

23857 Graven Hill, Bicester – Great Crested Newt eDNA Survey Report

LNT Care Property Development

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Executive Summary

Ecus Limited (Ecus Ltd), was commissioned by LNT Construction in June 2024 to undertake a Great Crested Newt (GCN) *Triturus cristatus* Environmental DNA (eDNA) presence / likely absence survey and a Habitat Suitability Index (HSI) assessment, in relation to proposed works required to facilitate the development of a care home and associated parking at Graven Hill, Bicester, OX25 2BF centred on OS Grid Reference (OS NGR): SP 58894 21247 hereafter referred to as 'the Site'. The Site extent is shown by the red line boundary in **Figure 1**.

A total of four waterbodies Pond 1 (P1), Pond 2 (P2), Ditch (D1) and Ditch (D2) were identified, at or within 250 metres of the Site as extant and relevant to the GCN survey. The ponds were deemed relevant based on their proximity, habitat connectivity and the terrestrial range of GCN (generally <250 metres, occasionally >500 metres, rarely >1 kilometre from their breeding site). D1 and D2 border the eastern and western extents of the Site. P1 and P2 are both located outside of the Site boundary, P1 is located 20 m south of the Site and P2 200 m west of the Site, respectively.

The terrestrial habitats at the Site were assessed during the survey visit, for their suitability to support GCN and were considered to offer GCN with minimal shelter, foraging and dispersal opportunities categorised as 'poor'.

The GCN eDNA survey water sampling was undertaken at P1, P2, D1 and D2 on 21st June 2024. The result of the survey was negative for P1 & D2 (GCN likely absent) and positive for P2 & D1 (GCN likely present).

Given the established likely presence of GCN at P2 and D1 it is considered reasonable to conclude that GCN may potentially be encountered within, and dependant, at least in part, on, terrestrial habitats at the Site. Therefore, the species poses a constraint to the proposed works.

The proposed works at the Site will involve the destruction of extensive areas of existing onsite terrestrial habitats encompassing approximately 1.1 ha and the temporary disturbance of P1, D1 and D2.

Based on the predicted development effect on the habitat at the Site, and with consideration for the English Nature Great Crested Newt Mitigation Guidelines (2001) and current GCN guidance issued by Natural England, the scale of impact of the proposed works on GCN has been assessed as 'medium'.

The Site is located within a Nature Space Partnership (NSP) GCN Amber Risk Zone. Nature Space state: 'Amber zones contain suitable habitat and GCN are likely to be present'. P2 which is located 200 m west of the Site falls within the Nature Space Red Risk Zone. Red zones contain suitable habitat and most important areas for GCN.

Consequently, it has been concluded that the unlicensed and/or unmitigated commencement of the proposed works at the Site will result in a breach of current wildlife legislation relating to GCN and will have a negative impact on the conservation status of the GCN population identified to be associated with P2 and D1 and the Site.

Therefore, it is deemed appropriate and proportionate to recommend that a formal enquiry to enter the Site into the NSP and West Oxfordshire District Council Great Crested Newt District Licencing (NSP GCN DL) scheme is prepared and submitted to NSP for determination, as soon as is reasonably practicable.

It is recommended that Amphibian Best Practice Measures should be implemented at the Site (in addition to entry into the NSP DL scheme) in order to protect common amphibians should they be encountered onsite during the proposed works.

1. Introduction

1.1 Scope of this Report

1.1.1 Ecus was commissioned by LNT Construction in June 2024 to undertake great crested newt (GCN) *Triturus cristatus* surveys at Graven Hill, Bicester, OX25 2BF (central National Grid Reference (NGR): SP 58894 21247), hereafter referred to as the ‘Site’. Habitat Suitability Index (HSI) assessments and eDNA surveys were undertaken at four waterbodies located within 250 m of the Site following on from recommendations provided within the Preliminary Ecological Appraisal (PEA) undertaken by Ecus Ltd in May 2024 (see **Table 1**).

Table 1. Waterbodies within 250 m of the Site subject to HSI and eDNA Surveys.

Ref.	Waterbody Type	Waterbody Name	NGR	w3w
1	Pond	Pond 1 (P1)	SP 58947 21194	///mash.cherry.spite
2	Ditch	Ditch 1 (D1)	SP 58826 21241	/// dairy.spider.truth
3	Ditch	Ditch 2 (D2)	SP 58940 21260	/// kings.laying.necks
4	Pond	Pond 2 (P2)	SP 58644 21182	///organs.lime.lined

1.2 Site Description

1.2.1 The Site (approximately 1.1 ha) mainly comprised of a large area of bare ground with sparse vegetation scattered throughout. Vegetated habitats within the Site included areas of bramble scrub *Rubus fruticosus* interspersed with scattered trees. A line of trees was also recorded along the western extent of the Site. Some scattered semi-mature/ immature trees were recorded within the Site boundary with a single mature oak *Quercus* spp. at SP 58918 21202 located on the southern boundary of the Site. Two ditches D1 and D2 were recorded bordering the eastern and western boundaries of the Site. Two ponds were situated outside of the Site boundary, one large pond P1 located 20 m south of the Site and a moderately sized pond P2 200 m west of the Site. A map of waterbodies is displayed in **Figure 1**.

1.2.2 In addition to the four waterbodies a small section of stream runs 249 m north of the Site. This stream was not considered to be relevant to the scope of works due to its unsuitability for GCN (fast flowing with limited suitability for breeding). This watercourse is not considered further in this GCN survey report.

1.2.3 The Site is located on the edge of a widely urban area of Bicester. The surrounding area to the north of the Site comprised mainly of residential and industrial buildings with associated roads. To the south of the Site and in the wider area are arable fields with pockets of woodland and hedgerows.

1.3 Project Scope

1.3.1 The current proposals for this project include the development of a care home with associated parking spaces and gardens.

1.4 Purpose of the Surveys

1.4.1 The purpose of the survey was to establish the presence or likely absence of GCN and if present, to assess the potential impact of the proposed maintenance works on the species.

1.4.2 This report details the methods and findings of the survey work and a subsequent assessment. The need for further survey work, licence application and/or mitigation measures is detailed, where appropriate.

1.5 Quality Assurance

1.5.1 The eDNA survey and HSI assessment was completed by GCN licenced Ecologists Molly-Marie Mills BSc (Hons) MSc and Nicole Bell BSc (Hons) MSc.

1.5.2 The eDNA and HSI report was completed by Graduate Ecologist Jessica Sams.

1.5.3 This report was reviewed by Consultant Ecologist Molly-Marie Mills in accordance with Ecus' Quality Assurance policy.

1.5.4 The report has been approved by Senior Ecologist Claire Evans BSc (Hons) MSc ACIEEM..

2. Wildlife Legislation

2.1.1 GCN are designated and protected as European protected species (EPS). EPS are protected under the Conservation of Habitats and Species Regulations 2017. It is an offence to:

- deliberately kill, injure, disturb, or capture them,
- deliberately take or destroy their eggs,
- damage or destroy their breeding sites and resting places - even if GCN are not present, and,
- possess, control, or transport them (alive or dead).

2.1.2 It is also an offence under the Wildlife and Countryside Act 1981 to intentionally or recklessly:

- disturb GCN while they occupy a structure or place used for shelter or protection,
- obstruct access to a place of shelter or protection.

3. Methodology

3.1 Data Consultation

- 3.1.1 Thames Valley Environmental Records Centre (TVERC) was approached for data consultation in May 2024, to provide recent (within the past 10 years) biological records within 2 km of the Site. This Search Area was considered appropriate due to the small spatial nature of any effects arising from the proposed works.
- 3.1.2 The Multi-Agency Geographic Information for the Countryside (MAGIC) website (<http://magic.defra.gov.uk>) was reviewed in August 2024 for information on the presence of granted European Protected Species (EPS) mitigation licences for great crested newt *Triturus cristatus* (GCN) within 2 km of the Sites. MAGIC was also used to search for information relating to GCN Class Survey Licence Returns and GCN pond survey data (2017-2019) within 2 km of the Sites.

3.2 Habitat Suitability Index Assessment

- 3.2.1 Each waterbody was assessed for its potential to support GCN following the Habitat Suitability Index Assessment (HSI) methodology (Oldham *et al.*, 2000) by ecologists from Ecus during the Site visit on 21st June 2024.
- 3.2.2 The GCN is a habitat specialist and its status in a given waterbody is influenced by the existence of particular features (e.g. fish, heavy shading) and/or the absence of others (e.g. suitable terrestrial habitat within 250 m). The HSI provides a numerical value (ranging from 0 to 1) that indicates the suitability of a waterbody for GCN. The higher the HSI score, the more suitable the waterbody may be considered for GCN. It should be noted that the HSI score should be verified by an experienced surveyor.

3.3 Environmental DNA (eDNA) sampling

- 3.3.1 The eDNA survey visit at P1, P2, D1 & D2 was undertaken on 21st June 2024. The survey was led by Ecus Consultant Ecologist Molly Mills BSc (Hons) MSc under a Natural England GCN survey class level 1 licence CL08 (Registration: 2023-11776-CL08-GCN) with support from Assistant Ecologist Nicole Bell BSc (hons) MSc (Registration: 2023-11777-CL08-GCN).
- 3.3.2 During the GCN eDNA survey visit twenty water samples were taken from evenly spaced locations around the surveyed pond margins, ensuring the sediment on the bed of the pond was not disturbed. The samples were mixed together and from this, six samples were taken and preserved in ethanol. Samples were kept cool at around 4°C before being sent to the laboratory for analysis. The whole procedure was undertaken with sterile gloves and equipment and without entering the water, to prevent contamination of the samples. Footwear was cleaned and disinfected with Virkon

S disinfectant solution before visiting the pond to prevent the spread of amphibian diseases and pathogens.

- 3.3.3 The water sample collection kit was supplied by ADAS Biotechnology, who also carried out the laboratory analysis for GCN eDNA in the water samples collected by Ecus, during the survey visit.
- 3.3.4 The GCN eDNA survey water sampling and subsequent laboratory analysis was carried out in strict accord with the Biggs *et al.* 'Analytical and methodological development for improved surveillance of the Great Crested Newt (WC1067) - Appendix 5 Technical advice note for field and laboratory sampling for great crested newt (*Triturus cristatus*) environmental DNA' (Freshwater Habitats Trust, Oxford) and current relevant guidance from Natural England.

3.4 Limitations

- 3.4.1 Samples could not be taken from around the entire margin for P1, D1 and P2 due to the presence of dense vegetation preventing access to the waterbody. However, it was possible for Ecus to collect water samples from approximately 20-80% of the margin for each of these waterbodies during the survey visit. The constraint encountered was not considered to have had a significant impact on the survey result obtained at these waterbodies.

4. Results

4.1.1 The following section provides the results of the desk study, HSI assessment and eDNA testing at each of the two ponds and two ditches within 250 m of the Graven Hill site in Bicester. The location of each waterbody can be seen on **Figure 1**.

4.2 Desk Study

4.2.1 Waterbodies P1, D1 and D2 fall within the 'Amber' zone under The NatureSpace Impact Risk Map. Amber zones contain suitable habitat and GCN are likely to be present. P2 falls within the 'Red' zone. Red zones contain suitable habitat and most important areas for GCN.

4.2.2 Thames Valley Environmental Records Centre (TVERC) returned 111 recent records (within the last 10 years) for amphibians within 2 km of the Site. These records related to common toad *Bufo bufo* and smooth newt *Lissotriton vulgaris*. The closest record related to a common toad located 860 m northeast of the Site in 2017.

4.2.3 TVERC also returned 219 records for Great Crested Newt (GCN) within 2 km of the Site. The closest record related to a GCN located 90 m south of the Site in 2014.

4.2.4 According to MAGIC, there were 20 granted European Protected Species (EPS) licences relating to GCN located within 2 km of the Site. Details of the GCN EPS licences are provided in **Appendix 2**.

4.2.5 MAGIC also identified 27 GCN class survey licence returns within 2 km of the Site. The closest record related to a class licence return for the ditch along the western extent of the Site in 2014. GCN confirmed as present.

4.3 HSI and eDNA Survey Results

4.3.1 HSI assessments and eDNA sampling was undertaken on four waterbodies (P1, P2, D1 & D2) situated within 250 m of the Site. Results of the HSI Assessments and eDNA sampling are provided below in **Tables 2, 3, 4 & 5**.

Pond 1 HSI and eDNA Results

Table 2: HSI results for the Pond 1

Variants	Pond Description
Geographical Location	Zone A: SP 58947 21194
Pond Area	3040 m ²
Permanence	Never dries
Water Quality	Good
Shade	100%
Waterfowl	Minor
Fish	Possible
Pond Count	28
Habitat	Good
Macrophyte Cover	35%
HSI Score	0.73 = Good

4.3.2 The eDNA survey returned a **negative** result. eDNA analysis achieves approximately 95% detection rate, therefore it is highly **unlikely** that GCN were present at the pond this breeding season.

Pond 2 HSI and eDNA Results

Table 3: HSI results for Pond 2

Variants	Pond Description
Geographical Location	Zone A: SP 58644 21182
Pond Area	195 m ²
Permanence	Never dries
Water Quality	Poor
Shade	100%
Waterfowl	Minor
Fish	Possible
Pond Count	22
Habitat	Moderate
Macrophyte Cover	100%
HSI Score	0.60 = Below Average

4.3.3 The eDNA survey returned a **positive** result. eDNA analysis achieves approximately 95% detection rate, therefore it is highly **likely** that GCN were present at the pond this breeding season.

Ditch 1 HSI and eDNA Results

Table 4: HSI results for Ditch 1

Variants	Pond Description
Geographical Location	Zone A: SP 58826 21241
Pond Area	18 m ²
Permanence	Rarely dries
Water Quality	Moderate
Shade	60%
Waterfowl	Absent
Fish	Possible
Pond Count	24
Habitat	Moderate
Macrophyte Cover	55%
HSI Score	0.65 = Average

4.3.4 The eDNA survey returned a **positive** result. eDNA analysis achieves approximately 95% detection rate, therefore it is highly **likely** that GCN were present at the pond this breeding season.

Ditch 2 HSI and eDNA Results

Table 5: HSI results for Ditch 2.

Variants	Pond Description
Geographical Location	Zone A: SP 58940 21260
Pond Area	15 m ²
Permanence	Dries annually
Water Quality	Moderate
Shade	100%
Waterfowl	Absent
Fish	Absent
Pond Count	24
Habitat	Moderate
Macrophyte Cover	40%
HSI Score	0.45 = Poor

4.3.5 The eDNA survey returned a **negative** result. eDNA analysis achieves approximately 95% detection rate, therefore it is highly **unlikely** that GCN were present at the pond this breeding season.

4.4 Results assessment

4.4.1 The terrestrial habitats at the Site were assessed during the survey visit, for their suitability to support GCN and were considered to offer GCN with minimal shelter, foraging and dispersal opportunities around the perimeter of the Site categorised as ‘poor’.

4.4.2 The proposed works will involve the temporary disturbance of P1. Given that GCN absence has

- been established at P1, impacts to GCN within 50 m associated with this pond are not predicted.
- 4.4.3 The proposed works will involve the temporary disturbance of D1. Given that GCN presence has been established at D1, impacts to GCN within 50 m associated with this ditch are predicted.
- 4.4.4 The proposed works are not expected to cause disturbance or destruction to P2 given the nature of the works, lack of hydrological connectivity and distance from the Site.
- 4.4.5 The proposed works will involve the temporary disturbance of D2. Given that GCN absence has been established at D2, impacts to GCN within 50 m associated with this ditch are not predicted.
- 4.4.6 The works will result in the predicted partial destruction, modified management and temporary disturbance of both Immediate Terrestrial Habitat and Intermediate Terrestrial Habitat associated with waterbodies P1, D1 and D2. Distant Terrestrial Habitat is not expected to be impacted by the works.
- 4.4.7 Based on the predicted development effect on the habitat features, as detailed below, and with consideration for the English Nature Great Crested Newt Mitigation Guidelines (2001) and current GCN guidance issued by Natural England, the scale of impact of the proposed works on GCN has been assessed as **medium**. See the highlighted text in **Table 6**.

Table 6. Impact Assessment Summary

Habitat Feature	Development Effect	Scale of Impact		
		Low	Medium	High
Confirmed GCN breeding pond/water body	Destruction			✓
	Isolation caused by fragmentation			✓
	Partial destruction; modification		✓	
	Temporary disturbance	✓		
	Post-development interference			✓
Other pond or water body	Destruction		✓	
	Isolation caused by fragmentation		✓	
	Partial destruction; modification	✓		
	Temporary disturbance	✓		
	Post-development interference	✓		
Immediate Terrestrial Habitat (less than 50m from a GCN/breeding pond or other waterbody identified to be potentially used by the species)	Destruction			✓
	Isolation caused by fragmentation			✓
	Partial destruction		✓	
	Modified management, resurfacing etc.		✓	
	Temporary disturbance	✓		
	Post-development interference		✓	
	Temporary destruction & reinstatement	✓		
Intermediate Terrestrial Habitat: (at a distance of 50 m up to 250 m from a GCN/breeding pond or other	Destruction		✓	
	Isolation caused by fragmentation		✓	
	Partial destruction	✓		
	Modified management, resurfacing, etc.	✓		

waterbody identified to be potentially used by the species	Temporary disturbance	✓		
	Post-development interference	✓		
	Temporary destruction & reinstatement	✓		
Distant Terrestrial Habitat (more than 250 m from a GCN/breeding pond or other water body potentially used by the species)	Destruction	✓		
	Isolation caused by fragmentation	✓		
	Partial destruction	✓		
	Modified management, resurfacing etc.	✓		
	Temporary disturbance	✓		
	Post-development interference	✓		
	Temporary destruction & reinstatement	✓		

4.4.8 Given the established likely presence of GCN at waterbody P2 and D1 it is considered reasonable to conclude that GCN may potentially be encountered within, and dependant, at least in part, on, terrestrial habitats at the Site. Therefore, the species poses a constraint to the proposed works.

4.5 Anecdotal Evidence

4.5.1 During the eDNA survey no incidental sightings of GCN (including adults, juveniles and eggs) were identified within any of the waterbodies.

4.5.2 An incidental sighting of a juvenile common frog *Rana temporaria* was identified on Site within D2.

5. Conclusions

- 5.1.1 GCN are included on Schedule 2 of The Conservation of Habitats and Species Regulations 2017 as amended by the Conservation of Habitats and Species (Amendment) (EU Exit) Regulations 2019. Under the Regulations GCN are protected from deliberate capture, injury or killing, from deliberate disturbance and from deliberate damage or destruction of a breeding Site or resting place.
- 5.1.2 Under the Wildlife and Countryside Act 1981 (as amended) it is also an offence to intentionally or recklessly disturb GCN while they are using a structure or place used for shelter or protection or obstruct access to or damage any such structure or place. The provisions of the Act (1981) also protect great crested newts from activities associated with their sale.
- 5.1.3 Smooth newt, palmate newt, common frog and common toad are included in Section 9(5) of the Wildlife and Countryside Act 1981 (as amended) which prohibits sale, barter, exchange, transporting for sale and advertising to sell or to buy these species.
- 5.1.4 Consequently, it has been concluded that the unlicensed and/or unmitigated commencement of the proposed works at the Site will result in a breach of current wildlife legislation relating to GCN and will have a negative impact on the conservation status of the GCN population identified to be associated with P2, D1 and the Site.

Nature Space District Level Licensing

- 5.1.5 P1, D1 and D2 fall within the 'Amber' zone under The NatureSpace Impact Risk Map. Amber zones contain suitable habitat and GCN are likely to be present. P2 falls within the 'Red' zone. Red zones contain suitable habitat and most important areas for GCN.
- 5.1.6 Therefore, it is deemed appropriate and proportionate to recommend that a formal enquiry to enter the Site into the NSP and West Oxfordshire, District Council, Great Crested Newt District Licencing (NSP GCN DL) scheme is prepared and submitted to NSP for determination, as soon as is reasonably practicable.
- 5.1.7 Each development is assessed on a case-by-case basis and both development zone and scale will be considered. Following the assessment of the Site a NatureSpace District Licensing report is issued (within 10 working days). This report details the proportionate outcome of the site assessment, including the second stage fee and any mitigation requirements, this of which can be submitted to the planning case officer.
- 5.1.8 Once the second stage fee has been paid, a NatureSpace Certificate which confirms that GCN compensation has been paid for will be issued.

- 5.1.9 The acceptance of the Site into the NSP DLL scheme will negate the legal risks associated with encountering GCN at the Site during the proposed works, will appropriately and proportionately address the predicted development impacts on GCN and will enable the proposed works at the Site to proceed lawfully, in a timely manner without the need to undertake further GCN survey effort at P2 and D2 or provide onsite GCN mitigation or habitat compensation.
- 5.1.10 In the event that the NSP GCN DL scheme is not available or viable for the Site the GCN survey findings and this report may be used to inform a non-licensed GCN Reasonable Avoidance Measures Method Statement (RAM MS).
- 5.1.11 It is recommended that amphibian Best Practice Measures (BPM) should be implemented at the Site (in addition to entry into the GCN DLL scheme) in order to protect individual amphibians including smooth newt, palmate newt, common frog and common toad should they be present/encountered onsite during the proposed works.
- 5.1.12 According to the Natural England's GCN survey guidance table (see **Appendix 2**), the survey data collected for this report is valid for three seasons. Therefore, if the proposed works have not been completed by June 2027, it is recommended that the Site is re-surveyed to determine if there have been any significant changes.

6. References




Biggs, J., et al. (2014). *Analytical and methodological development for improved surveillance of the Great Crested Newt. Appendix 5. Technical advice note for field and laboratory sampling of great crested newt (Triturus cristatus) environmental DNA*. Freshwater Habitats Trust, Oxford.



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English Nature (2001). *Great crested newt mitigation guidelines*. English Nature, Peterborough.

Oldham, 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, pp. 143-155.

Figure 1: Waterbodies Location Map

-  Site boundary
-  250 m buffer
-  Waterbody

- eDNA Results
-  Positive
 -  Negative



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Great Crested Newt Surveys

Figure 1
eDNA results for waterbodies within
250 m of Site

Unit 4, Prisma Park, Berrington Way,
Basingstoke, RG24 8GT

© Microsoft
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Appendix 1. Site Photographs



- 1 Temporary pool of water on Site.
- 2 Ditch 2 (D2) at eastern aspect of Site, GCN absent.
- 3 Pond 1 (P1), GCN absent.
- 4 Pond 1 (P1), GCN absent.

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Great Crested Newts Surveys
23857 Graven Hill, Bicester

Appendix 1: Site Photographs

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Unit 4 Prisma Park, Berrington Way, Wade Road, Basingstoke, RG24 8GT



- 5 Pond 1 (P1), GCN absent.
- 6 Pond 1 (P1), GCN absent.
- 7 Pond 2 (P2), GCN present.
- 8 Pond 2 (P2), GCN present.

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Appendix 1: Site Photographs

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Unit 4 Prisma Park, Berrington Way, Wade Road, Basingstoke, RG24 8GT

Appendix 2. GCN EPS Mitigation Licences from within 2 km of the Site

Licence number	Licence cover	Distance and direction	Date
EPSM2011-3350	Destruction of a resting place.	240 m South East	08.09.11 – 31.03.12
2016-20166-EPS-MIT-1	Destruction of a resting place and breeding site	310 m South West	25.05.16 – 30.06.27
2015-10019-EPS-MIT	Destruction of a resting place and breeding site	830 m South West	12.08.15 – 30.06.18
2018-38205-EPS-MIT	Destruction of a resting place and breeding site.	830 m South West	11.02.19 – 31.12.20
2015-10019-EPS-MIT-1	Destruction of a resting place and breeding site	830 m South West	01.04.16 – 30.06.18
2014-1173-EPS-MIT	Destruction of a resting place and breeding site	885 m West	17.07.14 – 31.10.17
2014-1173-EPS-MIT-1	Destruction of a resting place and breeding site	885 m West	23.09.14 – 31.10.17
2014-5541-EPS-MIT	Destruction of a resting place and breeding site	885 m West	12.12.14 – 31.10.17
2014-5541-EPS-MIT-2	Damage of a resting place	885 m West	14.10.15 – 31.10.17
2014-5541-EPS-MIT-1	Destruction of a resting place and breeding site.	885 m West	16.12.14 – 31.10.17
2016-20166-EPS-MIT-2	Destruction of a resting place and breeding site	885 m West	06.09.16 – 30.06.27
2019-39004-EPS-MIT	Unknown.	885 m West	11.02.19 –

			31.12.20
2019-39711-EPS-NSIP2	Destruction of a resting place and breeding site.	1.2 km North	29.05.20 – 31.12.30
2019-39711-EPS0NSIP2-1	Destruction of a resting place and breeding site.	1.2 km North	22.12.20 – 31.12.30
2020-46994-EPS-MIT	Unknown.	1.2 km North	29.05.20 – 01.06.22
2020-46994-EPS-MIT-1	Destruction of a resting place and breeding site.	1.2 km North	22.12.20 – 22.12.22
2014-1362-EPS-MIT-2	Destruction of a resting place and breeding site	1.3 km North East	21.10.15 – 31.03.16
2014-1362-EPS-MIT	Unknown	1.3 km North East	23.06.14 – 31.10.15
2014-1362-EPS-MIT-1	Damage of a resting place.	1.3 km North East	27.06.14 – 31.10.15
EPSM2012-5282	Destruction of a resting place.	1.3 km North East	05.12.13 – 01.10.15

Appendix 3. eDNA Survey Results

Client: Molly Mills,
Ecus



ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

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

www.adas.uk

Sample ID: ADAS-6139 Condition on Receipt: Low Sediment Volume: Passed
Client Identifier: P2, Graven Hill 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	04/07/2024
Degradation Control [§]	Within Limits	Real Time PCR	04/07/2024
Great Crested Newt*	12 of 12 (GCN positive)	Real Time PCR	04/07/2024
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:



Signed:



Position: Director: Biotechnology

Position: MD: Biotechnology

Date of preparation: 04/07/2024

Date of issue: 04/07/2024

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.

Client: Molly Mills,
Ecus



ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

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

www.adas.uk

Sample ID: ADAS-6140 Condition on Receipt: Low Sediment Volume: Passed
Client Identifier: D2, Graven Hill 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: Dr Helen Rees Report Issued by: Dr Ben Maddison

Signed:



Signed:



Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 04/07/2024 Date of issue: 04/07/2024

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.

Client: Molly Mills,
Ecus



ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

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

www.adas.uk

Sample ID: ADAS-6141 Condition on Receipt: White Precipitate Volume: Passed
Client Identifier: P1, Graven Hill 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: Dr Helen Rees Report Issued by: Dr Ben Maddison

Signed:



Signed:



Position: Director: Biotechnology

Position: MD: Biotechnology

Date of preparation: 04/07/2024

Date of issue: 04/07/2024

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.

Client: Molly Mills,
Ecus



ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

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

www.adas.uk

Sample ID: ADAS-6142 Condition on Receipt: Medium Sediment Volume: Passed
Client Identifier: D1, Graven Hill 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	02/07/2024
Degradation Control [§]	Within Limits	Real Time PCR	02/07/2024
Great Crested Newt*	2 of 12 (GCN positive)	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: Dr Helen Rees Report Issued by: Dr Ben Maddison

Signed:



Signed:



Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 04/07/2024 Date of issue: 04/07/2024

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)

Appendix 4. Natural England Survey Guidance Table

Survey guidance in relation to impact type, location and area. The guidance relevant to the Site highlighted in blue.

Impact type and location	Potential terrestrial habitat - loss or damage (ha)	Presence/ likely absence survey	Population size class assessment*	HSI	Maximum age of survey data (breeding seasons)
Permanent habitat loss or damage					
Pond(s) lost or damaged, with or without other habitat loss or damage	≥0	YES	YES	YES	2
No ponds lost or damaged, development within 50m of nearest pond	≤0.01	YES	NO	YES	3
	>0.01	YES	YES	YES	2
No ponds lost or damaged, development 50-100m from nearest pond	≤0.2	YES	NO	NO	3
	>0.2	YES	YES	YES	2
No ponds lost or damaged, development 100-250m from nearest pond	≤0.5	YES	NO	NO	4
	>0.5	YES	YES	YES	3
No ponds lost or damaged, development >250m from nearest pond (NB see notes)	≤5	YES	NO	NO	4
	>5	YES	NO	YES	3
Temporary habitat loss or damage					

Pond(s) lost or damaged, with or without other habitat loss or damage	≥0	YES	YES	YES	2
No ponds lost or damaged, development within 50 m of nearest pond	≤0.05	YES	NO	YES	3
	>0.05	YES	YES*	YES	3
No ponds lost or damaged, development 50-100m from nearest pond	≤0.5	YES	NO	NO	4
	>0.5	YES	YES	YES	3
No ponds lost or damaged, development >100m from nearest pond	≤5	YES	NO	NO	4
	>5	YES	NO	YES	4

* Population size class assessment deemed unsuitable for this Site due to lack of accessibility of the waterbodies with a positive eDNA result (D1 & P2), the scale of impact of the works and current 'poor' condition of terrestrial habitat on Site.

