial imagery and ordnance survey plans to further assess the potential presence of ponds/waterbodies within the Ecological Study Area, in particular within an area at least extending to a 250m radius of the Survey Area (as shown on Figure 7C.1). Whilst great crested newt can disperse into terrestrial habitats located up to 500m from a given breeding pond, based upon the landscape and topography of the Study Area, combined with presence of barriers to dispersal for amphibians within the Survey Area i.e. the River Don and local roads, it is considered that a buffer of 250m is appropriate when determining the potential presence of waterbodies and great crested newt populations which may be present.

3.2 Field Survey

3.2.1 Habitat Suitability Index (HSI) Assessment

The presence of ponds was identified from a mixture of aerial photography, during the initial PEA walkover or subsequent species/habitat surveys.

A Habitat Suitability Index (HSI) assessment was undertaken in 2016/2017 on ten water bodies located within the Survey Area. Two large reservoirs were scoped out (See Section 4.2.1) and were not subject to detailed HSI assessment The HSI assessment was not updated in 2019 however changes in the status or suitability of ponds since the previous survey were recorded.

The HSI assessment was devised to quantitatively assess the suitability of water bodies to support great crested newt. The HSI for great crested newts was developed by Oldham et al. (2000) and is a numerical index between 0 (indicating unsuitable habitat) and 1 (representing optimal habitat). The HSI incorporates ten suitability factors, all of which are thought to affect great crested newts. These factors are:

- Geographical location (SI1). The UK is divided into three zones A, B and C which illustrate decreasing potential for great crested newt in regard to their geographical range. The majority of England, including South Yorkshire is located within zone A; representing the most temperate climate and therefore highest potential zone.
- Pond area (SI2). In general pond sizes between 500 m^2 and 750 m^2 are optimal for great crested newt.
- Pond drying per decade (SI3). The occasional drying of a pond (once per decade) is optimal as this reduces numbers of predatory fish. However permanent water retention is preferable to annual drying up.
- Water quality (SI4). Good water quality is optimal and is measured through invertebrate diversity and the condition of the pond.
- Shade cover (SI5). Represented as a percentage of the bank. Unshaded ponds are preferred to those with heavily shaded ponds.
- Waterfowl (SI6). Heavy use by waterfowl can deteriorate the suitability of a pond for great crested newts, although minor use e.g., by moorhens is likely to have a negligible impact.

- Fish (SI7). In general, greater numbers of fish result in a higher level of predation upon great crested newt eggs and larvae and thus fewer numbers of fish in a pond increases the potential viability of a great crested newt population.
- Density of ponds within km^2 (SI8). The presence of ponds within the local area increases the chances of newts becoming established from nearby ponds. (and also the suitability of the local area to support a meta-population).
- Terrestrial habitat (SI9). A higher proportion of suitable terrestrial habitat located within 500 m of a breeding site (waterbody), increases the likelihood of great crested newt presence within a particular area.
- Macrophyte cover (SI10). The greater the proportion of the pond that is covered by aquatic vegetation, the opportunities for shelter and egg laying by great crested newt increases. Where macrophyte cover reaches 80% or above, the effect of a reduction in light and oxygen reaching the deeper water can adversely reduce the suitability of the pond for great crested newt.

The ten field scores are converted into suitability index scores which are multiplied together then calculated to the power of 0.1, to give a HSI score between 0 and 1.

HSI scores are categorised in terms of pond suitability for great crested newt as below:

- <0.5 = poor
- 0.5 – 0.59 = below average
- 0.60 – 0.69 = average
- 0.7 – 0.79 = good
- >0.8 = excellent

The system is not sufficiently precise enough to conclude that any particular waterbody with a high score will support great crested newt or that a waterbody with a low score will not support great crested newt. Oldham et al. (2000) reported that the lowest scoring pond supporting great crested newts in their study was 0.43.

3.2.2 Environmental DNA Survey

Environmental DNA (eDNA) survey is a relatively recent established technique approved by Defra and Natural England as an alternative technique to establish the presence/ absence of the great crested newt within aquatic habitats.

In aquatic environments, eDNA is diluted and distributed in the water. Sources of eDNA in pond water derive from faeces, mucous, gametes, shed skin and carcasses. The eDNA survey involves the collection of water samples for laboratory analysis for the DNA of species of interest, in this case great crested newt. The sampling procedure is prescribed in the published method and requires that the water sample be taken between mid-April and end of June.

eDNA sampling was conducted across nine ponds between April and the end of May 2016 and on a further pond (Pond 10) in May 2017 (see Table 4.2). Updated eDNA sampling was then conducted at six ponds in May 2019 (see Table 4.3). Only six out of the ten ponds were subject to eDNA sampling in 2019 as two ponds (Pond 4 and Pond 5) were dry; one was too shallow to sample and deemed unsuitable for great crested newt (Pond 10); and one was deemed unsuitable for great crested newt due to its size and presence of large Koi carp (Pond 7).The eDNA surveys were undertaken in accordance with the published method (Biggs et al. 2014) using sampling kits and analysis services provided by one of the approved eDNA suppliers.

Once a negative result was obtained from the eDNA analysis, further newt surveys using the traditional methods (as detailed in Section 3.2.3) ceased, as a negative result from this method provides a detection rate of 99%. Where a positive eDNA result was returned, ponds were subject to six surveys using the traditional methods. This is because eDNA can only be used in establishing presence or absence and does not provide a population estimate.

3.2.3 Great Crested Newt Presence/Absence Survey

All water bodies which were considered to have potential to support great crested newt as a result of the HSI Assessment or could not be scoped out for any other reason were subject to presence / absence surveys. No new ponds were identified for survey in 2019.

A total of nine ponds (Ponds 1 – 9; detailed in Table 4.1 below) were subject to traditional presence/absence surveys in 2016/2017. In 2019 non eDNA presence absence surveys were commenced (one survey visit only was conducted in combination with eDNA sampling survey) at six ponds; 1, 2, 3, 6, 8 & 9 as detailed in Table 4.1.

Pond 10 was not subject to presence/absence surveys in 2017 based upon the receipt of a negative eDNA result (detailed below), furthermore the results of the surveys from 2016 which indicated that Pond 10 wold be unlikely to support great crested newt. The eDNA survey was therefore considered sufficient to assess the status of great crested newt within this waterbody.

In 2019, Ponds 4 and 5 were dry so could not be surveyed; Pond 10 was practically dry and had limited aquatic vegetation and so was deemed unsuitable for great crested newt and Pond 7 was deemed unsuitable due to the presence of large koi carp and lack of aquatic vegetation.

In the event that great crested newts are identified a population assessment was undertaken based on the numbers of great crested newts that were found. Figure 7C.1 shows the location of the waterbodies subject to survey.

The survey methodology followed that outlined in the English Nature (now Natural England) Great Crested Newt Mitigation Guidelines (English Nature, 2001) and the standing advice guidance on the Natural England website (https://www.gov.uk/great-crested-newts-surveys-and-mitigation-for-development-projects, accessed April 2019). A combination of survey methods can be used to determine the presence of great crested newts in a pond. The guidance recommends that three survey methods should be utilised during each survey visit.

The survey methods utilised during these great crested newt surveys were: egg searching, torch surveys, bottle trapping and netting. In addition, items of natural and man-made refugia where present (logs, bricks, reptile mats etc) were carefully lifted to check for terrestrial amphibians. As outlined in section 3.2.2 the surveys involved the use of eDNA sampling as a survey technique. Based upon the receipt of a robust negative eDNA analysis result presence/absence surveys were ceased (or continued for positive results) before the full four survey visits were completed (except Pond 5 in 2016 as discussed in Section 4.2.3). A description of the survey methods used is provided in Appendix A.

During the surveys, any other species of amphibians or aquatic mammals observed within the water bodies were recorded.

3.3 Evaluation Methodology

The method of evaluation that has been utilised has been developed with reference to the Chartered Institute of Ecology and Environmental Management (CIEEM) 'Guidelines for Ecological Impact Assessment in the UK and Ireland – Terrestrial, Freshwater, Coastal and Marine (CIEEM, 2018). These give advice on scoping and carrying out environmental assessments and place appraisal in the context of relevant policies. Data received through consultation, desk-based studies and field-based surveys are used to allow relevant ecological features (i.e. designated sites, ecosystems, habitat and species) of importance (or potential value) to be identified, and the main factors contributing to their value described and related to available guidance.

Ecological features; in this case legally protected species i.e. the great crested newt, may be important for multiple different reasons (e.g. rarity in a particular geographic context; role in habitat connectivity; or a species on the edge of their range). Relevant reasons for which an ecological feature is important are described and considered in order to assign each relevant ecological feature an overall value in accordance with the following geographical frames of reference:

- international (i.e. European);
- national (i.e. England);
- regional (i.e. Northern England);
- county (i.e. South Yorkshire/Derbyshire);
- district (i.e. Barnsley Metropolitan Borough /Dark Peak)
- local (i.e. Survey Area and up to 2km radius); and
- negligible (used where the value is lower than the Local level).

3.4 Survey Limitations

No significant limitations to the surveys were encountered. Minor limitations include the presence of a high density of duckweed (*Lemna* sp.) on the water's surface at Pond 6 in 2016 and 2019 which limited torching. However as other survey techniques were also used in combination with torching, this is not considered a major limitation.

4. Results

4.1 Desk Study

No records for great crested newt (nor any other newt species) were provided from within the Ecological Study Area from Barnsley Records Centre/BMBC or the Peak District National Park Authority in 2016/2017 or 2019.

Two waterbodies partially present within the Survey Area and 250m buffer are the Winscar and Lower Windleden Reservoirs (shown on Figure 7C.1). They are both large impounding reservoirs (approximately 500,000 m^2 and 50,000m^2 in size respectively) used for water supply and they were deemed unsuitable to support great crested newt for a number of reasons including their size, likelihood of supporting fish, commercial activity (Winscar has a sailing club), large rocky substrate and presence of large numbers of wildfowl.

No additional waterbodies were identified in 2019 within 250m of the Survey Area which are suitable to support great crested newt. A waterbody is marked on OS plans being located east of Wogden Bottom (approximate grid reference SE183027) which confirmed during walkover surveys as not being present. A number of areas within the Survey Area and 250m buffer zone marked as 'Sinks' were also assessed and were found to form part of a running water drain or did not hold any water or aquatic vegetation. A total of ten waterbodies were identified for survey in 2016/2017 and 2019.

4.2 Field Survey

4.2.1 Habitat Suitability Index Assessment

Results of the HSI assessment in 2016/2017 are provided in Table 4.1, in addition to descriptions from 2016/2017 and 2019. The individual scores for each of the ten HSI components for each pond are further detailed in in Appendix B; with supporting photos in Appendix C. The locations of waterbodies subject to assessment are shown on Figure 7C.1.

Table 4.1: Details of the waterbodies identified within the Survey Area (2016/2017 and 2019)

Pond Number	Location (Easting, Northing)	Description (2016/2017)	Description (2019)	Photograph Reference (see Appendix C)	HSI Score/Suitability – 2016/2017 (see Appendix B)
Pond 1	417554 402574	This pond was located within Wogden Foot Local Wildlife Site (LWS) and is approximately 75m^2 and 0.5 m deep. It was of artificial origin and was lined. The margins supported great willowherb (*Epilobium hirsutum*), hairy sedge (*Carex hirta*), creeping bent (*Agrostis stolonifera*) and hoary willowherb (*Epilobium parviflorum*). Although no macrophytes were present within the waterbody a number of these marginal species had invaded into the margins. The LWS is subject to 'conservation grazing' as part of the LWS management, although no evidence of poaching around the pond was observed.	This pond was of similar dimensions to 2016/2017 and was lined. The same marginal vegetation was observed, with an increase in the cover of emergent macrophytes since the 2016/2017 surveys. Sheep were observed within close proximity to the pond, however there was no evidence of poaching.	Photograph 1	0.61 Average
Pond 2	417787 402886	This pond occurs at the east of the Survey Area within a small area of woodland between fields of grassland pasture. It was approximately 430m^2 and was fed by a small stream with an outflow of a similar size. Wildfowl were observed on the waterbody on a number of survey visits.	This pond had not changed in appearance since 2016/2017, however there was evidence of waterfowl on a number of survey visits and some bank erosion on the eastern sides and deep mud which made trap placement unsafe in areas of the margins.	Photograph 2	0.53 Below Average
Pond 3	415689 402379	Pond located within a residential garden on Don View and is approximately 160 m^2. This lined pond appeared to have some issues retaining water, as it was very low at the time of survey. The pond was unshaded with grass banks.	This pond had a water level of approximately 30cm, higher than the level in 2016/2017. Cover of emergent macrophytes made torching difficult in some areas of the margins, and banks were still grassed. An artificial pile of rocks to one end provides some refugia/shelter opportunities (albeit limited).	Photograph 3	0.67 Average
Pond 4	415517 402281	The pond occurs within a residential garden adjacent to an area of woodland and the existing Sealing End Compound (SEC). It was fed by a small surface runoff drain, although this was dry while the surveys were being conducted. The pond	This pond was dry in 2019 and was therefore not surveyed.	Photograph 4 & 5	0.64 Average

Pond Number	Location (Easting, Northing)	Description (2016/2017)	Description (2019)	Photograph Reference (see Appendix C)	HSI Score/Suitability – 2016/2017 (see Appendix B)
		was shaded from the surrounding woodland and was surrounded by rushes (*Juncus* sp.).			
Pond 5	415494 402255	A small pond which was at the end of a section of drainage channel was located within grassland adjacent to the existing National Grid SEC. It appeared to be of artificial origin. The pond was approximately 15m^2 and very shallow, particularly during the latter surveys, with only around 10cm of water. The lack of significant water had resulted in surrounding vegetation encroaching into the pond, although rush (*Juncus* sp.) and water mint (*Mentha aquatic*) were present in the wetter areas.	This pond was dry in 2019 and was therefore not surveyed in 2019.	Photograph 6 & 7	0.65 Average
Pond 6	416663 402767	A circular ornamental type pond located within allotments/garden areas adjacent to an area of scrub and young woodland. The pond is approximately 15 m2 with shallow grass banks with the occasional stand of rush (*Juncus* sp), marsh marigold (*Caltha palustris*), bulrush (*Typha latifolia*) and sedge (*Carex* sp.). The surface of the pond was dominated by a significant coverage of duckweed (*Lemna* sp.) throughout the survey period.	The vegetation surrounding the pond was identical to that in 2016/2017. As in 2016/2017, the surface of the pond was dominated by a significant coverage of duckweed (*Lemna* sp.) throughout the survey period.	Photograph 8	0.54 Below Average
Pond 7	415736 402253	A very small, artificially created pond located within a residential garden. It is approximately 2 m^2 in size and 0.4m deep. The pond is lined with a small fountain within it to circulate the water. At least six large ornamental fish were observed during the surveys.	This pond was likewise stocked with 6-8 large Koi carp in 2019 and was lined but the small fountain was no longer present. The pond was deemed unsuitable for great crested newts based on its size and the presence of fish and lack of aquatic vegetation and was therefore not subject to further survey.	Photograph 9	0.42 Poor
Pond 8	415723 402241	Located within the same residential garden as Pond 7, this artificially created pond is separated by a stone wall, from the property and concrete yard areas. The pond is also lined and	The vegetation surrounding the pond was identical to that in 2016/2017. There was a large amount of	Photograph 10	0.55 Below Average

Pond Number	Location (Easting, Northing)	Description (2016/2017)	Description (2019)	Photograph Reference (see Appendix C)	HSI Score/Suitability – 2016/2017 (see Appendix B)
		is approximately 15m^2 and 0.6m deep. No macrophytes were present.	burnt debris and wood surrounding the pond due to a fire at the property in early 2019.		
Pond 9	415770 402261	Pond 9 is a lined pond with shallow grass banks located adjacent to grazing pasture. It is approximately 20m^2 in size and 0.6m deep. It is fed from surface run off and appeared to have a water circulation system. Yellow flag iris (*Iris pseudacorus*) was present around 50% of the margin.	Yellow Flag Iris was not as dominant in 2019, with perhaps 30% coverage at the margins. Sheep were present next to the pond area during the survey in 2019, and there was some evidence of poaching at the pond margins. The water circulation system was still in evidence.	Photograph 11	0.63 Average
Pond 10	415136 401845	Pond 10 is a small area of standing water located within an indentation within moorland to the west of the Winscar car parking and access road. It appears to be an area of water associated with a small marshy temporal stream, and as such may be subject to drying out. Limited aquatic vegetation is present, apart from soft rush (*Juncus effusus*) and algae (indicating nutrient enrichment). The pond is oblong in shape with a circumference of 20 m and a depth of no more than 0.1 m and is heavily poached with a silty bottom.	In 2019 this pond was practically dry (no more than 5cm of water) and was scoped out as being unsuitable for GCN. The area surrounding the pond was highly silty and muddy, making access to the shallow area of water present unsafe. Algae and soft rush *(Juncus effusus)* was dominant. There was no other macrophytic vegetation present.	Photograph 12 & 13	0.52 Below Average

4.2.2 Environmental DNA Analysis

The results of the eDNA analyses are detailed in Tables 4.2 and 4.3 below. Full analysis reports as received from the external eDNA analysis organisations are provided as Appendix E.

Table 4.2: Environmental DNA Survey Results (2016)

Pond	Sample Collection Date	Result
1	26 April 2016	Negative
2	26 April 2016	Negative
3	26 April 2016	Negative
4	26 April 2016	Negative
5	23 May 2016	Negative*
6	6 May 2016	Negative
7	23 May 2016	Negative
8	23 May 2016	Negative
9	23 May 2016	Negative
10	18 May 2017	Negative

Table 4.3: Environmental DNA Survey Results (2019)

Pond	Sample Collection Date	Result
1	16 April 2019	Negative
2	16 April 2019	Negative
3	16 April 2019	Negative
4	N/A	Pond dry at time of survey.
5	N/A	Pond dry at time of survey.
6	16 April 2019	Negative
7	N/A	Pond scoped out from further survey (unsuitable)
8	16 April 2019	Negative
9	16 April 2019	Negative
10	N/A	Pond scoped out from further survey (unsuitable)

*Pond 5 – An initial eDNA sample taken on 26 April 2016 in combination with those conducted on Ponds 1 – 4. The analysis raised a positive result on this pond. Therefore presence/absence surveys were continued and also included 2 additional population assessment surveys; resulting in a total of 6 survey visits being conducted on this

pond. No evidence of the presence of great crested newt was identified during any of the six surveys visits, all of which included at least three survey methods with no limitations. A secondary eDNA sample was also taken on 23 May 2016 (following 4 survey visits) which concluded a negative result. Considering all factors; i.e. that a total of six traditional method survey visits were undertaken (using four survey methods on the majority of the visits), lack of survey restrictions, the result of the second eDNA analysis and overall lack of evidence of ponds within the surrounding area it is concluded that the initial positive eDNA result is deemed to be a false positive anomaly. The eDNA method and analysis process is not entirely accurate/guaranteed and the results may be due to contamination or other factor e.g. water quality. The result is superseded by the results of the traditional survey particularly given the number of survey visits and survey effort conducted on each visit.

4.2.3 Great Crested Newt Presence/Absence Surveys

The results of the presence / absence surveys are provided in Table 4.4 and 4.5. Further details containing field conditions and survey methods are provided in Appendix D.

Table 4.4: Great Crested Newt Survey Results (2016)

Pond	Survey Visit	Survey Date (2016)	Survey Methods*	Results
1	1	18/19 April	BT, T, E	13 toads, frog and toad spawn and toadlets
	2	25/26 April	T, N, E, (eDNA)	1 toad, frog and toad tadpoles
2	1	18/19 April	BT, T, E	5 toads, toad spawn and toadlets
	2	25/26 April	T, N, E (eDNA)	Frog and toad spawn
3	1	18/19 April	BT, T, E	6 toads and toad spawn
	2	25/26 April	T, N, E (eDNA)	Toad spawn
4	1	18/19 April	BT, T, E	2 toads, toad spawn and toadlets
	2	25/26 April	N, E, (eDNA)	Frog spawn, toad tadpoles
5	1	18/19 April	BT, T, E	Toadlets
	2	9/10 May	BT, T, E (eDNA)	Toad tadpoles and 1 dead toad
	3	16/17 May	BT, T, E, R	Toad tadpoles
	4	23/14 May	BT, T, N, E, R	No amphibians recorded
	5	1/2 June	BT, T, N, E, R	Tadpoles
	6	2/3 June	BT, T, E, R	No amphibians recorded
6	1	5/6 May	BT, T, R, E, (eDNA)	No amphibians recorded
	2	9/10 May	BT, T, R, E	No amphibians recorded
7	1	23/24 May	T, N, R, (eDNA)	No amphibians recorded , fish present
	2	1/2 June	T, N, R	No amphibians recorded , fish present
	3	2/3 June	T, N, R	No amphibians recorded , fish present
8	1	23/24 May	BT, T, N, R (eDNA)	1 immature frog

Pond	Survey Visit	Survey Date (2016)	Survey Methods*	Results
	2	1/2 June	BT, T, N, R	No amphibians recorded
	3	2/3 June	BT, T, N, R	No amphibians recorded
9	1	23/24 May	BT, T, R (eDNA)	Frog tadpoles
		1/2 June	BT, T, E, R	Frog tadpoles
	2	2/3 June	BT, T, E	Frog tadpoles

*Survey methods: BT – Bottle Trapping, T- Torching, N – Netting, E – egg search, R – refugia search,

Table 4.5: Great Crested Newt Survey Results (2019)

Pond	Survey Visit	Survey Date (2019)	Survey Methods*	Results
1	1	16/17 April	BT, T, E (eDNA)	1 toad, 5000 tadpoles
2	1	16/17 April	BT, T, E (eDNA)	4 frogs, 1 toad
3	1	16/17 April	T, R, E (eDNA)	250 tadpoles
4	1	16/17 April	N/A	POND DRY
5	1	16/17 April	N/A	POND DRY
6	1	16/17 April	T, R, E (eDNA)	No amphibians recorded
8	1	16/17 April	BT, T, E (eDNA)	No amphibians recorded
9	1	16/17 April	BT, T, R (eDNA)	20 tadpoles
10	1	16/17 April	N/A	Pond Dry

*Survey methods: BT – Bottle Trapping, T- Torching, N – Netting, E – egg search, R – refugia search,

Only one presence/likely absence survey visit to Ponds 1, 2, 3, 6, 8 and 9 was conducted in 2019 as the Negative eDNA sampling results were received prior to the planned second visit occurring, and therefore further surveys were no longer required.

No great crested newts or other newt species were found within any of the survey visits in 2016/2017 or 2019. Common frog (*Rana temporaria*) and Common Toad (*Bufo bufo*) were the only amphibian species encountered during the surveys.

5. Evaluation

In summary, no great crested newts were recorded during the presence / likely absence surveys (using both traditional and eDNA methods) conducted on the ten ponds within the Survey Area in 2016/2017 and 2019.

A small population size class of breeding common frog and toad was identified within the Survey Area., utilising ponds 1, 2 ,3 ,4, 5 and 9 in 2016/2017 and ponds 1, 3 and 9 in 2019.

It should be noted that reptile surveys were carried out across the site in 2016; artificial refugia (pieces of roofing felt and bitumen corrugated sheeting) were laid out in areas of suitable habitat around the site. This type of refugia could also be utilised by amphibians for shelter. A total of seven reptile survey visits were made to check the refugia during 2016. No great crested newts were identified terrestrially during these visits. Small numbers (<10) of common toad were found utilising the refugia for shelter across the site.

As great crested newt has been confirmed to be absent from the Survey Area, it is concluded that this species can be scoped out as a constraint to the Proposed Project and as a relevant ecological feature to the Ecological Impact Assessment (EcIA).

Whilst the presence of common species of amphibians should be considered by the Proposed Project were waterbodies to be affected, they do not present a significant legal constraint to the Proposed Project. The value of the Survey Area for common species of amphibian is not considered to be more than Local value in accordance with CIEEM evaluation methodology.

6. References

AECOM (2016) Peak District Visual Impact Provision Preliminary Ecological Appraisal. AECOM.

Barnsley Biodiversity Trust (2019) *Barnsley Biodiversity Action Plan; Second Edition*. [Online] Available from: http://www.barnsleybiodiversity.org.uk/biodiversityplan.html Accessed: 01.05.2019]

Biggs, J., Ewald, N., Valentini, A., Gaboriaud, C., Griffiths, R.A., Foster, J., Wilkinson, J., Arnett, A., Williams, P. & 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.

CIEEM (2018) Guidelines for Ecological Impact Assessment in the UK and Ireland: Terrestrial, Freshwater, Coastal and Marine. Chartered Institute of Ecology and Environmental Management, Winchester.

English Nature (2001) *Great Crested Newt Mitigation Guidelines*. English Nature, Peterborough

Joint Nature Conservation Committee (2003) Herpetofauna Workers' Manual. 2nd edition. Peterborough, JNCC.

Ministry of Housing, Communities and Local Government (2018) National Planning Policy Framework. Published on the UK Government website: https://www.gov.uk/government/publications/national-planning-policy-framework--2

Natural England (2015) *Great Crested Newts: Surveys and Mitigation for Development Projects Guidance* [Online] https://www.gov.uk/great-crested-newts-surveys-and-mitigation-for-development-projects (accessed 02.03.2016)

Oldham, R.S., Keeble, J., Swan, M.J.S. & Jeffcote, M. (2000) Evaluating the suitability of habitat for the Great Crested Newt (*Triturus cristatus*). *Herpetological Journal* 10 (4), 143 – 155.

Peak District National Park (2016) *Biodiversity Action Plan* [Online]. Available from: http://www.peakdistrict.gov.uk/looking-after/biodiversity/biodiversity-action-plan [Accessed: 01.05.2019]

Figure 7C.1 - Great Crested Newt Survey Area

THIS DRAWING IS TO BE USED ONLY FOR THE PURPOSE OF ISSUE THAT IT WAS ISSUED FOR AND IS SUBJECT TO AMENDMENT

KEY

- Great Crested Newt Survey Area
- Great Crested Newt Survey Area plus 250m Buffer
- Pond Subject to GCN Survey
- Scoped out from GCN Survey

Copyright
Contains Ordnance Survey Data © Crown Copyright and database right 2015
Reproduced from Ordnance Survey digital map data © Crown copyright 2015. All rights reserved. Licence number National Grid Electricity - 100024241

Purpose of Issue
ISSUE 1

Client
NATIONAL GRID

Project Title
VISUAL IMPACT PROVISION (VIP) PEAK DISTRICT (EAST) PROJECT

Drawing Title
GREAT CRESTED NEWT SURVEY

Drawn	Checked	Approved	Date
JW	CN	CM	AUG 2018

AECOM Internal Project No.	Scale @ A3
60495529	1:11,000

THIS DOCUMENT HAS BEEN PREPARED PURSUANT TO AND SUBJECT TO THE TERMS OF AECOM'S APPOINTMENT BY ITS CLIENT. AECOM ACCEPTS NO LIABILITY FOR ANY USE OF THIS DOCUMENT OTHER THAN BY ITS ORIGINAL CLIENT OR FOLLOWING AECOM'S EXPRESS AGREEMENT TO SUCH USE, AND ONLY FOR THE PURPOSES FOR WHICH IT WAS PREPARED AND PROVIDED.

AECOM
Scott House
Alencon Link, Basingstoke
Hampshire, RG21 7PP
Telephone (01256) 310200
Fax (01256) 310201
www.aecom.com

Drawing Number
FIGURE 7C.1

Rev
03

File Name:K:\5004 - Information Systems\60478116_National_Grid_VIP\project_files\MXDs\Peak District\Phase 2\Appendix 7C - GCN\Figure 7C-1 GCN Survey.mxd

Appendix A : Great Crested Newt Ecology and Survey Method Descriptions

Ecology

Great crested newt is the largest of the three native newt species. Great crested newts are like all British amphibians in that they rely on still water bodies for breeding but otherwise spend much of their lives on land. They are ectotherms and have permeable skins, so most terrestrial movement occurs when the air temperature is above 5ºC and there is, or has recently been, rain (English Nature, 2001).

The peak courtship and egg-laying period is normally from mid-March to mid-May. The female folds the leaves of aquatic plants around individual eggs – hence the need for still aquatic conditions. The larvae hatch out after about 3 weeks, and then take another 2 to 3 months to complete larval development. The larvae emerge from the pond upon completion of metamorphosis, which usually begins in early August and lasts for about 2 months. Adult newts generally leave the breeding ponds from late May onwards.

Adults and immature newts spend the winter in places where they will be protected from frost and flooding. Whilst on land outside the hibernation period, great crested newts require refuge from extremes of weather, hence during the day, they will often rest in dense vegetation, under refuges or underground.

Great crested newts in a given area often form a meta-population (a series of sub-populations that are linked by dispersal of individuals). Newts function in this way since they depend on habitats that vary in quality over time, and where the distribution of suitable habitats often changes. This meta-population concept complicates the study and conservation of this species, since impacts to a single pond may have knock-on effects on newts in nearby ponds. Great crested newts commonly move between ponds that are within around 250-500 m of each other.

English Nature (2001) now Natural England, considers the following pond characteristics as being favourable for breeding great crested newt populations:

1) Small to medium sized breeding ponds (100-300 m^2).
2) Variable depth, but preferably not so deep that aquatic and emergent vegetation is unable to take root.
3) A maximum depth around 4 m is acceptable.
4) Substantial cover of submerged and marginal vegetation.
5) Open areas to facilitate courtship behaviour.
6) Good populations of invertebrates and other amphibians as prey.
7) Ponds in clusters rather than in isolation.
8) Absence of shading on the south side.
9) Absence of fish.
10) Absence or low density of waterfowl.

Survey teams comprised at least one licensed great crested newt surveyor with an additional surveyor (on most occasions also a great crested newt licence holder) on each visit.

Survey Techniques

Egg Search

An egg search survey involves the searching of all vegetation within the waterbodies for the presence of newt eggs. Newts create a characteristic fold in the leaf in order to wrap the leaf around the egg, protecting it from predation and desiccation. It is possible to distinguish newt species by the size and colour of eggs. This method only assesses ponds for presence/absence and no population size data can be gathered from it. In addition, when eggs are confirmed this survey method is not repeated in subsequent visits to the same water body due to the destructive nature of this survey method.

Torchlight Survey

Torchlight surveys are undertaken at night and involve slowly walking around the water body with a powerful hand torch to identify any newts in the ponds. One million candle-power Clulite torches were used during each survey visit. When a newt is encountered, it is identified to species level, sexed and approximately aged.

Bottle Trapping Survey

Bottle trapping comprises strategically placing a number of 'newt bottle traps' (modified 2-litre plastic drinks bottles with ends removed and inverted which are attached to a cane) within vegetated areas around the margins of the pond, at approximately 2 m intervals where the water body is accessible. Newts swim into the bottles but cannot escape. The traps are placed at a 45° angle so that a bubble of air is present at the top end of the trap, allowing the newts' access to oxygen throughout the night. The traps are set at dusk and are checked and removed the next morning between 6 am and 11 am, and they will not be left longer than 12 hours after being first set the previous evening. All newts collected in the bottle traps are identified, sexed and approximately aged before being released. A record is kept of the number of traps placed in the pond and the number of newts collected in each trap.

Refuge Search

Refuge search involves lifting and searching underneath refuges such as logs, bark, rocks, and debris in close proximity to the pond. When a newt is encountered, it is identified to species level, sexed and approximately aged.

Netting

Netting uses a long-handled dip-net to capture great crested newts from around the pond margins. Netting can be conducted during the day or night, but better results may be obtained at night when adult newts are more likely to be in open water. When a newt is encountered, it is identified to species level, sexed and approximately aged.

Appendix B : Habitat Suitability Index Assessment

Appendix B: Detailed Habitat Suitability Index Assessment Score (2016/2017)

Factor	Pond 1		Pond 2		Pond 3		Pond 4		Pond 5	
	Value	Score	Value	Score	Value	Score	Value	Score	Value	Score
Location (SI 1)	A	1	A	1	A	1	A	1	A	1
Surface Area (SI 2)	75	0.1	430	0.9	160	0.3	25	0.05	15	0.05
Pond Drying (SI 3)	Sometimes	0.5	Rarely	1	Rarely	1	Sometimes	0.5	Sometimes	0.5
Water Quality (SI 4)	Moderate	0.67	Moderate	0.67	Moderate	0.67	Moderate	0.67	Moderate	0.67
Shade (SI 5)	0%	1	10%	1	5%	1	70%	0.9	5%	1
Water Fowl (SI 6)	Absent	1	Major	0.01	Minor	0.67	Absent	1	Absent	1
Fish (SI 7)	Absent	1	Possible	0.67	Possible	0.67	Absent	1	Absent	1
Pond Density per km^2(SI 8)	0.96	0.66	0.64	0.55	2.23	0.85	2.23	0.85	2.23	0.85
Terrestrial Habitat (SI 9)	Good	1	Good	1	Moderate	0.67	Good	1	Good	1
Macrophyte Cover (SI 10)	10%	0.35	50%	0.8	10%	0.35	60%	0.9	60%	0.9
HSI Score	0.61		0.53		0.67		0.64		0.65	
Suitability	Average		Below Average		Average		Average		Average	

Factor	Pond 6		Pond 7		Pond 8		Pond 9		Pond 10	
	Value	Score	Value	Score	Value	Score	Value	Score	Value	Score
Location (SI 1)	A	1	A	1	A	1	A	1	A	1
Surface Area (SI 2)	15	0.05	2	0.01	15	0.05	20	0.05	15	0.05
Pond Drying (SI 3)	Never	0.9	Never Dries	0.9	Never Dries	0.9	Never Dries	0.9	sometimes	0.5
Water Quality (SI 4)	Moderate	0.67	Poor	0.33	Poor	0.33	Moderate	0.67	Poor	0.33
Shade (SI 5)	0%	1	0%	1	0%	1	0%	1	0%	1
Water Fowl (SI 6)	Minor	0.67	Absent	1	Absent	1	Absent	1	Absent	1
Fish (SI 7)	Absent	1	Minor	0.33	Absent	1	Absent	1	Absent	1
Pond Density per km^2(SI 8)	0.32	0.38	1.59	0.78	1.59	0.78	1.59	0.78	2.23	0.85
Terrestrial Habitat (SI 9)	Moderate	0.67	Moderate	0.67	Moderate	0.67	Moderate	0.67	Moderate	0.67
Macrophyte Cover (SI 10)	10%	0.4	0%	0.3	0%	0.3	30%	0.6	5%	0.3
HSI Score	0.54		0.42		0.55		0.63		0.52	
Suitability	Below Average		Poor		Below Average		Average		Below Average	

Appendix C : Pond Photographs



Photograph 1 – Pond 1 in 2016 (no notable change in 2019)



Photograph 2 – Pond 2 in 2016 (no notable change in 2019)



Photograph 3 – Pond 3 in 2016 (no notable change in 2019)



Photograph 4 – Pond 4 in 2016



Photograph 5 – Pond 4 in 2019 (dry)



Photograph 6 – Pond 5 in 2016



Photograph 7 – Pond 5 in 2019 (dry)



Photograph 8 – Pond 6 in 2016 (no notable change in 2019)



Photograph 9 – Pond 7 in 2016 (no notable change in 2019)



Photograph 10 – Pond 8 in 2016 (no notable change in 2019)



Photograph 11 – Pond 9 in 2016 (no notable change in 2019)



Photograph 12 - Pond 10 in 2016



Photograph 13 – Pond 10 in 2019 (predominantly dry).

Appendix D : Presence / Absence Survey Details

Appendix D: Presence/Absence Survey Details

2016							
Survey Visit	**Date**	**Weather**	**Temperature (°C)**	**Pond No.**	**Turbidity (0 - 5)**	**Vegetation Cover (0 - 5)**	**Survey Methods**
1	18th April 16	Dry, Clear	Torching: 5.5 Bottle Traps Set: 7.5 Bottle Traps Collected: 7.5 Minimum Overnight Temperature: 4	1	0	1	Bottle trapping, Torchlight, Egg Search
				2	3	2	Bottle trapping, Torchlight, Egg Search
				3	5	0	Bottle trapping, Torchlight, Egg Search
				4	0	3	Bottle trapping, Torchlight, Egg Search
				5	3	4	Bottle trapping, Torchlight, Egg Search
2	5th May 16	Dry and warm	Torching: 12 Bottle Traps Set: 12 Bottle Traps Collected:12 Minimum Overnight Temperature: 8	6	2	2	Bottle trapping, Torchlight, Egg Search
3	9th May 16	Dry, warm and clear.	Torching: 12 Bottle Traps Set: 12 Bottle Traps Collected: 12 Minimum Overnight Temperature: 8	5	3	5	Bottle trapping, Torchlight, Egg Search, Refuge search
				6	2	2	Bottle trapping, Torchlight, Egg Search, Refuge search
4	16th May 16	Clear mild night, clear during the day and overcast. No rain for the previous 12 hours	Torching: 6 Bottle Traps Set: 6 Bottle Traps Collected:10 Minimum Overnight Temperature: 6	5	2	3	Bottle trapping, Torchlight, Egg Search, Refuge search
5	23rd May 16	Dry and warm	Torching: 10 Bottle Traps Set:10 Bottle Traps Collected:10 Minimum Overnight Temperature: 8	5	4	0	Bottle Trapping, Egg search, Netting
				7	0	0	Torchlight, Netting, Refuge
				8	0	2	Bottle traps, Netting , Refuge search , Torchlight
				9	4	0	Bottle traps, Netting , Refuge search , Torchlight
6	1st June 16	Mild evening and during the day. Fog in the evening, no rain throughout day or evening	Torching: 7.7 Bottle Traps Set:10 Bottle Traps Collected: 9.3 Minimum Overnight Temperature: 6	5	2	3	Bottle trapping, Torchlight, Egg Search, Refuge search
				7	4	0	Bottle trapping, Torchlight, Egg Search, Refuge search
				8	0	0	Bottle trapping, Torchlight, Egg Search, Refuge search
				9	0	2	Bottle trapping, Torchlight, Egg Search, Refuge search
7	2nd June 16	Mild evening and during the day. Air temp 8 no wind clear.	Torching: 10 Bottle Traps Set:10 Bottle Traps Collected:10 Minimum Overnight Temperature: 8	5	2	3	Bottle trapping, Torchlight, Egg Search, Refuge search
				7	4	0	Bottle trapping, Torchlight, Egg Search
				8	0	0	Bottle trapping, Torchlight, Egg Search
				9	0	2	Bottle trapping, Torchlight, Egg Search

2019							
Survey Visit	**Date**	**Weather**	**Temperature (°C)**	**Pond No.**	**Turbidity (0 - 5)**	**Vegetation Cover (0 - 5)**	**Survey Methods**
1	16th April 19	Dry, Clear	Torching: 8 Bottle Traps Set: 8 Bottle Traps Collected: 5 Minimum Overnight Temperature: 5	1	1	1	Bottle trapping, Torchlight, Egg Search
				2	1	1	Bottle trapping, Torchlight, Egg Search, Refuge Search
				3	1	2	Torching, Refugia Search, Egg Search
				6	1	2	Torching, Refugia Search, Egg Search
				8	1	1	Bottle trapping, Torchlight, Egg Search, Refuge Search
				9	1	1	Bottle trapping, Torchlight, Egg Search, Refuge Search

*Vegetation cover: 0 = no vegetation obscuring visibility, 5 = visibility completely obscured
**Turbidity: 0 = water completely clear, 5 = very turbid and no visibility

Appendix E : Supplier Environmental DNA analysis reports

ADAS Wolverhampton HQ
Pendeford House
Pendeford Business Park
Wobaston Road
Wolverhampton
WV9 5AP

Tel: 01159 516747
Fax: 01159 516415
Email: Helen.Rees@adas.co.uk

www.adas.co.uk

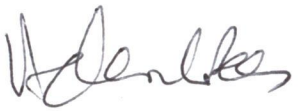
Client: [redacted]
AECOM ,
2 City Walk ,
Leeds,
LS11 9AR

Sample/Report ID: 2016-461
Client Identifier: Pond 1
Date of Receipt: 28/04/16

Condition on Receipt: Good
Description: 6x50mL - pond water samples in preservatives
Material Tested: DNA extracted from pond water samples

Visual Inspection of Volume: Passed

Determinant	Result	Method	Date of Analysis
Great Crested Newt	Negative	Real time PCR	04/05/16

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:		Signed:	
Position:	Senior Research Scientist	Position:	Team Leader: Biotechnology
Date of preparation:	05/05/16	Date of issue:	05/05/16

Notes: eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

ADAS is a trading name of ADAS UK Limited. Registered in England No. 3296903. Registered Office: ADAS Wolverhampton HQ, Pendeford House, Pendeford Business Park, Wobaston Road, Wolverhampton, WV9 5AP.
ADAS UK Limited is a member of the ADAS group of companies

Client: [redacted]
AECOM ,
2 City Walk ,
Leeds,
LS11 9AR

ADAS Wolverhampton HQ
Pendeford House
Pendeford Business Park
Wobaston Road
Wolverhampton
WV9 5AP

Tel: 01159 516747
Fax: 01159 516415
Email: Helen.Rees@adas.co.uk

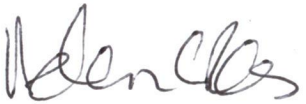
www.adas.co.uk

Sample/Report ID: 2016-462
Client Identifier: Pond 2
Date of Receipt: 28/04/16

Condition on Receipt: Good
Description: 6x50mL - pond water samples in preservatives
Material Tested: DNA extracted from pond water samples

Visual Inspection of Volume: Passed

Determinant	Result	Method	Date of Analysis
Great Crested Newt	Negative	Real time PCR	04/05/16

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:		Signed:	
Position:	Senior Research Scientist	Position:	Team Leader: Biotechnology
Date of preparation:	05/05/16	Date of issue:	05/05/16

Notes: eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

ADAS is a trading name of ADAS UK Limited. Registered in England No. 3296903. Registered Office: ADAS Wolverhampton HQ, Pendeford House, Pendeford Business Park, Wobaston Road, Wolverhampton, WV9 5AP.
ADAS UK Limited is a member of the ADAS group of companies

Client: [redacted]
AECOM ,
2 City Walk ,
Leeds,
LS11 9AR

ADAS Wolverhampton HQ
Pendeford House
Pendeford Business Park
Wobaston Road
Wolverhampton
WV9 5AP

Tel: 01159 516747
Fax: 01159 516415
Email: Helen.Rees@adas.co.uk

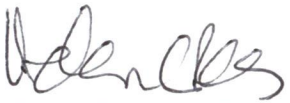
www.adas.co.uk

Sample/Report ID: 2016-463
Client Identifier: Pond 3
Date of Receipt: 28/04/16

Condition on Receipt: Good
Description: 6x50mL - pond water samples in preservatives
Material Tested: DNA extracted from pond water samples

Visual Inspection of Volume: Passed

Determinant	Result	Method	Date of Analysis
Great Crested Newt	Negative	Real time PCR	04/05/16

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:		Signed:	
Position:	Senior Research Scientist	Position:	Team Leader: Biotechnology
Date of preparation:	05/05/16	Date of issue:	05/05/16

Notes: eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

ADAS is a trading name of ADAS UK Limited. Registered in England No. 3296903. Registered Office: ADAS Wolverhampton HQ, Pendeford House, Pendeford Business Park, Wobaston Road, Wolverhampton, WV9 5AP.
ADAS UK Limited is a member of the ADAS group of companies

Client: [redacted]
AECOM ,
2 City Walk ,
Leeds,
LS11 9AR

ADAS Wolverhampton HQ
Pendeford House
Pendeford Business Park
Wobaston Road
Wolverhampton
WV9 5AP

Tel: 01159 516747
Fax: 01159 516415
Email: Helen.Rees@adas.co.uk

www.adas.co.uk

Sample/Report ID: 2016-460
Client Identifier: Pond 4
Date of Receipt: 28/04/16

Condition on Receipt: Good
Description: 6x50mL - pond water samples in preservatives
Material Tested: DNA extracted from pond water samples

Visual Inspection of Volume: Passed

Determinant	Result	Method	Date of Analysis
Great Crested Newt	Negative	Real time PCR	04/05/16

Report Prepared by: Dr Helen Rees
Signed:
Position: Senior Research Scientist
Date of preparation: 05/05/16

Report Issued by: Dr Ben Maddison
Signed:
Position: Team Leader: Biotechnology
Date of issue: 05/05/16

Notes: eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

ADAS is a trading name of ADAS UK Limited. Registered in England No. 3296903. Registered Office: ADAS Wolverhampton HQ, Pendeford House, Pendeford Business Park, Wobaston Road, Wolverhampton, WV9 5AP.
ADAS UK Limited is a member of the ADAS group of companies

Appendix 1: qPCR GCN eDNA analysis results breakdown

q-PCR GCN eDNA analysis Results

Experimental Samples	**Number of Positive Replicate Reactions***
Extraction Blank	0 of 12
Pond 4	0 of 12
Pond 1	0 of 12
Pond 2	0 of 12
Pond 3	0 of 12

Controls	**Number of Positive Replicate Reactions***
Negative PCR Control (Nuclease Free Water)	0 of 4
Positive Control GCN DNA 10^{-1} ng/µL	4 of 4
Positive Control GCN DNA 10^{-2} ng/µL	4 of 4
Positive Control GCN DNA 10^{-3} ng/µL	4 of 4
Positive Control GCN DNA 10^{-4} ng/µL	3 of 4

Inhibition Control	**Number of Positive Replicate Reactions at Expected C_t value†**
Pond 4	2 of 2
Pond 1	2 of 2
Pond 2	2 of 2
Pond 3	2 of 2

Degradation Control	
Expected rate of decay	None expected within time frame
Pond 4	Within Limits
Pond 1	Within Limits
Pond 2	Within Limits
Pond 3	Within Limits

* A sample is considered as positive for great crested newt if any of the replicates are positive.
† If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

ADAS is a trading name of ADAS UK Limited. Registered in England No. 3296903. Registered Office: ADAS Wolverhampton HQ, Pendeford House, Pendeford Business Park, Wobaston Road, Wolverhampton, WV9 5AP.
ADAS UK Limited is a member of the ADAS group of companies

Client: [redacted]
AECOM ,
2 City Walk ,
Leeds,
LS11 9AR

ADAS Wolverhampton HQ
Pendeford House
Pendeford Business Park
Wobaston Road
Wolverhampton
WV9 5AP

Tel: 01159 516747
Fax: 01159 516415
Email: Helen.Rees@adas.co.uk

www.adas.co.uk

Sample/Report ID: 2016-726 Condition on Receipt: Medium Sediment Visual Inspection of Volume: Passed

Client Identifier: 5 Description: 6x50mL - pond water samples in preservatives

Date of Receipt: 25/05/16 Material Tested: DNA extracted from pond water samples

Determinant	Result	Method	Date of Analysis
Great Crested Newt	Negative	Real time PCR	02/06/16

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:		Signed:	
Position:	Senior Research Scientist	Position:	Team Leader: Biotechnology
Date of preparation:	07/06/16	Date of issue:	07/06/16

Notes: eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

ADAS is a trading name of ADAS UK Limited. Registered in England No. 3296903. Registered Office: ADAS Wolverhampton HQ, Pendeford House, Pendeford Business Park, Wobaston Road, Wolverhampton, WV9 5AP.
ADAS UK Limited is a member of the ADAS group of companies

SureScreen Scientifics

Technical Report
Confidential

Folio No	**D1652**
Report No:	**1**
Client:	**AECOM Leeds**
Order No:	
Attn:	[redacted]
Date:	**11th May 2016**

TECHNICAL REPORT

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

[redacted]

Methodology

When Great Crested Newts (GCN) 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 traces to detect GCN inhabitation.

The laboratory testing is conducted in two phases. The sample first goes through an extraction process where all 6 tubes are pooled together to acquire as much eDNA as possible. The pooled sample is then tested via real time PCR (or q-PCR). This process amplifies select part of DNA allowing it to be detected and measured.

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 signal during the exponential phase of the reaction is measured for fast and objective data analysis.

The primers used in this process are specific to a part of mitochondrial DNA only found in GCN ensuring no other DNA is amplified.

Samples are tested in a clean room and the different phases of testing are kept separate to reduce any risk of cross contamination.

Each pooled sample is replicated 12 times to ensure results are accurate. If one of the twelve replicates tests positive the sample is declared positive. The sample is only declared negative if no replicates show amplification.

Inhibition and degradation checks are also carried out on each sample using a known DNA marker. Results of these quality control tests are recorded with each sample.

Results

Lab Ref	Sample	Co-Ordinates	Inhibition Check	Sample integrity	Result
22005	Pond 6, Lifespan Community Land, Dunford Bridge, South Yorkshire	SE16644 02779	Acceptable	Acceptable	**Negative**

Advice

Negative results may not indicate the absence of GCN just the presence of eDNA below the detection limits of the method. However this method is extremely sensitive. It is still advised to survey a pond using traditional methods within 2km of a positive result or a known habitat for GCN.

Positive results may be true positives but also may be due to contamination of samples from another pond or improper sampling technique. Please ensure traditional surveys are performed on positive ponds and care is taken to avoid spreading GCN DNA.

Samples undergo integrity scores to check for degradation post sampling. Samples which are not acceptable should be re-sampled. Sample integrity scores are based on the amount of degradation of an artificial DNA marker placed in the kits and analysed by qPCR.

PCR inhibitors can cause false results. 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.

Analysed and reported By: **J. Campbell BSc CEng.**

Checked and approved: **Troy Whyte BSc**

Client: [redacted]
AECOM ,
2 City Walk ,
Leeds,
LS11 9AR

ADAS Wolverhampton HQ
Pendeford House
Pendeford Business Park
Wobaston Road
Wolverhampton
WV9 5AP

Tel: 01159 516747
Fax: 01159 516415
Email: Helen.Rees@adas.co.uk

www.adas.co.uk

Sample/Report ID: 2016-728 | Condition on Receipt: Low Sediment | Visual Inspection of Volume: Passed

Client Identifier: 7 | Description: 6x50mL - pond water samples in preservatives

Date of Receipt: 25/05/16 | Material Tested: DNA extracted from pond water samples

Determinant	Result	Method	Date of Analysis
Great Crested Newt	Negative	Real time PCR	02/06/16

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:		Signed:	
Position:	Senior Research Scientist	Position:	Team Leader: Biotechnology
Date of preparation:	07/06/16	Date of issue:	07/06/16

Notes: eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

ADAS is a trading name of ADAS UK Limited. Registered in England No. 3296903. Registered Office: ADAS Wolverhampton HQ, Pendeford House, Pendeford Business Park, Wobaston Road, Wolverhampton, WV9 5AP.
ADAS UK Limited is a member of the ADAS group of companies

Appendix 1: qPCR GCN eDNA analysis results breakdown

q-PCR GCN eDNA analysis Results

Experimental Samples	**Number of Positive Replicate Reactions***
Extraction Blank	0 of 12
9	0 of 12
5	0 of 12
8	0 of 12
7	0 of 12

Controls	**Number of Positive Replicate Reactions***
Negative PCR Control (Nuclease Free Water)	0 of 4
Positive Control GCN DNA 10^{-1} ng/µL	4 of 4
Positive Control GCN DNA 10^{-2} ng/uL	4 of 4
Positive Control GCN DNA 10^{-3} ng/µL	4 of 4
Positive Control GCN DNA 10^{-4} ng/µL	4 of 4

Inhibition Control	**Number of Positive Replicate Reactions at Expected C_t value†**
9	2 of 2
5	2 of 2
8	2 of 2
7	2 of 2

Degradation Control	
Expected rate of decay	None expected within time frame
9	Within Limits
5	Within Limits
8	Within Limits
7	Within Limits

* A sample is considered as positive for great crested newt if any of the replicates are positive.
† If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

ADAS is a trading name of ADAS UK Limited. Registered in England No. 3296903. Registered Office: ADAS Wolverhampton HQ, Pendeford House, Pendeford Business Park, Wobaston Road, Wolverhampton, WV9 5AP.
ADAS UK Limited is a member of the ADAS group of companies

Client: [redacted]
AECOM ,
2 City Walk ,
Leeds,
LS11 9AR

ADAS Wolverhampton HQ
Pendeford House
Pendeford Business Park
Wobaston Road
Wolverhampton
WV9 5AP

Tel: 01159 516747
Fax: 01159 516415
Email: Helen.Rees@adas.co.uk

www.adas.co.uk

Sample/Report ID: 2016-727 | Condition on Receipt: Good | Visual Inspection of Volume: Passed

Client Identifier: 8 | Description: 6x50mL - pond water samples in preservatives

Date of Receipt: 25/05/16 | Material Tested: DNA extracted from pond water samples

Determinant	Result	Method	Date of Analysis
Great Crested Newt	Negative	Real time PCR	02/06/16

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:		Signed:	
Position:	Senior Research Scientist	Position:	Team Leader: Biotechnology
Date of preparation:	07/06/16	Date of issue:	07/06/16

Notes: eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

ADAS is a trading name of ADAS UK Limited. Registered in England No. 3296903. Registered Office: ADAS Wolverhampton HQ, Pendeford House, Pendeford Business Park, Wobaston Road, Wolverhampton, WV9 5AP.
ADAS UK Limited is a member of the ADAS group of companies

Client: [redacted]
AECOM ,
2 City Walk ,
Leeds,
LS11 9AR

ADAS Wolverhampton HQ
Pendeford House
Pendeford Business Park
Wobaston Road
Wolverhampton
WV9 5AP

Tel: 01159 516747
Fax: 01159 516415
Email: Helen.Rees@adas.co.uk

www.adas.co.uk

Sample/Report ID: 2016-725 | Condition on Receipt: Good | Visual Inspection of Volume: Passed

Client Identifier: 9 | Description: 6x50mL - pond water samples in preservatives

Date of Receipt: 25/05/16 | Material Tested: DNA extracted from pond water samples

Determinant	Result	Method	Date of Analysis
Great Crested Newt	Negative	Real time PCR	02/06/16

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:		Signed:	
Position:	Senior Research Scientist	Position:	Team Leader: Biotechnology
Date of preparation:	07/06/16	Date of issue:	07/06/16

Notes: eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

ADAS is a trading name of ADAS UK Limited. Registered in England No. 3296903. Registered Office: ADAS Wolverhampton HQ, Pendeford House, Pendeford Business Park, Wobaston Road, Wolverhampton, WV9 5AP.
ADAS UK Limited is a member of the ADAS group of companies

Client: [redacted]
AECOM,
2 City Walk,
Leeds,
LS11 9AR

RSK ADAS Ltd
Pendeford House
Pendeford Business Park
Wobaston Road
Wolverhampton
WV9 5AP

Tel: 01159 516747
Email: Helen.Rees@adas.co.uk

www.adas.co.uk

Sample/Report ID: 2017-684 | Condition on Receipt: Low Sediment | Visual Inspection of Volume: Passed

Client Identifier: VIP east peaks pond 10 | Description: 6x50mL - pond water samples in preservatives

Date of Receipt: 23/05/2017 | Material Tested: DNA extracted from pond water samples

Determinant	Result	Method	Date of Analysis
Great Crested Newt	Negative	Real time PCR	25/05/2017

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:	H Rees	Signed:	B. Maddison
Position:	Senior Research Scientist	Position:	Team Leader: Biotechnology
Date of preparation:	26/05/2017	Date of issue:	26/05/2017

Notes: eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

ADAS is a trading name of RSK ADAS Ltd. Registered in England No. 10486936. Registered Office: Spring Lodge, 172 Chester Road, Helsby, Cheshire, WA6 0AR.
RSK ADAS Ltd is part of RSK Group PLC.

Appendix 1: qPCR GCN eDNA analysis results breakdown

q-PCR eDNA analysis Results

Experimental Samples	GCN*	Inhibition Control†	Degradation Control§
Extraction Blank	0 of 12	N/A	N/A
VIP east peaks pond 10	0 of 12	2 of 2	Within Limits

Controls	Number of Positive Replicate Reactions*
Negative PCR Control (Nuclease Free Water)	0 of 4
Positive Control GCN DNA 10^{-1} ng/µL	4 of 4
Positive Control GCN DNA 10^{-2} ng/uL	4 of 4
Positive Control GCN DNA 10^{-3} ng/µL	4 of 4
Positive Control GCN DNA 10^{-4} ng/µL	4 of 4

* A sample is considered as positive for great crested newt if any of the replicates are positive.
† Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.
§ No decay is expected within time frame of kit preparation, sample collection and analysis.

DNA Analysis Report - Commercial in Confidence

Customer: Aecom

Address:

Contact:

Email:

Report date: 25-Apr-2019

Order Number: GCN19-1020 NG VIP PDE

Samples: Pond Water

Analysis requested: Detection of Great Crested Newt eDNA from pond water.

Thank you for submitting your samples for analysis with the Fera eDNA testing service. The details of the analysis are as follows:

Method:

The method detects pond occupancy from great crested newts (GCN) using traces of DNA shed into the pond environment (eDNA). The detection of GCN eDNA is carried out using real time PCR to amplify part of the cytochrome 1 gene found in mitochondrial DNA. The method followed is detailed in Biggs J., et al, (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.

The limits of this method are as follows: 1) the results are based on analyses of the samples supplied by the client and as received by the laboratory, 2) any variation between the characteristics of this sample and a batch will depend on the sampling procedure used. 3) the method is qualitative and therefore the levels given in the score are for information only, they do not constitute the quantification of GCN DNA against a calibration curve, 4) a 'not detected' result does not exclude presence at levels below the limit of detection.

The results are defined as follows:

Positive: DNA from the species was detected.

eDNA Score: Number of positive replicates from a series of twelve.

Negative: DNA from the species was not detected; in the case of negative samples the DNA extract is further tested for PCR inhibitors and degradation of the sample.

Inconclusive: Controls indicate degradation or inhibition of the sample, therefore the lack of detection of GCN DNA is not conclusive evidence for determining the absence of the species in the sample provided.

This test report may not be reproduced except in full, without the written approval of Fera. Fera hereby excludes all liability for any claim, loss, demands or damages of any kind whatsoever (whether such claims, loss, demands or damages were foreseeable, known or otherwise) arising out of or in connection with the preparation of any technical or scientific report , including without limitation, indirect or consequential loss or damage; loss of actual or anticipated profits (including loss of profits on contracts); loss of revenue; loss of business; loss of opportunity; loss of anticipated savings; loss of goodwill; loss of reputation; loss of damage to or corruption of data; loss of use of money or otherwise, and whether or not advised of the possibility of such claim, loss demand or damages and whether arising in tort (including negligence), contract or otherwise. This statement does not affect your statutory rights. Nothing in this disclaimer excludes or limits Fera liability for: (a) death or personal injury caused by Fera's negligence (or that of its employees, agents or directors); or (b) the tort of deceit; [or (c) any breach of the obligations implied by Sale of Goods Act 1979 or Supply of Goods and Services Act 1982 (including those relating to the title, fitness for purpose and satisfactory quality of goods);] or (d) any liability which may not be limited or excluded by law (e) fraud or fraudulent misrepresentation. The parties agree that any matters are governed by English law and irrevocably submit to the non-exclusive jurisdiction of the English courts.

DNA Analysis Report - Commercial in Confidence

CustomerReference	Fera Reference	GCN Detection	eDNA Score	Inhibition	Degradation
Pond 6	S19-015941	Negative	0	No	No
Pond 3	S19-015942	Negative	0	No	No
Pond 8	S19-015943	Negative	0	No	No
Pond 1	S19-015944	Negative	0	No	No
Pond 9	S19-015945	Negative	0	No	No
Pond 2	S19-015946	Negative	0	No	No

The results indicate that eDNA for great crested newts was not detected in any of the samples submitted. Analysis was conducted in the presence of the following controls: 1) extraction blank, 2) appropriate positive and negative PCR controls for each of the TaqMan assays (GCN, Inhibition, and Degradation). All controls performed as expected.

This test procedure was developed using research funded by the Department of Environment, Food and Rural Affairs.

Issuing officer: Steven Bryce
Tel: 01904 462 070
Email: e-dna@fera.co.uk

This test report may not be reproduced except in full, without the written approval of Fera. Fera hereby excludes all liability for any claim, loss, demands or damages of any kind whatsoever (whether such claims, loss, demands or damages were foreseeable, known or otherwise) arising out of or in connection with the preparation of any technical or scientific report , including without limitation, indirect or consequential loss or damage; loss of actual or anticipated profits (including loss of profits on contracts); loss of revenue; loss of business; loss of opportunity; loss of anticipated savings; loss of goodwill; loss of reputation; loss of damage to or corruption of data; loss of use of money or otherwise, and whether or not advised of the possibility of such claim, loss demand or damages and whether arising in tort (including negligence), contract or otherwise. This statement does not affect your statutory rights. Nothing in this disclaimer excludes or limits Fera liability for: (a) death or personal injury caused by Fera's negligence (or that of its employees, agents or directors); or (b) the tort of deceit; [or (c) any breach of the obligations implied by Sale of Goods Act 1979 or Supply of Goods and Services Act 1982 (including those relating to the title, fitness for purpose and satisfactory quality of goods);] or (d) any liability which may not be limited or excluded by law (e) fraud or fraudulent misrepresentation. The parties agree that any matters are governed by English law and irrevocably submit to the non-exclusive jurisdiction of the English courts.

AECOM Limited
5th Floor, 2 City Walk
Leeds LS11 9AR
United Kingdom

T: +44 (0)113 391 6800
aecom.com