

GREAT CRESTED NEWT (eDNA) REPORT

LAND NORTH OF A47 NORMANDY WAY AND WEST OF A447 ASHBY ROAD, HINCKLEY

ON BEHALF OF

RICHBOROUGH

REFERENCE: ZEL_477

DATE: June 2023

V3

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Report Data	
Report Title	Great Crested Newt (eDNA) Report
Report Reference	ZEL_477
Client	Richborough
Site	Land north of A47 Normandy Way and west of A447 Ashby Road, Hinckley
Surveyor	C. Needham MSc MCIEEM
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Reviewer	E. Seaton BSc (Hons) MCIEEM

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V1	Great Crested Newt (eDNA) Report	C. Needham MSc	17 November 2023
V2	Great Crested Newt (eDNA) Report	C. Needham MSc	22 January 2024
V3	Great Crested Newt (eDNA) Report	C. Needham MSc	01 March 2024

Disclosure:

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This report should not be copied or relied upon by any third party without the express prior written agreement of Zebra Ecology Ltd and the commissioning client/s.

The evidence gathered, and the opinions provided, have been prepared in accordance with the Chartered Institute of Ecology and Environmental Management (CIEEM) Code of Professional Conduct.

Where any appraisal is based upon information provided by third parties, it is assumed that this information is relevant, correct and complete; there has been no independent verification of information obtained from third parties unless otherwise stated. Where field investigations have been carried out these have been appropriate to the agreed scope of works and carried out to a level of detail required to achieve the stated objectives.



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EXECUTIVE SUMMARY

- Zebra Ecology Ltd was commissioned by Mr & Mrs Adcock to undertake a great crested newt (eDNA) presence/absence survey of the ponds (P1, P3, P4 and P6) located c.350m west, c.401m north, c.206m north and c.182m east of the site respectively (centred on Ordnance Survey grid reference SP 42751 95935).
- Access was requested to all off-site ponds within 500m of the application boundary. Access was granted to ponds P1, P3, P4 and P6. P1, P3 and P4 were subject to survey due to suitability. P6 was dry.
- The survey was completed on 28 June 2023 by an appropriately experienced great crested newt surveyor. Analysis was then undertaken by ADAS.
- The results of the great crested newt eDNA analysis has returned a result of 'eDNA absent' for the ponds (P1, P3 and P6). Therefore, there is no evidence to suggest that these ponds support great crested newts.
- No further work for great crested newts is recommended for this site. However, a Reasonable Avoidance Measure (RAMs) method Statement will need to be produced for the construction period of the development proposals to ensure that there is no breach of wildlife legislation (see Section 4).
- The Preliminary Ecological Appraisal (Zebra Ecology, 2023) should be referred to for other recommendations.

1.0 INTRODUCTION

Site Location and Summary

- 1.1 Zebra Ecology Ltd were commissioned by Richborough to undertake a presence/absence (eDNA) survey of the ponds within 500m of the land west of Ashby Road and north of Normandy Way (centred on Ordnance Survey grid reference SP 42751 95935).
- 1.2 The site is located immediately north of Hinckley and is c.16ha in area and comprises seven separate fields subject to cattle grazing with two centrally located farmhouse complexes and associated outbuildings (storage barns etc.). The site is further characterised by a mixture of native species-rich and species-poor hedgerows, with established scattered trees present across the site (confined within hedgerows).
- 1.3 Planning consent is being sought from Hinckley and Bosworth Council for '*Outline planning application with all matters reserved except for access, for the erection of up to 415 dwellings, associated open space, drainage, landscaping and infrastructure.*'
- 1.4 A pond is located along the northern boundary of the site on the Ordnance Survey map. However, no pond or remnants of a pond was observed in this location during the survey. A further six ponds (P1-P6) were identified within 500m of the application site.
 - Pond 1 – located c.350m west of the site.
 - Pond 2 – located c.288m north-west of the site.
 - Pond 3 – located c.401m north of the site.
 - Pond 4 – located c.206m north of the site.
 - Pond 5 – located c.436m east of the site.
 - Pond 6 – located c.182m east of the site.

1.5 The application site boundary and surrounding ponds are shown in Figure 1:

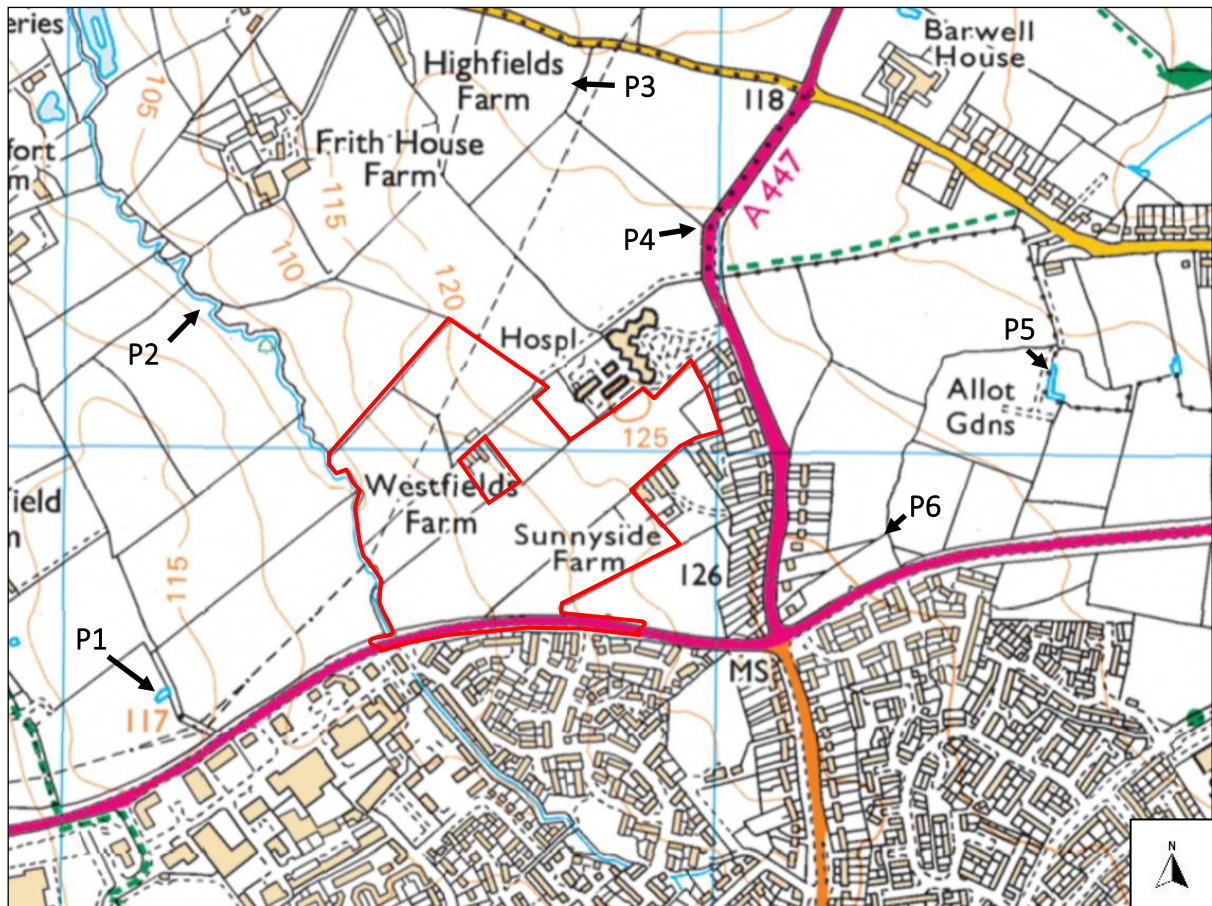


Figure 1: Ponds within 500m of the Application Site

2.0 METHODOLOGY

Desk Study

- 2.1 Existing ecological and nature conservation data relevant to the site was collated from various sources including the Multi Agency Geographic Information for the Countryside (MAGIC) online database (<http://magic.defra.gov.uk>) and Leicestershire and Rutland Environmental Records Centre (LRERC).
- 2.2 The Preliminary Ecological Appraisal (Zebra Ecology, 2023) included a biological records data search from Leicestershire and Rutland Environmental Records Centre (LRERC) to identify any designated sites and protected / notable species within a 2km radius of SP 42751 95935. It should be noted that the absence of biological records for an area does not imply that taxa are not present.

Field Protocol

- 2.3 A site visit was undertaken to sample the ponds on 28 June 2023. The sampling was undertaken by C. Needham (Natural England great crested newt licence ref: 2015-19015-CLS-CLS). The field sampling collection protocol was followed as per the '*Technical advice note for field and laboratory sampling of great crested newt (Triturus cristatus) environmental DNA*' endorsed by Natural England (2014).
- 2.4 Locations of where twenty 30mL samples could be sampled and taken from the ponds were identified. These were spaced as evenly as possible with areas targeted that may be used as egg-laying substrate and open water areas which newts may be utilising for displaying.
- 2.5 The samples were thoroughly mixed and six 15mL samples pipetted into six sterile tubes containing 35mL of ethanol to preserve the eDNA. The preserved samples were then immediately returned at ambient temperature for analysis or stored and refrigerated at 2-4°C until ready for analysis.

Laboratory Protocol

- 2.6 The kits were returned to ADAS (approved by Natural England in 2014) for analysis. Great crested newt (*Triturus cristatus*) DNA was amplified using the primers and probes listed in Appendix 2 of Technical Advice Note (DEFRA, 2014). DNA was then extracted using the DNA Blood and Tissue kit (Qiagen®) following the manufacturer's instructions. Polymerase chain reaction (PCR) was undertaken to amplify the DNA (if present).

Limitations

- 2.7 No significant limitations were encountered to negatively influence results.
- 2.8 The risk of aquatic animals (e.g. herons, water voles) transferring great crested eDNA between sites cannot be ruled out. Further research is currently ongoing to determine whether this is a significant risk; however, it is currently anticipated to be small.

3.0 RESULTS AND CONCLUSION

Desk Study

- 3.1 Fourteen records of great crested newts have been returned by the third-party data search. The closest record has been returned c.0.65km to the south-east of the site. All other records are located over 1km from the site.

eDNA Survey

- 3.2 The results of great crested newt eDNA analysis has returned a result of 'eDNA absent' for all ponds surveyed (P1, P3 and P4). P6 was dry. Therefore, there is no evidence to suggest that any of the surveyed ponds support great crested newts. A full copy of the results can be found in Appendix B.

4.0 RECOMMENDATIONS

- 4.1 Whilst no evidence of great crested newts was recorded during the survey, access was denied to Ponds 2 and 5, and therefore the presence of newts within these ponds cannot be ruled out.
- 4.2 A Natural England Rapid Risk Assessment (assuming the presence of great crested newts within the above ponds) has been undertaken and returned the following result 'AMBER: OFFENCE LIKELY'.

Table 2: Natural England Rapid Risk Assessment for great crested newts taken from the development licence method statement.

Component	Likely effect (select one for each component; select the most harmful option if more than one is likely; lists are in order of harm, top to bottom)	Notional offence probability score
Great crested newt breeding pond(s)	No effect	0
Land within 100m of any breeding pond(s)	No effect	0
Land 100-250m from any breeding pond(s)	No effect	0
Land >250m from any breeding pond(s)	5 - 10 ha lost or damaged	0.3
Individual great crested newts	No effect	0
Maximum:		0.3
Rapid risk assessment result:	AMBER: OFFENCE LIKELY	

- 4.3 The Natural England risk assessment tool has been developed as a general guide only and does not take into account site-specific factors such as terrestrial habitat quality. The vast majority of the site comprises cattle-grazed fields, which provide sub-optimal habitat for great crested newts. Furthermore, these ponds are located over 250m from the site. Research has shown that the majority of great crested newts stay within 50m of a pond, with very few exceeding 100m (Cresswell and Whitworth, 2004).
- 4.4 Given the above, providing the works are carried out under Reasonable Avoidance Measures for great crested newts, the development proposals are considered unlikely to have a negative impact on great crested newts and will ensure full compliance with the strict legislation afforded to this European Protected Species (EPS). Therefore, no further survey work is required, in line with published planning advice (see ODPM Circular 06/2005 Biodiversity and Geological Conservation – Statutory Obligations and Their Impact Within the Planning System, p.33):
- “...bearing in mind the delay and cost that may be involved, developers should not be required to undertake surveys for protected species unless there is a reasonable likelihood of the species being present and affected by the development”.
- 4.5 It is anticipated that the following will form key components of the strategy:

- Protection and enhancement of retained boundary hedgerows.
- Toolbox talk to be provided to contractors by a suitably qualified ecologist at the onset of works. All contractors to be made aware of the procedure to follow should a great crested newt be discovered (or suspected) on-site.
- Sensitive timing and duration of works. Hedgerow removal to be undertaken between March to June when great crested newts are more likely to be in ponds. Duration of ground works to be kept as short as possible.
- Hand and destructive searches to carried out by a licensed ecologist prior to groundworks.
- Implementation of additional precautionary working measures in association with machinery, trenches, work debris etc.
- **If it is suspected that a great crested newt has been found, work must be suspended and the advice of a suitably licensed ecologist sought.**

4.6 The Preliminary Ecological Appraisal (Zebra Ecology, 2023) should be referred to for other recommendations.

5.0 REFERENCES

- 4.8 Biggs, J., Ewald, N., Valentini, A., Gaboriaud, C., Griffiths, R.A., Foster, J., Wilkinson, J., Arnett, A., Williams, P. and 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, UK.
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- 4.10 English Nature (2001). *Great Crested Newt Mitigation Guidelines*. English Nature, Peterborough, UK.
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- 4.12 Gent, A. and Gibson, S. (1998). *Herpetofauna Workers Manual*. Joint Nature Conservation Committee, Peterborough, UK.
- 4.13 Jehle, R. and Arntzen, J.W. (2000). *Post-breeding migrations of newts (Triturus cristatus and T. marmoratus) with contrasting ecological requirements*. Journal of Zoology. 2000;251(3):297–306. doi: 10.1111/j.1469-7998.2000.tb01080.x.
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- 4.15 Langton, T., Beckett, C. and Foster, J. (2001). *Great Crested Newt Conservation Handbook*. Froglife, Suffolk, UK.
- 4.16 Malmgren, J.C. (2002). How does a newt find its way from a pond? Migration patterns after breeding and metamorphosis in great crested newts. *Herpetological Journal*. 2002;12:29–35.
- 4.17 Matos, C., Sillero, N., & Argaña, E. (2012). *Spatial analysis of amphibian road mortality levels in northern Portugal country roads*. *Amphibia-Reptilia*. 2012;33:469–483. doi: 10.1163/15685381-00002850
- 4.18 Natural England (2020). Template for Method Statement to support application for licence under Regulation 55(2)(e) of The Conservation of Habitats and Species Regulations 2017 (as amended) in respect of great crested newts *Triturus cristatus*. Form WML-A14-2 (Version April 2020). Natural England, Bristol, UK. <https://www.gov.uk/government/publications/great-crested-newts-apply-for-a-mitigation-licence> [Accessed August 2023].
- 4.19 Natural England (2022). Guidance – Great Crested Newts: Surveys and Mitigation for Development Projects. Standing advice for local planning authorities to assess impacts of development on great crested newts. <https://www.gov.uk/guidance/great-crested-newts-surveys-and-mitigation-for-development-projects> [Accessed August 2023]

- 4.20 **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.
- 4.21 **People's Trust for Endangered Species (2008).** *Hedgerows: a guide to wildlife and management*. People's Trust for Endangered Species, London, UK.
- 4.22 **Zebra Ecology Ltd (2023).** Preliminary Ecological Appraisal – Land west of Ashby Road (A447) and north of Normandy Way (A47). Zebra Ecology, Worcester, UK (unpublished)

APPENDIX A

Photographs



Photograph 1: view of Pond 1. Photo facing north-east.



Photograph 2: view of Pond 3. Photo facing south.



Photograph 3: view of Pond 4. Photo facing north-east.



Photograph 4: view of Pond 6 (dry). Photo facing north-west.

APPENDIX B

eDNA Results

Client: Andy Elliott,
Zebra Ecology



ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

Tel: 01159 229249
Email: Helen.Rees@adas.co.uk

www.adas.uk

Sample ID: ADAS-2634 Condition on Receipt: Medium Sediment Volume: Passed
Client Identifier: P3 Description: pond water samples in preservative
Date of Receipt: 06/07/2023 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	17/07/2023
Degradation Control [§]	Within Limits	Real Time PCR	17/07/2023
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	17/07/2023
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:		Signed:	
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	18/07/2023	Date of issue:	18/07/2023

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: Andy Elliott,
Zebra Ecology



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Tel: 01159 229249
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Sample ID: ADAS-2635 Condition on Receipt: Low Sediment Volume: Passed
Client Identifier: P1 Description: pond water samples in preservative
Date of Receipt: 06/07/2023 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	17/07/2023
Degradation Control [§]	Within Limits	Real Time PCR	17/07/2023
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	17/07/2023
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:		Signed:	
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	18/07/2023	Date of issue:	18/07/2023

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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.

Client: Andy Elliott,
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Sample ID: ADAS-2636 Condition on Receipt: Low Sediment Volume: Passed
Client Identifier: P4 Description: pond water samples in preservative
Date of Receipt: 06/07/2023 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	14/07/2023
Degradation Control [§]	Within Limits	Real Time PCR	14/07/2023
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	14/07/2023
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
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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.

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)

APPENDIX C

Legislation

The great crested newt is a 'European Protected Species' and is listed on both Annex II and IV of the EC Habitats Directive. The Directive is transposed into UK law through the Conservation of Habitats and Species Regulations 2017. They are also protected by the Wildlife and Countryside Act 1981, as amended by the Countryside and Rights of Way Act 2000. These pieces of legislation combine to give substantial protection to great crested newts and their breeding ponds and terrestrial habitat, making it an offence to:

- Deliberately capture, injure or kill a great crested newt;
- Intentionally or recklessly disturb a great crested newt in a structure or place that they use for shelter or protection or deliberately disturb a group of a great crested newts;
- Damage or destroy a great crested newt resting place/shelter (even if they are not occupying it at the time);
- Possess or advertise/sell/exchange a great crested newt (dead or alive) or any part of a great crested newt (including eggs and all life-stages);
- Intentionally or recklessly obstruct access to a great crested newt resting place/shelter.

APPENDIX D

Qualifications and Experience

Zebra Ecology Ltd is Registered Practice of the Chartered Institute of Ecology and Environmental Management (CIEEM). A comprehensive range of ecological services are offered including Preliminary Ecological Appraisal (PEA), Ecological Impact Assessment (EclA), Habitat Regulations Assessment (HRA), Biodiversity Impact Assessment (BIA) and European Protected Species (EPS) Surveys / Licensing.

The practice works closely with clients to achieve their aspirations alongside securing the best outcomes for the environment. With wildlife legislation and policy as its basis; commercial awareness, pragmatism and defensible advice is combined to form Zebra Ecology's approach.

As well as offering a wide range of ecological services, Zebra Ecology forms part of Zebra Group offering an in-house collaborative approach in conjunction with Zebra Architects, Zebra Landscape Architects, Zebra Trees and Zebra Land and Development.

Cassie Needham BSc (Hons) MSc MCIEEM

Cassie holds a BSc (Hons) degree in Geography with Ecology from the University of Sussex and an MSc in Conservation from University College London. Her ecological experience includes Preliminary Ecological Appraisals, Ecological Impact Assessments (EclA) and Habitat Regulations Assessments (HRA) as well as surveys for protected species; great crested newts, reptiles, white-clawed crayfish, bats, hazel dormice and water voles. She also holds Natural England survey licences for bats (Class 2), great crested newts, hazel dormice and white-clawed crayfish and is a Certificated Surveyor in Japanese Knotweed. Cassie has held Natural England Mitigation (development) licences for great crested newts and numerous Natural England licences to close or disturb badger setts. Cassie is a Full member of the Chartered Institute of Ecology and Environmental Management.

Emma Seaton BSc (Hons) MCIEEM

Emma holds a BSc (Hons) degree in Biology from the University of Sheffield and has since gained a postgraduate certificate in Ecological Consultancy. Her ecological experience includes Preliminary Ecological Appraisals, Ecological Impact Assessments (EclA), surveying for notable / European Protected Species, mitigation / licensing advice and providing Continued Professional Development (CPD) sessions for developers on Biodiversity Net Gain. She has held Natural England survey licences for bats (Class 2), great crested newts and white-clawed crayfish since 2015. She is also a Registered Consultant under the Bat Mitigation Class Licence (BMCL) licence. Emma is a Full member of the Chartered Institute of Ecology and Environmental Management.