

**M25 junction 28 improvement scheme
TR010029
6.3 Environmental Statement
Appendix 7.9: Great crested newt survey**

APFP Regulation 5(2)(a)
Planning Act 2008

Infrastructure Planning (Applications: Prescribed Forms and Procedure) Regulations 2009



Infrastructure Planning

Planning Act 2008

The Infrastructure Planning (Applications: Prescribed Forms and Procedure) Regulations 2009

M25 junction 28 scheme Development Consent Order 202[x]

6.3 ENVIRONMENTAL STATEMENT 7.9: GREAT CRESTED NEWT SURVEY

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Appendix 7.9 Great crested newt survey

7. Great crested newt survey

7.1 Background and survey objectives

- 7.1.1 Great crested newts are a European Protected Species subject to full protection under the Conservation of Habitats and Species Regulations 2017 and the Wildlife and Countryside Act 1981 (as amended). A summary of the relevant legislation can be found in Appendix 7.1.
- 7.1.2 The great crested newt survey was carried out in 2017, 2018 and 2019. The aim of the survey was to:
- determine the habitat suitability and presence/likely absence of great crested newts within waterbodies up to 250 m of the M25 junction 28 Improvement Scheme (the Scheme).
 - determine the distribution and population size-class of any breeding populations of great crested newts.

7.2 Methodology

Desk study

- 7.2.1 The following organisations were contacted in 2017 and 2019, for any desk study records of great crested newt within the last 10 years and within 2 km of the DCO boundary:
- Greenspace Information for Greater London (GIGL)
 - Essex Records Centre (ERC)
 - Essex Field Club (EFC)

Scoping

- 7.2.2 Great crested newt typically use suitable terrestrial habitat up to 500 m from a breeding pond. However, there is a notable decrease in great crested newt abundance beyond a distance of 250 m from a breeding pond¹. Therefore, taking into account the localised nature and potential impacts of the Scheme, and the presence of the M25 and A12 corridors which restrict the dispersal of great crested newts, a distance of 250 m from the Scheme was used in the initial scoping assessment.
- 7.2.3 Ordnance survey maps were used to identify all ponds within 250 m of the Scheme.
- 7.2.4 Construction activity associated with the Scheme is focused on land northwest of junction 28, with the exception of temporary works associated with the gas main diversion south of the A12. All other activities are restricted to the existing carriageway or the replacement of signs on existing gantries. Therefore, ponds were scoped out of further survey where no potential impacts on these ponds were identified due to the distance between the pond and proposed construction footprint.

¹ Cresswell, W. & Whitworth, R. (2004) English Nature Research Reports Number 576: An assessment of the efficiency of capture techniques and the value of different habitats for the great crested newt *Triturus cristatus*. English Nature, Peterborough.

Field survey

- 7.2.5 All ponds scoped in for further survey were subject to a Habitat Suitability Index (HSI) assessment and presence/likely absence surveys using water sampling (to check for environmental DNA (eDNA)) or conventional survey techniques using bottle-traps, torchlight surveys and egg searches. These surveys were carried out unless ponds were scoped out during the first field survey visit as either not present or unsuitable for breeding great crested newt. Where great crested newt were confirmed as present, six conventional survey visits were carried out to determine the population class-size present in each pond.
- 7.2.6 All great crested newt surveys were led by surveyors with relevant species survey licence.

Habitat Suitability Index assessment

- 7.2.7 Ponds were assessed for their potential to support great crested newt using the HSI assessment². This technique provides a standardised assessment of the potential of a waterbody to support great crested newt and is recognised by the licencing authorities. The HSI is calculated using ten habitat variables ('suitability indices') which are known to affect the survival of great crested newts. These are:
- Geographical location (i.e. with respect to the range of great crested newt)
 - Pond area
 - Permanence (how regularly does the pond dry out)
 - Water quality
 - Shade
 - Water fowl (population density)
 - Fish (stocking density)
 - Pond count (number of ponds within 1 km)
 - Terrestrial habitat (quality of terrestrial habitat local to the pond) and
 - Macrophytes (% cover of aquatic plant cover during the newt breeding season March-May)
- 7.2.8 Each habitat variable is assessed by experienced surveyors in the field. The ten suitability indices are combined to derive the final HSI score for the waterbody. The HSI, expressed as a value between 0.01 and 1.0, is then categorised as shown follows:
- <0.5 = poor
 - 0.5-0.59 = below average
 - 0.6-0.69 = average
 - 0.7-0.79 = good or
 - >0.8 = excellent

² Oldman, R. S., Keeble, J., Swan, M. J. S., and Jeffcote, M. (2000). Evaluating the Suitability of Habitat for the Great crested newt (*Triturus cristatus*) *Herpetological Journal* 10 (4), 143-155.

Presence/likely absence surveys - environmental DNA (eDNA) survey techniques

- 7.2.9 Presence/likely absence surveys followed the methodology provided by Department for Environment, Food & Rural Affairs (Defra) in Appendix 5 of the report for Defra project WC10673: *Analytical and methodological development for improved surveillance of the Great Crested Newt*³. The survey involved taking water samples at each suitable pond to send for laboratory analysis to test for the presence of great crested newt eDNA. This technique provides presence/likely absence only and is currently unable to provide an estimate of population size-class.
- 7.2.10 All field surveys, survey sample storage and analysis were conducted in accordance with the Defra protocol as provided with the eDNA test equipment which has been appended to this report (Annex 2).
- 7.2.11 Sampling eDNA kits were received on 5 June 2018, the surveys were completed by a licenced ecologist (reference 2017-28594-CLS-CLS) between 8 and 12 June 2018 and the kits were sent to the laboratory for analysis on 12 June 2018.
- 7.2.12 All laboratory work was conducted in accordance Defra methodology⁴, with samples analysed using a Real Time qPCR.

Presence/likely absence surveys and population size-class surveys – conventional survey techniques

- 7.2.13 All field surveys were undertaken in accordance with good practice great crested newt survey guidelines⁵.
- 7.2.14 A minimum of four surveys visits were undertaken between mid-March and mid-June with at least two of the surveys undertaken between mid-April and mid-May. In waterbodies where great crested newt presence was confirmed, a further two surveys were undertaken between mid-April and mid-May in order to obtain a population estimate. All surveys were conducted in suitable weather conditions.
- 7.2.15 The following three standard survey techniques were used:
- Egg searching: examining submerged vegetation and debris around the pond perimeter for folds where great crested newt eggs may be present.
 - Torching: involved walking around the pond perimeter after dusk and scanning the water for adult great crested newt within the marginal vegetation or in open potential display areas using a high-powered torch.
 - Bottle-trapping: involved setting specifically made traps around the pond perimeter of each pond in the evening and revisiting the ponds the following morning to check for any great crested newts.

³ Biggs J, Ewald N, Valentini A, Gaboriaud C, Griffiths RA, Foster J, Wilkinson J, Arnett A, Williams P and Dunn F (2014). Analytical and methodological development for improved surveillance of the Great Crested Newt. Defra Project WC1067. Freshwater Habitats Trust: Oxford

⁴ Biggs J, Ewald N, Valentini A, Gaboriaud C, Griffiths RA, Foster J, Wilkinson J, Arnett A, Williams P and Dunn F (2014). Analytical and methodological development for improved surveillance of the Great Crested Newt. Defra Project WC1067. Freshwater Habitats Trust: Oxford

⁵ English Nature (2001). Great Crested Newt Mitigation Guidelines, English Nature, Peterborough;

- 7.2.16 Where six surveys were carried out, the maximum adult count per pond is used to assess the population size-class as follows⁶:
- 'Small' for maximum counts up to 10
 - 'Medium' for maximum counts between 11 and 100
 - 'Large' for maximum counts over 100

Survey limitations

- 7.2.17 During the site visit in 2017 pond P3 was dry and therefore HSI and eDNA analysis surveys were not undertaken. Vegetation, largely nettle within the pond, indicated that it remained dry throughout the year. Pond P3 was revisited in 2018 and found to hold water and conventional survey work commenced (egg search, bottle trapping and torching). Great crested newt were recorded during the first visit, and therefore no water sample was taken for eDNA analysis. Pond P3 was only successfully surveyed on four visits, as it had dried up by survey visit five. The population size-class category has been determined based on the four successful visits.

7.3 Results

Desk study

- 7.3.1 In total, the desk study search returned 125 recent records of great crested newt within 2 km of the Scheme. GiGL provided 85 records of great crested newt, ERC provided 32 records of great crested newt and EFC eight records of great crested newt. No records of great crested newt were provided for land within the Scheme.
- 7.3.2 A search of MAGIC found records of two European Protected Species Mitigation (EPSM) licences for great crested newts were identified 1 km to the west of the Scheme. These licences ran from 2012 to 2013 (EPSM2011-2843) and 2012 to 2015 (EPSM2012-4454).
- 7.3.3 Data indicates that a meta-population of great crested newts is present northwest and west of the Scheme, in ponds around Dagnam Park, The Manor Local Nature Reserve (LNR) and Maylands Golf Club. The data provided was collected from survey work in 2010 and included peak counts of great crested newt per pond which ranged from 1 to 10. The location of these desk study records is provided on Figure 1, Annex 1. On-line resources indicate the meta-population of great crested newt present northwest of the Scheme is the largest known population of great crested newt in London Borough of Havering, and largest in Greater London⁷.

Scoping

- 7.3.4 A total of 16 ponds were identified within 250 m of the DCO boundary. These ponds are listed in Table 3.1. Eight of these ponds were initially scoped in for further survey. Of these, two were found to no longer be present and one was

⁶ Natural England guidance provided at <https://www.gov.uk/guidance/great-crested-newts-surveys-and-mitigation-for-developmentprojects> [accessed December 2019]

⁷ London Borough of Havering Biodiversity Supplementary Planning Document: <https://www3.havering.gov.uk/Documents/Planning/LDF/Protecting-Enhancing-Boroughs-Biodiversity-SPD.pdf> [accessed November 2019]

completely unsuitable for breeding great crested newts. The remaining five ponds were taken forward for detailed field survey.

Table 7.1: Summary of ponds within 250 m of the Scheme scoped in for further assessment

Pond number	Distance and direction from the Scheme	Scoping result
P1	Within	Potential impacts identified, further survey required
P2	Within	Potential impacts identified, further survey required
P3	20 m southwest (south of the A12)	Potential impacts identified, further survey required
P4	110 m northwest	Potential impacts identified, further survey required
P5	190 m northwest	Potential impacts identified, further survey required
P6	165 m west	Scoped out: unsuitable. Formal duck pond, heavily used by wildfowl including geese. Not within 250 m of Scheme during 2017 scoping surveys. Survey in 2019 (survey date 19/06/2019) found pond to be unsuitable for breeding great crested newt with HSI recorded as <0.5 (see Annex 3)
P7	5 m (within loop)	Scoped out: pond no longer exists (survey 08/06/2017)
P8	Within	Scoped out: pond no longer exists (survey 08/06/2017)
P9	30 m west	Scoped out: no potential impacts to pond or terrestrial habitat identified
P10	125 m northeast	Scoped out: no potential impacts to pond or terrestrial habitat identified
P11	245 m northeast	Scoped out: no potential impacts to pond or terrestrial habitat identified
P12	145 m northeast	Scoped out: no potential impacts to pond or terrestrial habitat identified
P13	205 m northeast	Scoped out: no potential impacts to pond or terrestrial habitat identified
P14	250 m northeast	Scoped out: no potential impacts to pond or terrestrial habitat identified
P15	110 m east	Scoped out: no potential impacts to pond or terrestrial habitat identified
P16	195 m west	Scoped out: no potential impacts to pond or terrestrial habitat identified

Field survey

7.3.5 A summary of the field survey results is provided in Table 3.2.

7.3.6 Water sampling from pond P1 returned a negative result. Taking the negative result and 'below average' HSI and condition of pond into account, great crested newts are assumed to be absent from P1.

- 7.3.7 Great crested newts are confirmed as present in one pond within the DCO boundary (P2) and three ponds within 250 m of the DCO boundary (P3, P4 and P5).
- 7.3.8 A small population of great crested newts is present to the south of the A12, in pond P3. This pond is south of the A12, 20 m from the DCO boundary.
- 7.3.9 Due to the proximity of ponds P2, P4 and P5, it is assumed these ponds form a metapopulation of great crested newt present in the northwest of the Scheme. The peak adult count for the metapopulation (data from the three ponds combined) for any one survey visit was 46, indicating a medium metapopulation. The great crested newts utilising pond P2, P4 and P5 are assumed to be part of the wider population present west and northwest of the Scheme around Dagnam Park and The Manor LNR.
- 7.3.10 The presence of the M25 and A12 corridors forms a barrier to the movement of great crested newts. Although dispersal across these features may be possible via culverts along Weald Brook and Ingrebourne River when conditions are suitable, it is considered likely that there is limited regular dispersal between ponds separated by these roads. Therefore, P2, P4 and P5 likely forms the most south and east extent of aquatic habitat utilised by the wider metapopulation present in this area, with populations which may be present east or south of the Scheme forming separate populations
- 7.3.11 Full details of the HSI, eDNA and population surveys are provided in Annex 3, Annex 5 and Annex 6 respectively.

Table 7.2: Summary of great crested newt survey results

Pond number	Survey result: GCN = great crested newt	Distance from Scheme	HSI category	Peak count of adult GCN (if present)
P1	GCN assumed absent – eDNA negative	Within Scheme	Below average	n/a
P2	GCN present – medium population	Within Scheme	Excellent	22
P3	GCN present – small population	20 m south	Average	2
P4	GCN present – small population	190 m northwest	Good	6
P5	GCN present – medium population	185 m west	Excellent	21

Annex 1: Great crested newt survey plan

Annex 2: eDNA survey protocol



Email: eDNA@adas.co.uk
www.adas.co.uk

eDNA Survey Protocol

Kits should be kept at room temperature in an appropriate solvent store, consistent with Home Office regulations. Use within two weeks of receipt.

Kit contents: 1 sterile Whirl-Pak bag; 2 pairs of sterile gloves; 1 sterile 30 mL sampling ladle; a sample box containing 6 x 50 mL sample tubes two thirds full of preserving fluid (contains alcohol); 1 sterile 10 mL pipette; 1 protocol sheet.

Please **keep all packaging** as you will require this for couriered return of samples (see instructions enclosed with your order)

Don't go in the water.

- Collect your eDNA water sample before you do any other surveys at the pond.
- Take the sample whilst standing on the pond bank.
- Don't tread in the pond water itself either before or during collection of the DNA water sample as there is a considerable risk of contaminating your pond sample by bringing in Great Crested Newt DNA in mud and water from other areas on your boots and equipment.

Walk around the pond, to identify areas where you can take your eDNA samples

Roughly plan where you will collect the 20 water samples from. The aim is to spread the samples out evenly around the pond edge. The samples should be taken from both open water and vegetated areas if present and if possible should avoid water that is less than 10 cm deep. If you cannot access all areas of the pond, spread the samples out as best you can without entering the water. Existing data shows that eDNA can be patchy depending on where the animals have been. Sampling in many areas considerably increases the chance of collecting their eDNA successfully.

NOTE: Before you take each ladle sample, be sure to mix the pond the water column by gently using the ladle

To stir the water from the surface to close to the pond bottom WITHOUT disturbing the mud in the bottom. DNA 'sinks' and so will often be present in larger amounts close to the pond bottom. **It is important not to collect sediment as this may cause inhibition of the PCR analysis which could lead to an inconclusive result** (please see examples of different sediment levels within sampling tubes at <http://www.adas.uk/Service/eDNA-analysis-for-great-crested-newt>).

Sample Collection

- Open your kit and put on a pair of gloves.
- Open the sterile Whirl-Pak bag by tearing off the clear plastic strip along the perforated line, then pull the tabs.

Collect 20 samples of 30 mL of pond water from around the pond (in the areas you have already identified) using the sampling ladle (fill the ladle), and empty each sample into the Whirl-Pak bag.



Email: eDNA@adas.co.uk
www.adas.co.uk

Sample Preservation

1. When you have collected your 20 samples, close the bag securely using the top tabs (fold over several times and bend tabs over) and shake the Whirl-Pak bag for 10 seconds. This mixes any DNA across the whole water sample.
2. Put on a fresh pair of gloves to keep the next stage as uncontaminated as possible.

Label each conical tube with the date, your name (sampler), and the pond name after the sample ID number.

3. Using the clear plastic pipette provided take 15 mL of water from the Whirl-Pak bag, and transfer into one of the six conical tubes containing preserving fluid (i.e. fill tube to the 50 mL mark).

NOTE: Please do not overfill or under fill the tubes.

4. Close the tube and ensure the cap is tight - leaky samples could later contaminate the laboratory with DNA.
5. Shake the tube vigorously for 10 seconds to mix the sample and preservative.
6. Repeat for each of the 6 conical tubes in the kit.
7. Double check that the lids are on tightly if they have leaked during shaking please also wipe the tubes.
8. Empty the remaining water from the whirl-Pak bag back into the pond.
9. Place all used gloves, pipettes, rubbish into the sampling bag and dispose.

If storage of samples is necessary prior to their return please store refrigerated (2-4°C). Samples can be stored in this way for up to 1 month prior to analysis.

Returning the kit - Drop off option

Should you wish to return your items directly to us, they can be dropped off at **Vet School Stores, SVMS, Nottingham University, Sutton Bonington Campus, Loughborough, LE12 5RD**. (please note opening times: 8.00am - 5.00pm Monday-Friday) Please clearly mark your box "FAO Helen Rees ADAS UK Ltd".

Booking your DHL Collection

Samples must be packed in accordance with the instructions provided at the time of delivery. You need to contact the ADAS office via email at eDNACouriering@adas.co.uk so we can arrange your collection.

We require the address of where the parcel will be, your contact details and the date of collection. Please have your parcel ready for collection BEFORE you contact us and give us at least 48 hours' notice, wherever possible we will try to book the requested date between 9am-5pm. Once we have booked your return we will email you the DHL collection documents, these will need to be printed off and attached to your parcel before your driver arrives. Should you have any problems please call the office on 01159 516747.

Annex 3: Habitat Suitability Index data

Table 7.3: HSI assessment result tables

Pond reference:	P1	Survey date:	08/06/2017
Suitability indices	Criteria	Value	Indices score
Geographic location	Location within Great Crested Newt Range A, B, C	A	1
Pond area	Pond surface area (m ²)	25	0.1
Pond permanence	Pond dries annually, sometimes, rarely, never	Rarely	1
Water quality	Good, Moderate, poor, bad	Poor	0.33
Percentage shade of pond margin	Percentage shaded to at least 1m from shore	100	0.2
Number of waterfowl	Absent, minor, major	Absent	1
Occurrence of fish	Absent, possible, minor, major	Absent	1
Waterbody density	Number of waterbodies within 1km (excluding pond surveyed)	1	0.6
Terrestrial habitat	Quality of terrestrial habitat. Good, moderate, poor, none	Moderate	0.67
Macrophyte cover	Percentage of pond surface occupied by macrophyte cover	0	0.3
HSI Score			0.5
Suitability category			Below average

Pond reference:	P2	Survey date:	05/04/2018
Suitability indices	Criteria	Value	Indices score
Geographic location	Location within Great Crested Newt Range A, B, C	A	1
Pond area	Pond surface area (m ²)	100	0.2
Pond permanence	Pond dries annually, sometimes, rarely, never	Rarely	1
Water quality	Good, Moderate, poor, bad	Moderate	0.67
Percentage shade of pond margin	Percentage shaded to at least 1m from shore	5	1
Number of waterfowl	Absent, minor, major	Absent	1
Occurrence of fish	Absent, possible, minor, major	Absent	1
Waterbody density	Number of waterbodies within 1km (excluding pond surveyed)	13+	1
Terrestrial habitat	Quality of terrestrial habitat. Good, moderate, poor, none	Good	1
Macrophyte cover	Percentage of pond surface occupied by macrophyte cover	70	1
HSI Score			0.82
Suitability category			Excellent

Pond reference:	P3	Survey date:	05/04/2018
Suitability indices	Criteria	Value	Indices score
Geographic location	Location within Great Crested Newt Range A, B, C	A	1
Pond area	Pond surface area (m ²)	100	0.2
Pond permanence	Pond dries annually, sometimes, rarely, never	Annually	0.1
Water quality	Good, Moderate, poor, bad	Moderate	0.67
Percentage shade of pond margin	Percentage shaded to at least 1m from shore	40	1
Number of waterfowl	Absent, minor, major	Minor	0.67
Occurrence of fish	Absent, possible, minor, major	Absent	1
Waterbody density	Number of waterbodies within 1km (excluding pond surveyed)	13+	1
Terrestrial habitat	Quality of terrestrial habitat. Good, moderate, poor, none	Moderate	0.67
Macrophyte cover	Percentage of pond surface occupied by macrophyte cover	60	0.9
HSI Score			0.59
Suitability category			Average

Pond reference:	P4	Survey date:	05/04/2018
Suitability indices	Criteria	Value	Indices score
Geographic location	Location within Great Crested Newt Range A, B, C	A	1
Pond area	Pond surface area (m ²)	400	0.8
Pond permanence	Pond dries annually, sometimes, rarely, never	Sometimes	0.5
Water quality	Good, Moderate, poor, bad	Moderate	0.67
Percentage shade of pond margin	Percentage shaded to at least 1m from shore	70	0.8
Number of waterfowl	Absent, minor, major	Minor	0.67
Occurrence of fish	Absent, possible, minor, major	Possible	0.67
Waterbody density	Number of waterbodies within 1km (excluding pond surveyed)	13+	1
Terrestrial habitat	Quality of terrestrial habitat. Good, moderate, poor, none	Moderate	0.67
Macrophyte cover	Percentage of pond surface occupied by macrophyte cover	30	0.6
		HSI Score	0.72
		Suitability category	Good

Pond reference:	P5	Survey date:	05/04/2018
Suitability indices	Criteria	Value	Indices score
Geographic location	Location within Great Crested Newt Range A, B, C	A	1
Pond area	Pond surface area (m ²)	450	0.9
Pond permanence	Pond dries annually, sometimes, rarely, never	Never	0.9
Water quality	Good, Moderate, poor, bad	Moderate	0.67
Percentage shade of pond margin	Percentage shaded to at least 1m from shore	50	1
Number of waterfowl	Absent, minor, major	Minor	0.67
Occurrence of fish	Absent, possible, minor, major	Possible	0.67
Waterbody density	Number of waterbodies within 1km (excluding pond surveyed)	13+	1
Terrestrial habitat	Quality of terrestrial habitat. Good, moderate, poor, none	Good	1
Macrophyte cover	Percentage of pond surface occupied by macrophyte cover	20	0.5
HSI Score			0.81
Suitability category			Excellent

Pond reference:	P6	Survey date:	19/06/2019
Suitability indices	Criteria	Value	Indices score
Geographic location	Location within Great Crested Newt Range A, B, C	A	1
Pond area	Pond surface area (m ²)	600	1
Pond permanence	Pond dries annually, sometimes, rarely, never	Rarely	1
Water quality	Good, Moderate, poor, bad	Poor	0.33
Percentage shade of pond margin	Percentage shaded to at least 1m from shore	50	1
Number of waterfowl	Absent, minor, major	Major	0.01
Occurrence of fish	Absent, possible, minor, major	Minor	0.3
Waterbody density	Number of waterbodies within 1km (excluding pond surveyed)	13+	1
Terrestrial habitat	Quality of terrestrial habitat. Good, moderate, poor, none	Poor	0.33
Macrophyte cover	Percentage of pond surface occupied by macrophyte cover	<5%	0.35
HSI Score			0.40
Suitability category			Poor

Annex 4: eDNA survey data

Table 7.4: Results of the eDNA surveys

Pond number	Date of survey	Positive / Negative	eDNA score	Inhibition control
P1	08/06/17	Negative	0 of 12	Within limits
P2	08/06/17	Positive	3 of 12	Within limits

Result sheets provided in Annex 5

Annex 5: eDNA result sheets

Client: [REDACTED]
ADAS,
Battlegate Road,
Boxworth,
Cambridge,
CB23 4NN



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

Email: [REDACTED]

www.adas.co.uk

Sample/Report ID: 2017-872

Condition on Receipt: Medium Sediment

Visual Inspection of Volume: Passed

Client Identifier: P2

Description: 6x50mL - pond water samples in preservatives

Date of Receipt: 12/06/2017

Material Tested: DNA extracted from pond water samples

Determinant	Result	Method	Date of Analysis
Great Crested Newt	Positive	Real time PCR	15/06/2017

Report Prepared by:



Report Issued by:



Signed:

Signed:

Position:

Senior Research Scientist

Position:

Team Leader: Biotechnology

Date of preparation:

16/06/2017

Date of issue:

16/06/2017

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.



Client: [REDACTED]
ADAS,
Battlegate Road,
Boxworth,
Cambridge,
CB23 4NN

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

Email: [REDACTED]

www.adas.co.uk

Sample/Report ID: 2017-876 Condition on Receipt: Good Visual Inspection of Volume: Passed
Client Identifier: P1 Description: 6x50mL - pond water samples in preservatives
Date of Receipt: 12/06/2017 Material Tested: DNA extracted from pond water samples

Determinant	Result	Method	Date of Analysis
Great Crested Newt	Negative	Real time PCR	15/06/2017

Report Prepared by:



Report Issued by:



Signed:

Position: Senior Research Scientist

Signed:

Position: Team Leader: Biotechnology

Date of preparation: 16/06/2017

Date of issue: 16/06/2017

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.

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
P2	3 of 12	2 of 2	Within Limits
P1	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/μL		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.

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Annex 6: Population size-class survey data

Table 7.5: Pond P2 – population size-class survey data

Date						Traps (15)						Torch						
	Air Temp °C	Wind	Cloud cover (%)	Rain	Egg search	Great crested newt			Smooth newt			Great crested newt			Smooth newt			
						M	F	Juv	M	F	Juv	M	F	Juv	M	F	Juv	
05/06 April 2018	11	2	10	N	Y	3	10	0	3	2	0	0	0	0	0	0	0	0
12/13 April 2018	9	3	40	N	Y	1	8	0	0	0	0	0	0	0	0	0	0	0
19/20 April 2018	20	2	5	N	N	9	10	0	0	0	0	0	0	0	0	0	0	0
26/27 April 2018	8	4	20	N	N	5	6	0	0	0	0	0	1	0	0	0	0	0
10/11 May 2018	13	3	30	N	N	<u>8</u>	<u>14</u>	0	0	0	0	0	0	0	0	0	0	0
16/17 May 2018	12	4	10	N	N	4	3	0	0	1	0	1	0	0	0	0	0	0

Comments/limitations: Egg search ceased after great crested newt eggs confirmed. Eggs found on float grass. Turbidity and vegetation cover consistent throughout survey. Turbidity = 4 and vegetation cover = 3 on each visit.

Table key:

- M = male, F = female and Juv = juvenile
- Y = Yes, N = No
- Wind described as Beaufort scale (1 – 12)

Peak count: 22 (11/05/18 – trapping survey)

Table 7.6: Pond P3 – population size-class survey data

Date						Traps (10)						Torch						
	AM	PM	AM	PM	Egg search	Great crested newt			Smooth newt			Great crested newt			Smooth newt			
						M	F	Juv	M	F	Juv	M	F	Juv	M	F	Juv	
05/06 April 2018	11	2	10	N	Y	0	0	0	0	1	0	0	0	0	0	0	0	0
12/13 April 2018	9	3	40	N	Y	<u>2</u>	0	0	0	2	0	0	0	0	0	0	0	0
19/20 April 2018	20	2	5	N	N	0	0	0	12	6	0	0	0	0	0	0	1	0
26/27 April 2018	8	4	20	N	N	1	0	0	4	4	0	0	0	0	0	0	2	0

Comments/limitations: Egg search ceased after great crested newt eggs confirmed.

Table key:

- M = male, F = female and Juv = juvenile
- Y = Yes, N = No
- Wind described as Beaufort scale (1 – 12)

Peak count: 2 (12/04/18 – trapping survey)

Table 7.7: Pond P4 – population size-class survey data

Date						Traps (15)						Torch					
	Air Temp °C	Wind	Cloud cover (%)	Rain	Egg search	Great crested newt			Smooth newt			Great crested newt			Smooth newt		
						M	F	Juv	M	F	Juv	M	F	Juv	M	F	Juv
05/06 April 2018	11	2	10	N	Y	0	0	0	0	0	0	1	0	0	0	0	0
12/13 April 2018	9	3	40	N	Y	0	0	0	0	0	0	0	0	0	0	0	0
19/20 April 2018	20	2	5	N	N	2	2	0	0	0	0	<u>2</u>	<u>4</u>	0	1	0	0
26/27 April 2018	8	4	20	N	N	2	1	0	1	0	0	0	0	0	0	0	0
10/11 May 2018	13	3	30	N	N	0	0	0	0	0	0	0	0	0	1	0	0
16/17 May 2018	12	4	10	N	N	0	0	0	0	0	0	0	0	0	0	0	0

Comments/limitations: Egg search ceased after great crested newt eggs confirmed. Eggs found on emergent plant. Female great crested newt seen egg-laying during torching survey on 19/04/2018.

Turbidity and vegetation cover consistent throughout survey. Turbidity = 3 and vegetation cover = 3 on each visit.

Table key:

- M = male, F = female and Juv = juvenile
- Y = Yes, N = No
- Wind described as Beaufort scale (1 – 12)

Peak count: 6 (19/04/18 – torching survey)

Table 7.8: Pond P5 – population size-class survey data

Date						Traps (15)						Torch						
	Air Temp °C	Wind	Cloud cover (%)	Rain	Egg search	Great crested newt			Smooth newt			Great crested newt			Smooth newt			
						M	F	Juv	M	F	Juv	M	F	Juv	M	F	Juv	
05/06 April 2018	11	2	10	N	Y	0	1	0	3	0	0	0	0	0	0	0	0	0
12/13 April 2018	9	3	40	N	Y	0	1	0	0	0	0	3	0	0	0	0	0	0
19/20 April 2018	20	2	5	N	N	7	8	0	5	2	0	17	4	0	4	7	0	0
26/27 April 2018	8	4	20	N	N	0	0	0	7	0	0	0	0	0	0	0	0	0
10/11 May 2018	13	3	30	N	N	1	0	0	0	0	0	0	0	0	0	0	0	0
16/17 May 2018	12	4	10	N	N	0	0	0	0	0	0	0	0	0	0	0	0	0

Comments/limitations: Egg search ceased after great crested newt eggs confirmed. Eggs found on forget-me-not. Two frogs recorded 19/04/2018.

Turbidity and vegetation cover consistent throughout survey. Turbidity = 4 and vegetation cover = 1 on each visit.

Table key:

- M = male, F = female and Juv = juvenile
- Y = Yes, N = No
- Wind described as Beaufort scale (1 – 12)

Peak count: 21 (12/04/18 – torching survey)

Annex 6: Site photographs

Pond P1



Pond P2



Pond P3



Pond P4



Pond P5



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