



UNIVERSAL DESTINATIONS & EXPERIENCES UK PROJECT

Former Kempston Hardwick Brickworks
and adjoining land, Bedford

Environmental Statement Volume 3

Appendix 6.7 - Great Crested Newt Survey Report

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1 INTRODUCTION

1.1 PROJECT BACKGROUND

- 1.1.1. This Appendix has been prepared in support of the planning proposal for the construction and operation of an Entertainment Resort Complex and associated development (hereafter referred to as the 'Proposed Development'). The Proposed Development is described in **Chapter 2: Description of the Proposed Development (Volume 1)** of the Environmental Statement.
- 1.1.2. The Proposed Development is located to the southwest of Bedford, Bedfordshire and will hereafter be referred to as 'the Site'. The Site equates to 268ha and comprises four zones; the Core Zone, Lake Zone, East Gateway Zone and the West Gateway Zone. The study area constitutes a 250m buffer around the Site boundary. The Site boundary and study area are shown in **Figure A.1: GCN Results within 250m of the Site boundary of Annex 1: Figures**.

1.2 ECOLOGICAL BACKGROUND

- 1.2.1. A Preliminary Ecological Appraisal (PEA) was undertaken by WSP in April 2024 (see **Appendix 6.1: Preliminary Ecological Appraisal Report (Volume 3)**). The initial desk study, to scope the presence of waterbodies using aerial mapping, identified 14 potentially suitable water bodies for great crested newts (GCN) *Triturus cristatus* within the Site, and a further 23 potentially suitable waterbodies within 250m of the Site (**Figure A.1: GCN Results within 250m of the Site boundary of Annex 1: Figures**). The PEA habitat survey identified suitable aquatic habitat for GCN within the Site, such as lakes, ponds and reedbeds, and terrestrial habitat including scrub, tall grassland, hedgerows and woodland.
- 1.2.2. The initial desk study (as detailed in **Appendix 6.1: Preliminary Ecological Appraisal Report (Volume 3)**) revealed 165 previous records of GCN within 2km of the Site (**Figure A.2: GCN Records Within 2km of the Site boundary of Annex 1: Figures**). Five records of GCN were returned from within the Site, one from the Lake Zone and four from the Core Zone.
- 1.2.3. The PEA recommended that Habitat Suitability Index (HSI) assessments and eDNA (environmental deoxyribonucleic acid) surveys should be undertaken on the ponds within and up to 250m of the Site boundary where impacts by the Proposed Development is deemed likely, to determine the presence or likely absence of GCN.

1.3 SCOPE OF THE REPORT

- 1.3.1. WSP UK Ltd was commissioned by UDX to complete GCN surveys, with the following objectives, the results of which are provided in this report:
- Complete a HSI assessment of water bodies within and up to 250m from the Site boundary, to assess their suitability of aquatic habitat for GCN and determine if further survey was required;
 - Complete eDNA surveys to determine the presence or likely absence of GCN from water bodies within and up to 250m from the Site boundary; and
 - Present the findings of the survey in a baseline report.

- 1.3.2. WSP has provided this report solely for the use of the recipient and accepts no liability to any third parties or any other party using or reviewing the report or any part thereof. WSP makes no warranties or guarantees, actual or implied, in relation to this report, or the ultimate commercial, technical, economic, or financial effect on the project to which it relates, and bears no responsibility or liability related to its use other than as set out within the scope of the contract under which it was supplied.

1.4 RELEVANT LEGISLATION AND POLICY

- 1.4.1. GCN are afforded a high level of protection under the *Conservation of Habitats and Species Regulations 2017*¹ (as amended) (the '*Habitat Regulations*'), which makes it an offence to:
- Deliberately capture, injure or kill a wild GCN;
 - Deliberately disturb wild GCN; "*disturbance of animals includes in particular any disturbance which is likely*":
 - "*To impair their ability to survive, to breed or reproduce, or to rear or nurture their young*"; or
 - "*In the case of animals of a hibernating or migratory species, to hibernate or migrate*"; or
 - To significantly affect "*the local distribution or abundance of the species to which they belong*"; and
 - Damage or destroy a breeding site or resting place used by this species.
- 1.4.2. Due to the high level of protection afforded to GCN and their habitat, mitigation for this species is governed by a strict licensing procedure administered by Natural England (normally, planning permission must be obtained before a licence can be sought). Licensing is subject to three tests, as defined under the *Habitats Regulations 2017*¹, these must also be applied by the planning authority before granting permission for activities affecting GCN. For permission to be granted the following criteria must be satisfied:
- 1.4.3. The proposal is necessary *to preserve "public health or public safety or other imperative reasons of overriding public interest including those of a social or economic nature and beneficial consequences of primary importance for the environment"*;
- "*There is no satisfactory alternative*"; and
 - The proposals "*will not be detrimental to the maintenance of the population of the species concerned at a favourable conservation status in their natural range*"¹.
- 1.4.4. GCN is also listed as a Species of Principal Importance (SPI) for the Conservation of Biodiversity in England in accordance with Section 41 of the *Natural Environment and Rural Communities (NERC) Act 2006*². Under Section 40 of the *NERC Act (2006)* public bodies (including local planning authorities) have a duty to have regard for the conservation of SPI when carrying out their functions, including determining planning applications.

1 ¹ HM Government (2017) *The Conservation of Habitats and Species Regulations 2017*. Available at: <https://www.legislation.gov.uk/ukxi/2017/1012/contents> [Accessed: 25 March 2025].

²HM Government (2006) *Natural Environment and Rural Communities Act 2006, Section 40*. Available at: <https://www.legislation.gov.uk/ukpga/2006/16/contents> [Accessed: 25 March 2025].

2 METHODS

2.1 DESK STUDY

- 2.1.1. Datasets including those from the local records centre and those included in data published on the Multi-Agency Geographic Information for the Countryside (Magic) mapping application³ were consulted for information regarding GCN Class Licence returns and European Protected Species Mitigation Licenses (EPSML) that have been granted within 2km of the Proposed Development.

2.2 HABITAT SUITABILITY INDEX (HSI) ASSESSMENT

- 2.2.1. Surveyed water bodies were assessed for their suitability to support GCN using the standard HSI assessment method (Amphibian and Reptile Groups (ARG) of the UK⁴, based on Oldham *et al.*⁵). Water bodies were identified using 1:25,000 OS mapping; this was also cross referenced against aerial photography.
- 2.2.2. Water bodies were assessed and scored on ten key variables which are known to influence breeding populations of GCN, in accordance with standard methods⁴. These variables are:
- Geographic location;
 - Water body area;
 - Water body permanence;
 - Water quality;
 - Water body shading;
 - Impact of waterfowl;
 - Fish stocks;
 - Number of water bodies within 1km;
 - Terrestrial habitat around the water body; and
 - Macrophyte cover of the water body.
- 2.2.3. Scores for each of the above variables were used to calculate an overall HSI value for each water body. This was then cross referenced with the guidelines⁴ to assign the water body one of five categories: poor, below average, average, good, or excellent (**Table 2-1**). Index calculation is not a failsafe method of identifying whether a water body supports GCN or not; therefore, professional judgement and availability of records in the locality have also been used to inform the requirement for further survey.

³ Department for Environment Food and Rural Affairs (n.d.) *Magic Map*. Available at: <https://magic.defra.gov.uk/> [Accessed: 25 March 2025].

⁴ Amphibian and Reptile Groups of UK (2010) *ARG UK Advice Note 5 – Great Crested Newt Habitat Suitability Index*. Available at: <https://www.arguk.org/info-advice/advice-notes/9-great-crested-newt-habitat-suitability-index-arg-advice-note-5/file> [Accessed: 25 March 2025].

⁵ Oldham, R.S., Keeble, J., Swan, M.J. and Jeffcote, M. (2010) 'Evaluating the suitability of habitat for the great crested newt (*Triturus cristatus*)', *The Herpetological Journal*, 10(4), pp. 143-155. Available at: <https://www.thebhs.org/publications/the-herpetological-journal/volume-10-number-4-october-2000/1617-03-evaluating-the-suitability-of-habitat-for-the-great-crested-newt-triturus-cristatus> [Accessed: 25 March 2025].

Table 2-1 – Categorisation of HSI scores

HSI score	Waterbody suitability
<0.5	Poor
0.5 – 0.59	Below average
0.6 – 0.69	Average
0.7 – 0.79	Good
>0.8	Excellent

- 2.2.4. As per the standard HSI assessment method, for ponds larger than 2000m², the pond area factor was omitted from the HSI calculation as there are no data for such large ponds⁴.

2.3 ENVIRONMENTAL DNA WATER SAMPLING

- 2.3.1. Following the desk study, water bodies that could be affected by the Proposed Development were subject to further Environmental DNA (eDNA) survey to determine the presence or likely absence of GCN.
- 2.3.2. The survey comprised of eDNA⁶ (Biggs et al., 2014) water sampling, undertaken concurrently with the HSI survey.
- 2.3.3. The eDNA surveys were undertaken following survey techniques described in Biggs *et al.*⁶:
- A single visit to each target waterbody was made between mid-April and late-June, during the newt breeding season;
 - Samples were collected in satisfactory weather conditions. Periods of heavy rain were avoided as this would have increased the risk of cross-contamination;
 - Twenty sub-samples of water were taken from each waterbody using sterile sampling equipment provided by the laboratory (ADAS);
 - The locations of the 20 sub-samples were spaced as evenly as possible around the waterbody margin, and where possible were targeted at areas of vegetation suitable for use as egg laying substrate, and at open water areas which GCN may use for displaying;
 - The sub-samples were mixed and pipetted into six sample tubes containing an alcohol and pH buffer solution;
 - The samples were sent to ADAS for laboratory testing using real time Polymerase Chain Reaction (PCR) to amplify part of the cytochrome 1 gene found in mitochondrial DNA; and
 - The water samples from each waterbody were assigned a positive or negative result as well as a score between 0 and 12 representing the number of positive replicates from a series of 12.

⁶ 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.* Oxford, England: Freshwater Habitats Trust.

- 2.3.4. GCN release eDNA into the water bodies in which they live when they deposit skin cells, faeces, mucus, sperm, or eggs into the water. The DNA in this material can persist, and be detected, in the water for several weeks. A positive eDNA result concludes that GCN DNA is present in the water sample, whilst a negative result concludes that the presence of GCN is considered unlikely within that water body. Negative eDNA results cannot conclusively say that GCN are not present within the water body, but rather that DNA from the species was not detected.

2.4 DATES OF SURVEY AND PERSONNEL

- 2.4.1. Lead surveyors were competent and experienced in conducting GCN HSI and eDNA surveys and hold a current Natural England Level 1 GCN Class Licence.
- 2.4.2. GCN eDNA surveys were completed in June 2024. Specific survey dates are provided in **Table D-1, Annex 4**.

2.5 INCIDENTAL SURVEYS

- 2.5.1. Pond 35 located in the Lake Zone was subject to further appraisal during a walkover by WSP ecologists in February 2025. The waterbody was previously identified as a section of ditch within habitat surveys previously conducted, however following further review was confirmed to have limited/no flow and was suitable to support amphibians with aquatic egg laying material present and moderate water depth and quality. Ponds 36, 37 and 38 located in the Core Zone were not previously subject to HSI/eDNA as were identified after the recognised survey period for conducting eDNA surveys (mid-April to June inclusive). Whilst outside the recommended survey period, incidental torchlight survey was conducted on waterbodies 36 and 37 on 24th/25th February 2025 by licenced WSP ecologists. No surveys have been conducted on Pond 38.
- 2.5.2. Pond 6 and 26 were also subject to incidental torchlight survey on 24th February 2025 by licenced WSP ecologists.

2.6 NOTES AND LIMITATIONS

- 2.6.1. Due to access constraints, of the 38 identified water bodies within 250m of the Site, 17 were not surveyed. Twelve of these waterbodies are located on the western side of the A421 which represents a moderate barrier to dispersal. Two ponds; 2 and 27 could not be accessed but comprise of stocked commercial fishing ponds. Where ponds could not be accessed to be surveyed a precautionary principle will be applied, considering dispersal linkages and distance to the Site.
- 2.6.2. Two ponds, previously labelled water body reference 8 and 30 in **Figure A.1: GCN Results within 250m of the Site boundary of Annex 1: Figures**, were confirmed via field survey to be hydrological connected and therefore they were surveyed as one combined waterbody for both the eDNA survey and HSI assessment.
- 2.6.3. Seven waterbodies within the Site; Ponds 7, 8, 9, 10, 31, 32, and 33 returned indeterminate results. These waterbodies are all located within the Lake Zone study area (**Figure A.1: GCN Results within 250m of the Site boundary of Annex 1: Figures**). Contaminants may be present in the water, which could have contributed to an inconclusive result. Due to this limitation, confirmed presence nor likely absence of GCN can be determined.

- 2.6.4. Three waterbodies, Ponds 5, 6, and 26 had limited access due to dense vegetation surrounding the bank and therefore surveyors were only able to collect an eDNA sample from one location (therefore not in accordance with the sampling guidance). Ponds 5 and 6 returned positive results, so this was not considered to represent a significant limitation to the use of eDNA at these ponds. However, whilst Pond 26 returned a negative result a precautionary approach has been applied and this has been assigned as an indeterminate eDNA result (see **Section 3.3.3**).
- 2.6.5. Records held by local biological record centres and local recording groups are generally collected on a voluntary basis; therefore, the absence of records does not demonstrate the absence of species, it may simply indicate a gap in recording coverage.
- 2.6.6. European Protected Species Mitigation Licence information is taken from Magic⁷ and spatial resolution is unknown.
- 2.6.7. Ecological survey data, including great crested newt survey data, is typically valid for 18 months to three years unless otherwise specified, for example if conditions are likely to change more quickly due to ecological processes or anticipated changes in management⁸.

⁷ Five EPSML located within proximity to the Site were requested from NE via a Freedom of Information request to gain an additional insight into the local status of GCN and potential location of mitigation licence receptor locations.

⁸ Chartered Institute of Ecology and Environmental Management (2019) Advice note on the lifespan of ecological reports and surveys. Available at: <https://cieem.net/wp-content/uploads/2019/04/Advice-Note.pdf> [Accessed: 24 March 2025].

3 RESULTS AND EVALUATION

3.1 DESK STUDY

- 3.1.1. A total of 18 European Protected Species Mitigation Licences (EPSML) for GCN were recorded within the 2km of the Site. Supplementary licence data on five of the 18 licences are provided in **Table 3-1** and their locations shown in **Figure A.2: GCN Records Within 2km of the Site boundary of Annex 1: Figures**. The desk study results also confirm that GCN have been present in the area since at least 2010. At least one pond had a medium population size during this period.

Table 3-1 - European Protected Species Mitigation Licence information

Licence number	Date	Distance from proposed development area	Summary
EPSM2010-1763	01/06/2010 to 31/05/2012	230m south	No information held by NE
EPSM2011-3277	26/03/2013 to 30/04/2023	90m north	Licence permitted for trapping. Receptor site is 110m northwest of Proposed Development Site.
EPSM2012-3926	10/04/2013 to 01/07/2023	660m south	No information held by NE
2015-15321-EPS-MIT-1	29/02/2016 to 01/07/2019	1.9km east	Licence granted for translocation in 2016 allowed for up to 250 newts to be translocated. Receptor pond located approximately 1.75km from Proposed Development area. A survey of the receptor site prior to translocation found a medium population (112) in 2015.
2020-49399-EPS-MIT	02/10/2020 to 31/12/2023	120m east	Licence permitted for pitfall trapping of up to 25 individuals away from pond in 2020. Receptor is approximately 100m east of Proposed Development area.

3.2 HABITAT SUITABILITY INDEX (HSI) ASSESSMENT

- 3.2.1. A summary of the HSI assessment and location information for the relevant water body is included in **Table 3-2** and the full HSI results are shown in **Table B-1, Annex 2**. Water body reference numbers correspond to those mapped in **Figure A.1: GCN Results within 250m of the Site boundary of Annex 1: Figures**.

- Poor – eight waterbodies (Pond 4, 7, 8, 9, 10, 31, 32, 34);
- Below average – seven waterbodies (Ponds 1, 5, 6, 25, 26, 33 and 37);
- Average – two waterbodies (Ponds 23 and 36);
- Good – three waterbodies (Ponds 21, 35 and 38); and

- Excellent – one waterbody (Pond 11).

3.2.2. Of these, fourteen ponds are located within the Site boundary; seven in the Core Zone (Pond 1, 6, 25, 26, 36, 37 and 38), six in the Lake Zone (Pond 8, 9, 10, 31, 33 and 35) and one in the West Gateway Zone on the western side of the A421; Pond 12.

3.3 ENVIRONMENTAL DNA WATER SAMPLING

- 3.3.1. Of the 37 water bodies identified within the study area, 17 of these water bodies were surveyed for eDNA (**Figure A.1: GCN Results within 250m of the Site boundary of Annex 1: Figures**).
- 3.3.2. Seven water bodies, Ponds 1, 5, 6, 21, 23, 25, and 34 returned positive eDNA results, indicating the presence of GCN within those waterbodies.
- 3.3.3. Three water bodies, Ponds 4, 11 and 26 returned a negative result, indicating the likely absence of GCN within these waterbodies. Due to the survey limitations associated with Pond 26 a precautionary approach should be applied, and this pond should be reassigned as 'indeterminate'. Pond 26 is therefore presented as 'Indeterminate' in **Figure A.1: GCN Results within 250m of the Site boundary of Annex 1: Figures**.
- 3.3.4. Seven waterbodies, Ponds 7, 8, 9, 10, 31, 32, and 33 returned indeterminate results, meaning neither confirmed presence nor likely absence of GCN could be determined.
- 3.3.5. Full eDNA laboratory certificates are available in **Annex 3**.
- 3.3.6. The remaining 20 waterbodies within the study area, (of which four are located within the Site) were unable to be accessed or were not subject to eDNA survey.
- 3.3.7. Throughout this document the water body reference numbers will be used to refer to individual water bodies. The ADAS Client ID, in **Table 3-2**, corresponds to the ID submitted for each waterbody sample presented in the eDNA laboratory result certificates (**Annex 3**).

Table 3-2 - Summary of HSI Results and eDNA Survey Results

Water body Ref.	ADAS client ID	Grid reference	Proximity to Site boundary (at closet point)	Connectivity to Site	HSI score (see Annex 2)	HSI category	eDNA results
1	JF07	TL 02416 43487	Within boundary	Good	0.53	Below average	Positive
4	JF13	TL02633 43350	140m	Good	0.37	Poor	Negative
5	5	TL 03093 44597	36m	Good	0.47	Poor	Positive
6	JF12	TL02929 44485	Within boundary	Good	0.54	Below average	Positive
7	JF14	TL 03142 44465	0m	Good	0.39	Poor	Indeterminate

Water body Ref.	ADAS client ID	Grid reference	Proximity to Site boundary (at closet point)	Connectivity to Site	HSI score (see Annex 2)	HSI category	eDNA results
8	JF04	TL 03763 45544	Within boundary	Good	0.39	Poor	Indeterminate
9	JF02	TL 03112 45459	Within boundary	Good	0.39	Poor	Indeterminate
10	JF10	TL03003 45028	Within boundary	Good	0.37	Poor	Indeterminate
11	11	TL 02608 45057	96m	Good	0.86	Excellent	Negative
21	21	TL 01998 43803	26m	Good	0.73	Good	Positive
23	23	TL 02302 43268	73m	Good	0.62	Average	Positive
25	JF06	TL 02282 43470	Within boundary	Good	0.58	Below average	Positive
26	JF08	TL 03131 44259	Within boundary	Good	0.53	Below average	Negative* (reassigned to Indeterminate)
31	JF03	TL 03246 45499	Within boundary	Good	0.39	Poor	Indeterminate
32	JF9	TL03610 45425	0m	Good	0.37	Poor	Indeterminate
33	JF05	TL 03101 45432	Within boundary	Good	0.54	Below average	Indeterminate
34	JF11	TL 03100 44611	98m	Good	0.58	Below average	Positive
35	N/A	TL 02883 45250	Within boundary	Good	0.86	Excellent	N/A (see section 3.4)
36	N/A	TL 03051 43949	Within boundary	Good	0.63	Average	N/A (see section 3.4)
37	N/A	TL0279 4371	Within boundary	Good	0.59	Below average	N/A (see section 3.4)
38	N/A	TL 02975 43831	Within boundary	Good	0.70	Good	N/A (see section 3.4)

* See Section 3.3.3.

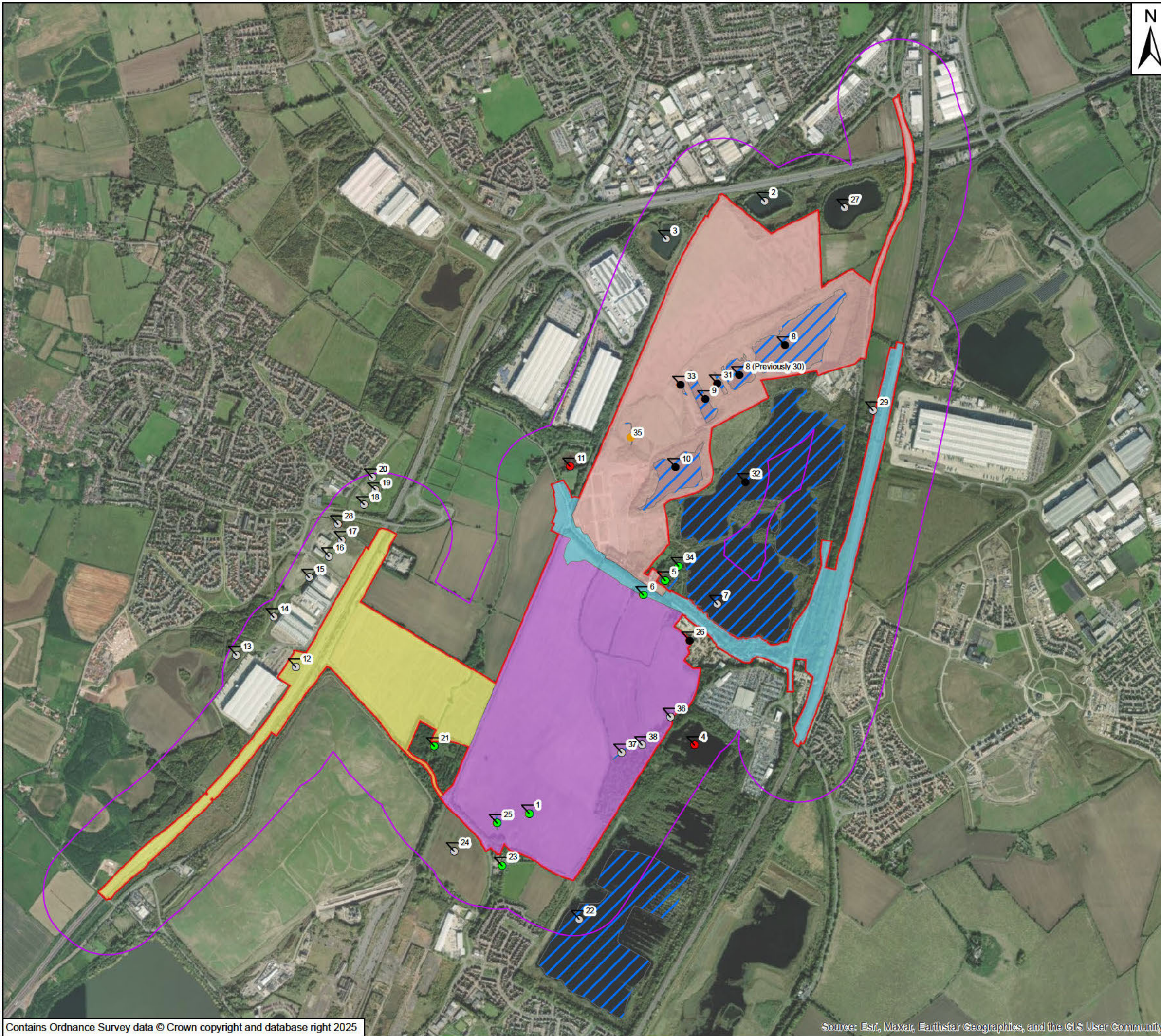
3.4 INCIDENTAL SURVEYS

- 3.4.1. Torchlight surveys conducted on Pond 35, whilst out of season, confirmed the presence of GCN with five adult individuals seen in the torchlight (four males and one female) alongside smooth newt. The presence of both species is confirmed in this waterbody.
- 3.4.2. A single adult male was identified within Pond 6 during torchlight survey; reaffirming the positive eDNA result from 2024.
- 3.4.3. No GCN were identified in Ponds 26 and 36 during the incidental torchlight surveys. However, this survey technique alone is not sufficient to confirm absence of species, and as stated in **Section 2.5**, these surveys were outside of the recommended survey period. Therefore, there is still potential for GCN to be present in these ponds.
- 3.4.4. Waterbodies 37 and 38 were subsequently identified within Coronation Pits woodland. Waterbody 37 comprises a section of drainage ditch where the waterflow was absent and contained evidence of aquatic vegetation. The section of ditch was subject to a torchlight survey in late February 2025. No amphibians were recorded at this location, nor within areas of ephemeral water within the adjacent woodland. It is acknowledged that this survey was conducted outside the recommended survey period.
- 3.4.5. Waterbody 38 is located within an area of dense scrub and previously had appeared to be an ephemeral area of water within the woodland, however held water in February 2025 and subsequently in May 2025 (following an extended period of dry weather) therefore was considered potentially suitable to support amphibians, albeit it is partially shaded and has scrub dominated banks with limited aquatic vegetation.

ANNEX 1

FIGURES:

Figure A.1: GCN Results within 250m of the Site boundary



Key

- Site boundary
- 250m buffer
- Core Zone
- East Gateway Zone
- Lake Zone
- West Gateway Zone

GCN eDNA Result

- Indeterminate
- Negative
- Positive
- Unsampled

GCN status

- GCN confirmed via torch survey



Client:	Universal Destinations & Experiences		
Project:	Universal Destinations & Experiences UK Project		
Title:	Figure A.1: GCN Results within 250m of the Site boundary		
Drawing No:	Figure A.1	Drawn:	SS
Date:	5/14/2025	Checked:	CM
Scale:	16,500 @ A3	Approved:	SB

Figure A.2: GCN Records within 2km of the Site boundary



- Key**
- Site boundary
 - 2km Buffer
 - ▲ Great Crested Newt (GCN) Biological Records 2014-2024
 - European Protected Species Mitigation Licence (EPSML)

0 0.5 1
Kilometres








Client:	Universal Destinations & Experiences		
Project:	Universal Destinations and Experiences UK Resort		
Title:	Figure A.2: GCN Records within 2km of the Site boundary		
Drawing No:	Figure A.2	Drawn:	SS
Date:	14/05/2025	Checked:	CM
Scale:	30,000 @ A3	Approved:	SB




ANNEX 2




HSI CALCULATIONS



Table B-1 – HSI calculations



Water body Ref.	Within the Site	S1: Geographic location	S2: Water body area (n/a indicates the pond area exceeded 2000m ²)	S3: Water body permanence	S4: Water quality	S5: % Shade (1m from bank)	S6: Impact of waterfowl	S7: Fish stocks	S8: Number of water bodies <1km	S9: Terrestrial habitat	S10: Macrophyte cover (%cover)	HSI SCORE	HSI CATEGORY	Photo
1	Yes	1	1	0.5	0.01	1	1	1	0.97	0.67	0.5	0.53	Below average	
4	No	1	n/a	0.9	1	1	0.01	0.01	0.97	1	0.5	0.37	Poor	
5	No	1	0.05	0.5	0.33	0.2	1	1	1	1	0.35	0.47	Poor	 


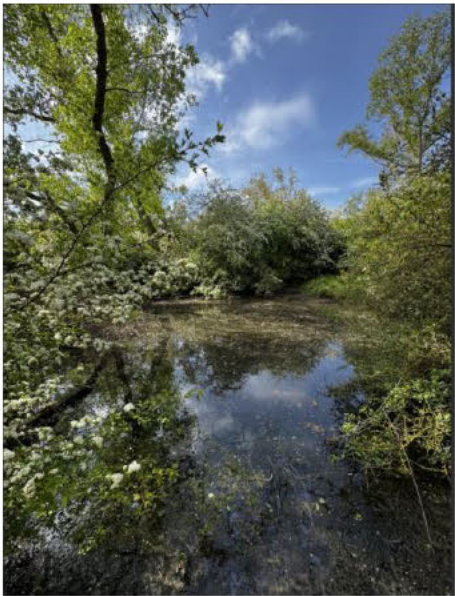
Water body Ref.	Within the Site	S1: Geographic location	S2: Water body area (n/a indicates the pond area exceeded 2000m ²)	S3: Water body permanence	S4: Water quality	S5: % Shade (1m from bank)	S6: Impact of waterfowl	S7: Fish stocks	S8: Number of water bodies <1km	S9: Terrestrial habitat	S10: Macrophyte cover (%cover)	HSI SCORE	HSI CATEGORY	Photo
5	No	1	0.05	0.5	0.33	0.2	1	1	1	1	0.35	0.47	Poor	
6	Yes	1	0.65	0.1	0.33	1	0.67	0.67	1	0.67	0.3	0.54	Below average	
7	No	1	n/a	0.9	1	1	0.01	0.01	0.95	1	0.9	0.39	Poor	

Water body Ref.	Within the Site	S1: Geographic location	S2: Water body area (n/a indicates the pond area exceeded 2000m ²)	S3: Water body permanence	S4: Water quality	S5: % Shade (1m from bank)	S6: Impact of waterfowl	S7: Fish stocks	S8: Number of water bodies <1km	S9: Terrestrial habitat	S10: Macrophyte cover (%cover)	HSI SCORE	HSI CATEGORY	Photo
8	Yes	1	n/a	0.9	1	1	0.01	0.01	1	1	0.9	0.39	Poor	
9	Yes	1	n/a	0.9	1	1	0.01	0.01	1	1	1	0.39	Poor	
10	Yes	1	n/a	0.9	1	1	0.01	0.01	1	1	0.5	0.37	Poor	

Water body Ref.	Within the Site	S1: Geographic location	S2: Water body area (n/a indicates the pond area exceeded 2000m ²)	S3: Water body permanence	S4: Water quality	S5: % Shade (1m from bank)	S6: Impact of waterfowl	S7: Fish stocks	S8: Number of water bodies <1km	S9: Terrestrial habitat	S10: Macrophyte cover (%cover)	HSI SCORE	HSI CATEGORY	Photo
11	No	1	n/a	0.9	1	0.6	0.67	0.67	1	1	0.9	0.86	Excellent	
21	No	1	1	0.9	0.33	0.4	1	1	0.96	1	0.4	0.73	Good	
23	No	1	0.2	0.5	0.33	1	0.67	1	0.97	0.67	0.6	0.62	Average	
25	Yes	1	0.2	0.1	0.33	0	0	0	0.97	0.67	1	0.58	Below average	

Water body Ref.	Within the Site	S1: Geographic location	S2: Water body area (n/a indicates the pond area exceeded 2000m ²)	S3: Water body permanence	S4: Water quality	S5: % Shade (1m from bank)	S6: Impact of waterfowl	S7: Fish stocks	S8: Number of water bodies <1km	S9: Terrestrial habitat	S10: Macrophyte cover (%cover)	HSI SCORE	HSI CATEGORY	Photo
26	Yes	1	0.25	0.5	0.33	0.2	1	1	0.96	0.67	0.3	0.53	Below average	
31	Yes	1	0.91	0.9	1	1	0.01	0.01	1	1	1	0.39	Poor	
32	No	1	n/a	0.9	1	1	0.01	0.01	1	1	0.5	0.37	Poor	
33	Yes	1	0.6	0.5	0.67	1	0.01	1	1	1	1	0.54	Below average	
34	No	1	0.1	1	0.33	0.6	0.67	0.67	1	1	0.5	0.58	Below average	

Water body Ref.	Within the Site	S1: Geographic location	S2: Water body area (n/a indicates the pond area exceeded 2000m ²)	S3: Water body permanence	S4: Water quality	S5: % Shade (1m from bank)	S6: Impact of waterfowl	S7: Fish stocks	S8: Number of water bodies <1km	S9: Terrestrial habitat	S10: Macrophyte cover (%cover)	HSI SCORE	HSI CATEGORY	Photo
35	Yes	1	0.60	1	0.67	1	0.67	1	1	1	0.8	0.86	Excellent	
36	Yes	1	1	0.4	1	0.33	0.6	0.67	0.97	0.67	0.4	0.63	Average	

Water body Ref.	Within the Site	S1: Geographic location	S2: Water body area (n/a indicates the pond area exceeded 2000m ²)	S3: Water body permanence	S4: Water quality	S5: % Shade (1m from bank)	S6: Impact of waterfowl	S7: Fish stocks	S8: Number of water bodies <1km	S9: Terrestrial habitat	S10: Macrophyte cover (%cover)	HSI SCORE	HSI CATEGORY	Photo
37	Yes	1	0.8	0.1	0.33	0.7	1	0.67	1	1	0.4	0.59	Below Average	
38	Yes	1	1	1	0.33	0.2	1	0.67	1	1	0.6	0.70	Good	

ANNEX 3

EDNA RESULTS – LABORATORY CERTIFICATES

Client: [REDACTED]
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Sample ID: ADAS-5307 Condition on Receipt: Good Volume: Passed
Client Identifier: JF06 Description: pond water samples in preservative
Date of Receipt: 18/06/2024 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	21/06/2024
Degradation Control [§]	Within Limits	Real Time PCR	21/06/2024
Great Crested Newt*	12 of 12 (GCN positive)	Real Time PCR	21/06/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: [REDACTED] Report Issued by: [REDACTED]

Signed:

[REDACTED]

Signed:

[REDACTED]

Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 25/06/2024 Date of issue: 25/06/2024

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.

** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more 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 degradation is expected within time frame of kit preparation, sample collection and analysis.

[#] Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

Client: [REDACTED]
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Sample ID: ADAS-5330 Condition on Receipt: White Precipitate Volume: Passed
Client Identifier: JF02 Description: pond water samples in preservative
Date of Receipt: 18/06/2024 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	0 of 2	Real Time PCR	20/06/2024
Degradation Control [§]	Evidence of degradation or residual inhibition	Real Time PCR	20/06/2024
Great Crested Newt*	Indeterminate	Real Time PCR	20/06/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: [REDACTED] Report Issued by: [REDACTED]

Signed: [REDACTED] Signed: [REDACTED]

Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 25/06/2024 Date of issue: 25/06/2024

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[†] 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 degradation is expected within time frame of kit preparation, sample collection and analysis.

[#] Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

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Sample ID: ADAS-5331 Condition on Receipt: Low Sediment Volume: Passed
Client Identifier: JF08 Description: pond water samples in preservative
Date of Receipt: 18/06/2024 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	21/06/2024
Degradation Control [§]	Within Limits	Real Time PCR	21/06/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	21/06/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: [REDACTED] Report Issued by: [REDACTED]

Signed: [REDACTED] Signed: [REDACTED]

Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 25/06/2024 Date of issue: 25/06/2024

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** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more 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 degradation is expected within time frame of kit preparation, sample collection and analysis.

[#] Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

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Sample ID: ADAS-5332 Condition on Receipt: White Precipitate Volume: Passed
Client Identifier: JF05 Description: pond water samples in preservative
Date of Receipt: 18/06/2024 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	0 of 2	Real Time PCR	21/06/2024
Degradation Control [§]	Evidence of degradation or residual inhibition	Real Time PCR	21/06/2024
Great Crested Newt*	Indeterminate	Real Time PCR	21/06/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: [REDACTED] Report Issued by: [REDACTED]

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Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 25/06/2024 Date of issue: 25/06/2024

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[†] 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 degradation is expected within time frame of kit preparation, sample collection and analysis.

[#] Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

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Sample ID: ADAS-5333 Condition on Receipt: White Precipitate Volume: Passed
Client Identifier: JF04 Description: pond water samples in preservative
Date of Receipt: 18/06/2024 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	0 of 2	Real Time PCR	21/06/2024
Degradation Control [§]	Evidence of degradation or residual inhibition	Real Time PCR	21/06/2024
Great Crested Newt*	Indeterminate	Real Time PCR	21/06/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: [REDACTED] Report Issued by: [REDACTED]

Signed: [REDACTED] Signed: [REDACTED]

Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 25/06/2024 Date of issue: 25/06/2024

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.

** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more 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 degradation is expected within time frame of kit preparation, sample collection and analysis.

[#] Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

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Sample ID: ADAS-5334 Condition on Receipt: White Precipitate Volume: Passed
Client Identifier: JF03 Description: pond water samples in preservative
Date of Receipt: 18/06/2024 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	0 of 2	Real Time PCR	21/06/2024
Degradation Control [§]	Evidence of degradation or residual inhibition	Real Time PCR	21/06/2024
Great Crested Newt*	Indeterminate	Real Time PCR	21/06/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: [REDACTED] Report Issued by: [REDACTED]

Signed:

[REDACTED]

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[REDACTED]

Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 25/06/2024 Date of issue: 25/06/2024

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[#] Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

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Sample ID: ADAS-5335 Condition on Receipt: Good Volume: Passed
Client Identifier: JF07 Description: pond water samples in preservative
Date of Receipt: 18/06/2024 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	21/06/2024
Degradation Control [§]	Within Limits	Real Time PCR	21/06/2024
Great Crested Newt*	12 of 12 (GCN positive)	Real Time PCR	21/06/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: [REDACTED] Report Issued by: [REDACTED]

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[REDACTED]

Signed:

[REDACTED]

Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 25/06/2024 Date of issue: 25/06/2024

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[#] Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

Appendix 1: Interpretation of results

Sample Condition

Upon sample receipt we score your samples according to quality: good, low sediment, medium sediment, high sediment, white precipitate, and presence of algae.

There are three reasons as to why sediment should be avoided:

1. It is possible for DNA to persist within the sediment for longer than it would if it was floating in the water which could lead to a false positive result i.e. in this case GCN not recently present but present a long time ago
2. In some cases sediment can cause inhibition of the PCR analysis used to detect GCN eDNA within samples which could lead to an indeterminate result.
3. In some cases sediment can interfere with the DNA extraction procedure resulting in poor recovery of the eDNA which in turn can lead to an indeterminate result.

Algae can make the DNA extraction more difficult to perform so if it can be avoided then this is helpful.

Sometimes samples contain a white precipitate which we have found makes the recovery of eDNA very difficult. This precipitate can be present in such high amounts that it interferes with the eDNA extraction process meaning that we cannot recover the degradation control (nor most likely the eDNA itself) at sufficient levels for the control to be within the acceptable limits for the assay, therefore we have to classify these type of samples as indeterminate.

What do my results mean?

A positive result means that great crested newts are present in the water or have been present in the water in the recent past (eDNA degrades over around 7-21 days).

A negative result means that DNA from the great crested newt has not been detected in your sample.

On occasion an inconclusive result will be issued. This occurs where the DNA from the great crested newt has not been detected but the controls have indicated that either: the sample has been degraded and/or the eDNA was not fully extracted (poor recovery); or the PCR inhibited in some way. This may be due to the water chemistry or may be due to the presence of high levels of sediment in samples which can interfere with the DNA extraction process. A re-test could be performed but a fresh sample would need to be obtained. We have successfully performed re-tests on samples which have had high sediment content on the first collection and low sediment content (through improved sample collection) on the re-test. If water chemistry was the cause of the indeterminate then a re-test would most likely also return an inconclusive result.

The results will be recorded as indeterminate if the GCN result is negative and the degradation result is recorded as:

1. evidence of decay - meaning that the degradation control was outside of accepted limits
2. evidence of degradation or residual inhibition - meaning that the degradation control was outside of accepted limits but that this could have been due to inhibitors not being removed sufficiently by the dilution of inhibited samples (according to the technical advice note)

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Sample ID: ADAS-5302 Condition on Receipt: White Precipitate Volume: Passed
Client Identifier: P320-JF9 Description: pond water samples in preservative
Date of Receipt: 26/06/2024 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	0 of 2	Real Time PCR	03/07/2024
Degradation Control [§]	Evidence of degradation or residual inhibition	Real Time PCR	03/07/2024
Great Crested Newt*	Indeterminate	Real Time PCR	03/07/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: [REDACTED] Report Issued by: [REDACTED]

Signed:

[REDACTED]

Signed:

[REDACTED]

Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 03/07/2024 Date of issue: 03/07/2024

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.

** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.*

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[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#] Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

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Sample ID: ADAS-5303 Condition on Receipt: White Precipitate Volume: Passed
Client Identifier: P320-JF10 Description: pond water samples in preservative
Date of Receipt: 26/06/2024 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	0 of 2	Real Time PCR	03/07/2024
Degradation Control [§]	Evidence of degradation or residual inhibition	Real Time PCR	03/07/2024
Great Crested Newt*	Indeterminate	Real Time PCR	03/07/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

Signed:

[REDACTED]

Signed:

[REDACTED]

Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 03/07/2024 Date of issue: 03/07/2024

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[#] Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

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Sample ID: ADAS-5304 Condition on Receipt: Good Volume: Passed
Client Identifier: P320-JF11 Description: pond water samples in preservative
Date of Receipt: 26/06/2024 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	03/07/2024
Degradation Control [§]	Within Limits	Real Time PCR	03/07/2024
Great Crested Newt*	12 of 12 (GCN positive)	Real Time PCR	03/07/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: [REDACTED] Report Issued by: [REDACTED]

Signed:

[REDACTED]

Signed:

[REDACTED]

Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 03/07/2024 Date of issue: 03/07/2024

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.

** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more 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 degradation is expected within time frame of kit preparation, sample collection and analysis.

[#] Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

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Sample ID: ADAS-5305 Condition on Receipt: White Precipitate Volume: Passed
Client Identifier: P320-JF14 Description: pond water samples in preservative
Date of Receipt: 26/06/2024 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	0 of 2	Real Time PCR	02/07/2024
Degradation Control [§]	Evidence of degradation or residual inhibition	Real Time PCR	02/07/2024
Great Crested Newt*	Indeterminate	Real Time PCR	02/07/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: [REDACTED] Report Issued by: [REDACTED]

Signed:

[REDACTED]

Signed:

[REDACTED]

Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 03/07/2024 Date of issue: 03/07/2024

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[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#] Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

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Sample ID: ADAS-5312 Condition on Receipt: White Precipitate Volume: Passed
Client Identifier: P320-JF13 Description: pond water samples in preservative
Date of Receipt: 26/06/2024 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	03/07/2024
Degradation Control [§]	Within Limits	Real Time PCR	03/07/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	03/07/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: [REDACTED] Report Issued by: [REDACTED]

Signed: [REDACTED] Signed: [REDACTED]

Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 03/07/2024 Date of issue: 03/07/2024

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.

** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more 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 degradation is expected within time frame of kit preparation, sample collection and analysis.

[#] Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

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Sample ID: ADAS-5314 Condition on Receipt: Good Volume: Passed
Client Identifier: P320-JF12 Description: pond water samples in preservative
Date of Receipt: 26/06/2024 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	03/07/2024
Degradation Control [§]	Within Limits	Real Time PCR	03/07/2024
Great Crested Newt*	1 of 12 (GCN positive)	Real Time PCR	03/07/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: [REDACTED] Report Issued by: [REDACTED]

Signed: [REDACTED] Signed: [REDACTED]

Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 03/07/2024 Date of issue: 03/07/2024

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.

** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more 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 degradation is expected within time frame of kit preparation, sample collection and analysis.

[#] Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

Appendix 1: Interpretation of results

Sample Condition

Upon sample receipt we score your samples according to quality: good, low sediment, medium sediment, high sediment, white precipitate, and presence of algae.

There are three reasons as to why sediment should be avoided:

1. It is possible for DNA to persist within the sediment for longer than it would if it was floating in the water which could lead to a false positive result i.e. in this case GCN not recently present but present a long time ago
2. In some cases sediment can cause inhibition of the PCR analysis used to detect GCN eDNA within samples which could lead to an indeterminate result.
3. In some cases sediment can interfere with the DNA extraction procedure resulting in poor recovery of the eDNA which in turn can lead to an indeterminate result.

Algae can make the DNA extraction more difficult to perform so if it can be avoided then this is helpful.

Sometimes samples contain a white precipitate which we have found makes the recovery of eDNA very difficult. This precipitate can be present in such high amounts that it interferes with the eDNA extraction process meaning that we cannot recover the degradation control (nor most likely the eDNA itself) at sufficient levels for the control to be within the acceptable limits for the assay, therefore we have to classify these type of samples as indeterminate.

What do my results mean?

A positive result means that great crested newts are present in the water or have been present in the water in the recent past (eDNA degrades over around 7-21 days).

A negative result means that DNA from the great crested newt has not been detected in your sample.

On occasion an inconclusive result will be issued. This occurs where the DNA from the great crested newt has not been detected but the controls have indicated that either: the sample has been degraded and/or the eDNA was not fully extracted (poor recovery); or the PCR inhibited in some way. This may be due to the water chemistry or may be due to the presence of high levels of sediment in samples which can interfere with the DNA extraction process. A re-test could be performed but a fresh sample would need to be obtained. We have successfully performed re-tests on samples which have had high sediment content on the first collection and low sediment content (through improved sample collection) on the re-test. If water chemistry was the cause of the indeterminate then a re-test would most likely also return an inconclusive result.

The results will be recorded as indeterminate if the GCN result is negative and the degradation result is recorded as:

1. evidence of decay - meaning that the degradation control was outside of accepted limits
2. evidence of degradation or residual inhibition - meaning that the degradation control was outside of accepted limits but that this could have been due to inhibitors not being removed sufficiently by the dilution of inhibited samples (according to the technical advice note)

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Sample ID: ADAS-5308 Condition on Receipt: Good Volume: Passed
Client Identifier: 21 (Parcel D), P320 Description: pond water samples in preservative
Date of Receipt: 04/07/2024 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	17/07/2024
Degradation Control [§]	Within Limits	Real Time PCR	17/07/2024
Great Crested Newt*	12 of 12 (GCN positive)	Real Time PCR	17/07/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: [REDACTED] Report Issued by: [REDACTED]

Signed: [REDACTED] Signed: [REDACTED]

Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 18/07/2024 Date of issue: 18/07/2024

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.

** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more 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 degradation is expected within time frame of kit preparation, sample collection and analysis.

[#] Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

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Sample ID: ADAS-5310 Condition on Receipt: Good Volume: Passed
Client Identifier: 11 (Parcel A), P320 Description: pond water samples in preservative
Date of Receipt: 04/07/2024 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	17/07/2024
Degradation Control [§]	Within Limits	Real Time PCR	17/07/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	17/07/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: [REDACTED] Report Issued by: [REDACTED]

Signed: [REDACTED] Signed: [REDACTED]

Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 18/07/2024 Date of issue: 18/07/2024

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.

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Sample ID: ADAS-5311 Condition on Receipt: Low Sediment Volume: Passed
Client Identifier: 23 (Parcel B), P320 Description: pond water samples in preservative
Date of Receipt: 04/07/2024 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	18/07/2024
Degradation Control [§]	Within Limits	Real Time PCR	18/07/2024
Great Crested Newt*	1 of 12 (GCN positive)	Real Time PCR	18/07/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: [REDACTED] Report Issued by: [REDACTED]

Signed: [REDACTED] Signed: [REDACTED]

Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 18/07/2024 Date of issue: 18/07/2024

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Sample ID: ADAS-5313 Condition on Receipt: Low Sediment Volume: Passed
Client Identifier: 5 (Parcel A), P320 Description: pond water samples in preservative
Date of Receipt: 04/07/2024 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	17/07/2024
Degradation Control [§]	Within Limits	Real Time PCR	17/07/2024
Great Crested Newt*	12 of 12 (GCN positive)	Real Time PCR	17/07/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: [REDACTED] Report Issued by: [REDACTED]

Signed: [REDACTED] Signed: [REDACTED]

Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 18/07/2024 Date of issue: 18/07/2024

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ANNEX 4

SURVEY DATES

Table D-1 - Survey Dates

Water Body Ref.	Date of eDNA Survey	Date of HSI	Comments
1	13.06.2024	13.06.2024	
4	21.06.2024	21.06.2024	
5	20.06.2024	20.06.2024	Limited access, only able to sample from one location
6	20.06.2024	20.06.2024	Limited access, only able to sample from one location
7	20.06.2024	20.06.2024	
8	13.06.2024	13.06.2024	
9	10.06.2024	10.06.2024	
10	20.06.2024	20.06.2024	
11	27.06.2024	27.06.2024	
21	27.06.2024	27.06.2024	
23	27.06.2024	27.06.2024	
25	13.06.2024	13.06.2024	
26	13.06.2024	13.06.2024	Limited access, only able to sample from one location
31	10.06.2024	10.06.2024	
32	20.06.2024	20.06.2024	
33	13.06.2024	13.06.2024	
34	27.06.2024	27.06.2024	
35	N/A	24.02.2025	
36	N/A	25.02.2025	
37	N/A	07.05.2025	
38	N/A	07.05.2025	



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