Technical Briefing Note

Ref: 1006638 BN01 GCN vf

Land East of J11, M40, Banbury (1006638)

Great Crested Newt Survey Results

Date: November 2023

1. Introduction

1.1. Aspect Ecology is advising Greystoke CB Ltd in respect of ecological issues relating to land located east of Junction 11 of the M40 motorway at Banbury, Oxfordshire, centered at OS grid reference SP 476 422 (hereafter referred to as the site).

1.2. In particular, Aspect Ecology was commissioned in March 2023 to undertake specific Great Crested Newt eDNA survey work in regard to the site.

1.3. The site is proposed for development to provide a new large scale logistics development.

1.4. The site contains a number of ponds, with further offsite ponds and waterbodies present within the vicinity of the site, whilst the habitats present within the site are likely to provide potentially suitable terrestrial opportunities for amphibian species. Accordingly, the habitats present provide potentially suitable opportunities to support the fully protected amphibian species Great Crested Newt, which (if present) could therefore potentially represent a relevant consideration in regard to the proposed development of the site. As such, in order to confirm presence or likely absence of this species from the sites (and inform the development proposals), further survey work was undertaken of the ponds located within 250m of the sites, the results of which are set out below.

2. Previous Survey Information

2.1. The site was originally subject to ecological survey work by others in 2021, in order to inform the proposed development, including as set out within the documents submitted to inform the previous planning application for the site (ref: 22/01488/OUT). The previous survey work included initial assessment of the potential suitability for Great Crested Newt along with eDNA sampling of ponds considered suitable and where access was permitted.

3. Identification of Ponds

3.1. On the basis of the information available, including the previous survey information, available mapping and aerial imagery, a total of five ponds/waterbodies (P1, P4, P4, P6 and P7) were identified within the site, whilst a further five (P2, P5, P8, P9 and P10) were identified within 250m of the survey area (see attached Plan 6638/PP1). The locations of each of the identified ponds/waterbodies was surveyed by Aspect Ecology during March to June 2023 in order to establish any potential for individual waterbodies to support Great Crested Newt.

3.2. Pond P1 (Figure 1.) is located within the north east of the site and forms a permanent waterbody, which was recorded to support frequent aquatic and marginal vegetation including Common Reed.

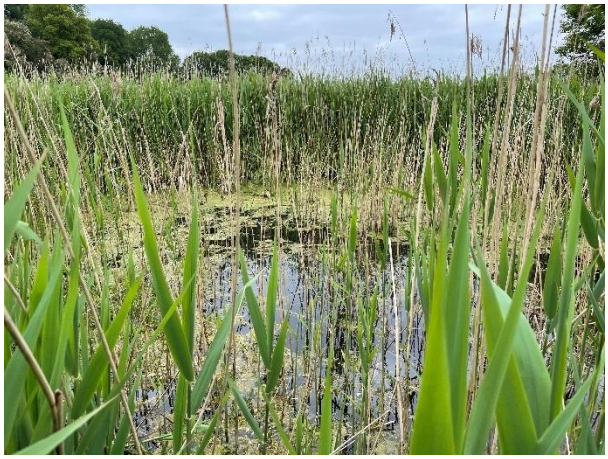


Figure 1. Pond P1.

3.3. P2 was previously (2021) reported to remain completely dry during the amphibian breeding season. During the current survey work, the location of pond P2 was similarly recorded to remain completely dry. Accordingly, P2 clearly does not provide potentially suitable breeding opportunities for Great Crested Newt and is not further considered.

3.4. Pond P3 is located within the centre of the site, within an area of cattle-grazed pasture and represents a field pond, approximately 10m in diameter with a number of associated trees (including substantial deadwood - see Figure 2). The pond was recorded to be heavily poached by grazing cattle, with extremely turbid water and lacking in aquatic or marginal vegetation.

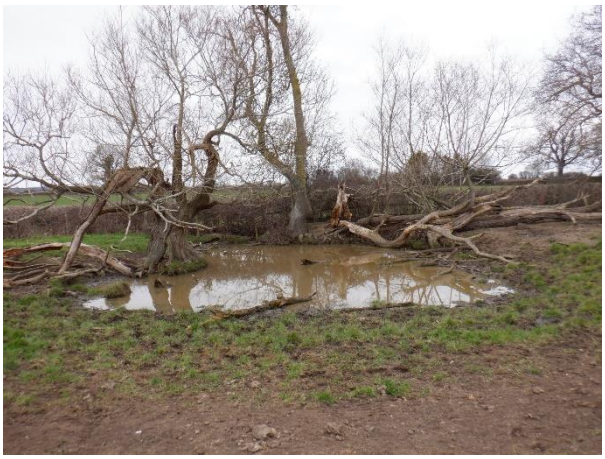


Figure 2. Pond P3.

3.5. P4 is located at the junction of a number of hedgerows, within the south east of the site. Previously (2021) P4 was recorded to be heavily poached by cattle, visibly nutrified and very shallow. At the time of the current (2023) surveys, P4 was recorded to represent a dry depression, overgrown with scrub, which therefore clearly does not provide potentially suitable breeding opportunities for Great Crested Newt. Accordingly, P4 has been scoped out of further survey and is not considered further.

3.6. Pond P5 (Figure 3.) is located offsite, approximately 250m south east of the site boundary. The pond was recorded to support deep water, with frequent emergent and marginal vegetation, dominated by Yellow Flag Iris *Iris pseudacorus*, within a private curtilage. A small ornamental fish pond was also noted within the same curtilage during the survey visit, supporting high densities of Koi Carp, which was not recorded to provide suitable breeding opportunities for Great Crested Newt and accordingly was excluded from further consideration.

Figure 3. Pond P5

3.7. Pond P6 represent a shallow depression supporting small amounts of water (approximately 5m diameter and no more than a few cm deep), which was recorded to be heavily poached and turbid, albeit with Flote-grass *Glyceria fluitans* present (see Figure 4).

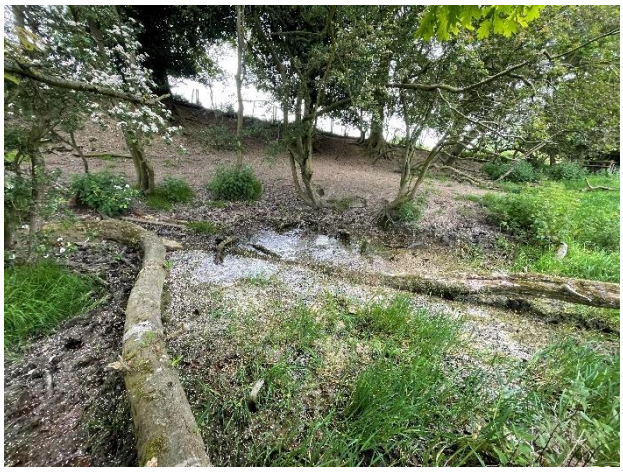


Figure 4. Pond P6

3.8. Pond P7 is located within an area of cattle grazed pasture in the south west of the site, with a margin dominated by scrub and individual trees. The pond was recorded to support very turbid water, with heavily poached banks lacking in aquatic vegetation, albeit a number of Willows and associated deadwood are present around the margins.

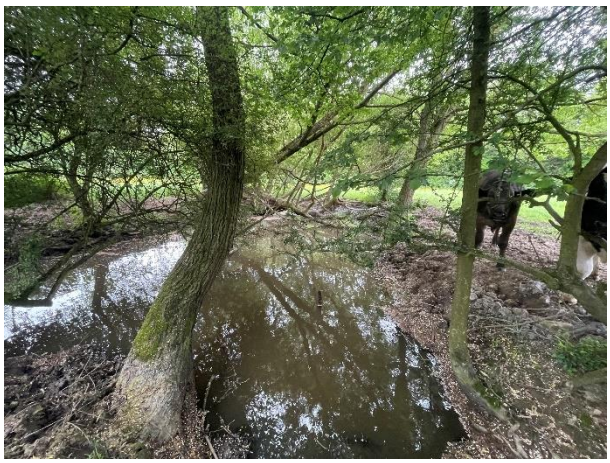


Figure 5. Pond P7

3.9. P8 is located offsite, within a private residential curtilage, approximately 130m south of the site, from which it is separated by the main. Review of historical aerial imagery indicates a previous pond present around 2017, which appears to have subsequently been filled in by 2021 (ref: Google Earth). Further, the location of the former pond was viewed during 2023 from the adjacent Banbury Lane, which confirmed the absence of any existing waterbody at this location, having been back-filled by the previous owner (pers. comm. Resident). Accordingly, P8 has been scoped out of further survey and is not considered further.

3.10. P9 represents an existing open air swimming pool located approximately 270m east of the proposed development site boundary within Carrdus School. Accordingly, P9 clearly does not represent potentially suitable breeding opportunities for Great Crested Newt and has been scoped out of further survey or consideration.

3.11. Pond P10 is located approximately 260m south of the proposed development site boundary, beyond the main A422 dual carriageway, which forms a further significant barrier to potential amphibian movement in the direction of the site. Accordingly, P10 is clearly well-removed and separated from the site. The pond represents a small ornamental pond with butyl liner albeit supporting aquatic and emergent vegetation (see Figure 6).

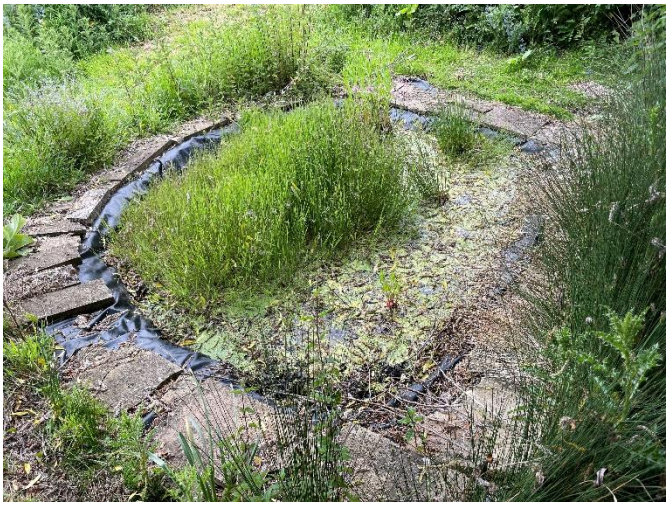


Figure 6. Pond P10

3.12. Overall therefore, following updated inspections during 2023, a total of six waterbodies (P1, P3, P5, P6, P7 and P10) are present within the site and surrounding areas which were considered to provide potentially suitable breeding opportunities for amphibians and were therefore subject to further survey/consideration in regard to Great Crested Newt.

4. Survey Methodology

Habitat Suitability Index (HSI) Assessment

4.1. As a first step in identifying the potential presence of Great Crested Newt at the site, a Habitat Suitability Index (HSI) study was undertaken of all relevant water bodies within 250m[1] of the site, along with ponds P5 and P10 (which are located just outside of the 250m buffer from the site).

4.2. Guidance set out within Natural England's Method Statement template, to be used when applying for a Great Crested Newt development licence, states that surveys of ponds within 500m of the

[1] 250m is the typical maximum migratory range of this species, see English Nature (2004) '*An assessment of the efficiency of capture techniques and the value of different habitats for the great crested newt* Triturus cristatus'. English Nature Research Report 576

site boundary are only required when '(a) data indicates that the pond(s) has potential to support a large Great Crested Newt population, (b) the footprint contains particularly favourable habitat, (c) the development would have a substantial negative effect on that habitat and (d) there is an absence of dispersal barriers.'

4.3. An HSI study is used to assess the potential of water bodies to support Great Crested Newt. It is undertaken by attributing a score to a number of factors that can affect the presence or absence of this species. Ten factors are utilised in an HSI assessment, as described below:

- *SI1 Location.* The location of the water body within Great Britain;
- *SI2 Pond area.* The size of the water body;
- *SI3 Permanence.* How often the water body dries out;
- *SI4 Water Quality.* The water quality, based primarily on invertebrate diversity;
- *SI5 Shade.* The percentage of the perimeter of the water body that is shaded;
- *SI6 Fowl.* The presence or absence of water fowl;
- *SI7 Fish.* The presence or absence of fish;
- *SI8 Pond Count.* The number of water bodies within 1km of the surveyed water body (not counting those on the far side of major barriers such as roads);
- *SI9 Terrestrial.* The quality of terrestrial habitat surrounding the water body; and
- *SI10 Macrophytes.* The percentage cover of the surface area of the water body covered by macrophytes (aquatic plants).

4.4. The overall suitability of the water body is then determined by entering these figures into an equation devised by Oldham *et al*. (2000)[2]. The suitability of water bodies is classed into one of five categories, either 'poor', 'below average', 'average', 'good' or 'excellent'.

4.5. This HSI study was undertaken in line with the guidelines developed by Oldham *et al*. and subsequently adapted by ARG UK (2010)[3]. A suitably experienced ecologist undertook the assessment in line with these guidelines, with the study also supplemented by desktop research where appropriate.

Environmental DNA (eDNA) Sampling

4.6. An eDNA survey was carried out to determine the presence/absence of Great Crested Newt within ponds P1, P3, P5, P6, P7 and P10 (see attached Plan 6638/PP1).

4.7. Water samples were collected during June 2023, following the procedure outlined in the methods manual prepared for DEFRA by Biggs *et al*. (2014)[4]. The survey fell within the acceptable seasonal window set out by Natural England (15th April to 30th June)[5]. Samples were collected by suitably licensed Aspect Ecology staff. The water samples were sent for laboratory analysis which was conducted by 'Cellmark' and also followed the procedure set out by Biggs *et al.* (2014)[14].

[2] Oldham RS, Keeble J, Swan MJS & Jeffcote M (2000) '*Evaluating the suitability of habitat for the Great Crested Newt (Triturus cristatus)'*. Herpetological Journal 10 (4), 143-155

[3] Amphibian & Reptile Groups of the UK (2010) '*ARG UK Advice Note 5: Great Crested Newt Habitat Suitability Index*'

[4] 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.

[5] Natural England (2015) '*Great crested newts: surveys and mitigation for development projects. Standing advice for local planning authorities who need to assess the impacts of development on great crested newts*'. Last updated at www.gov.uk on 24/12/2015.

5. Results and Discussion

5.1. As set out above, ponds P1, P3, P5, P6, P7 and P10 were subject to inspection and specific presence/absence survey (eDNA sampling) during June 2023 in order to determine the presence or likely absence of Great Crested Newt.

5.2. The results of the HSI assessment and eDNA survey in relation to ponds P1, P3, P5, P6, P7 and P10 are summarised in Table 5.1., below, with the associated laboratory analysis results appended to this report.

Table 5.1. *Great Crested Newt eDNA and HSI assessment results summary.*

Pond	Suitability Indices										HSI Score	Suitability	eDNA Survey
	SI 1 Location	**SI 2** Pond Area	**SI 3** Pond Drying	**SI 4** Water Quality	**SI 5** Shade	**SI 6** Water Fowl	**SI 7** Fish	**SI 8** Ponds	**SI 9** Terrestrial Habitat	**SI 10** Macrophytes			
P1	*1*	*0.6*	*0.9*	*0.67*	*1*	*1*	*1*	*1*	*1*	*0.8*	***0.88***	*Excellent*	***Negative (GCN Absent)***
P3	*1*	*0.6*	*1*	*0.01*	*1*	*1*	*1*	*1*	*0.67*	*0.3*	***0.51***	*Below Average*	***Negative (GCN Absent)***
P5	*1*	*0.4*	*1*	*1*	*1*	*1*	*1*	*1*	*0.67*	*0.9*	***0.87***	*Excellent*	***Positive (GCN Present)***
P6	*1*	*0.01*	*0.1*	*0.01*	*1*	*1*	*1*	*1*	*0.67*	*0.3*	***0.27***	*Poor*	***Negative (GCN Absent)***
P7	*1*	*0.8*	*0.9*	*0.01*	*1*	*1*	*1*	*1*	*0.67*	*0.3*	***0.52***	*Below Average*	***Negative (GCN Absent)***
P10	*1*	*0.05*	*0.5*	*0.67*	*1*	*1*	*1*	*1*	*1*	*0.8*	***0.65***	*Average*	***Negative (GCN Absent)***

5.3. The results of the survey work identified no evidence for the presence of Great Crested Newt within ponds P1, P3, P6, P7 and P10, such that this species is unlikely to be present within these ponds.

5.4. However, the eDNA sampling returned a positive result (indicating the presence of Great Crested Newt) within offsite pond P5. Based on the HSI result (confirmed by experienced surveyors), pond P5 was identified to provide excellent suitability for Great Crested Newt. Pond P5 is located approximately 250m east of the site boundary, over 500m from the closest proposed development area (with enhanced habitats proposed within the intervening land within the site boundary).

6. Summary and Conclusions

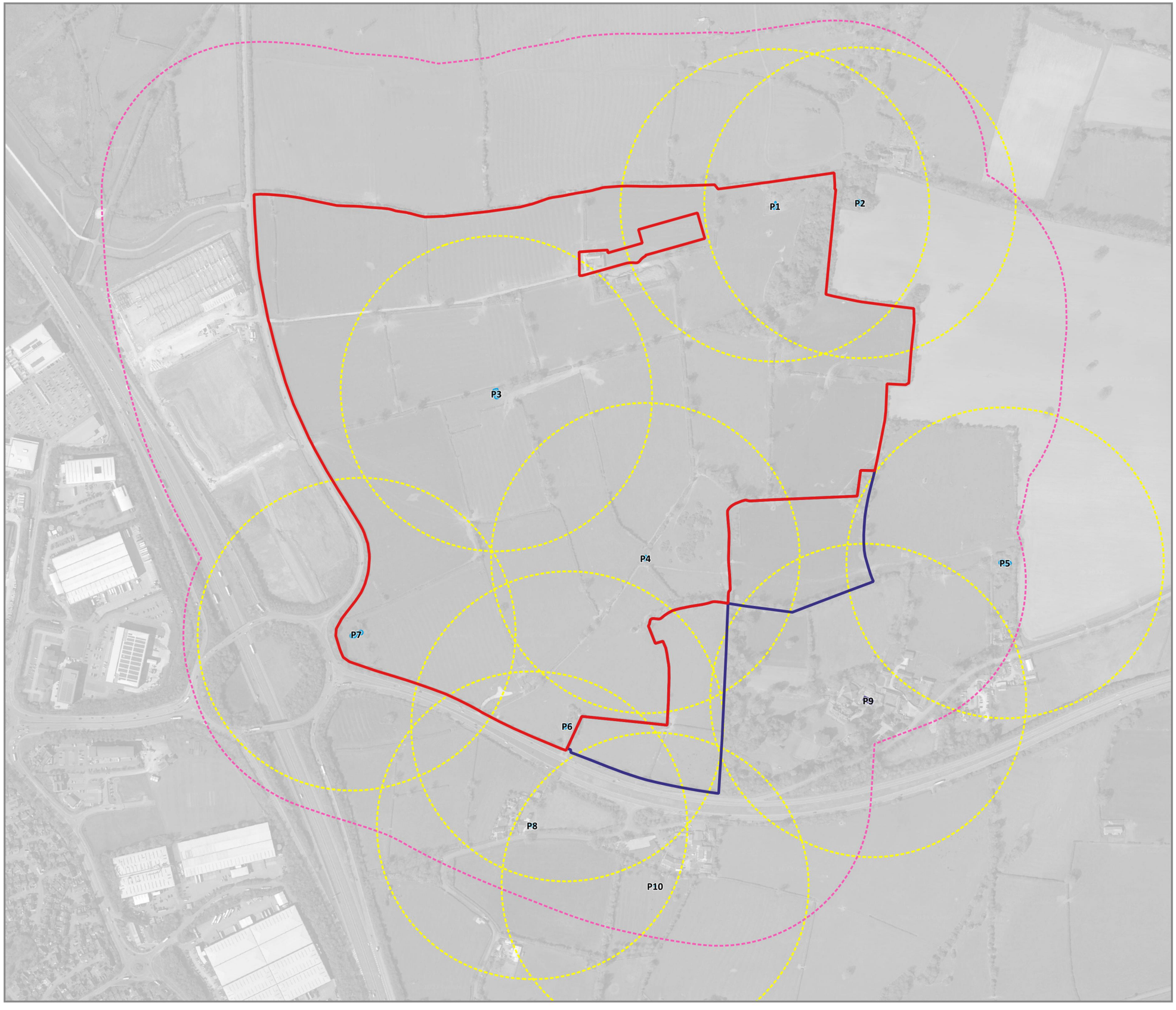
6.1. This note presents the findings of specific Great Crested Newt (eDNA) surveys and habitat suitability assessment in respect of Great Crested Newt, in order to confirm the presence or likely absence of this species within identified ponds/waterbodies in the vicinity of the site and inform the proposed development.

6.2. Ponds P1, P3, P5, P6, P7 and P10 were subject to eDNA survey for Great Crested Newt during June 2023. No evidence was recorded for the presence of Great Crested Newt within ponds P1, P3, P6, P7 or P10, however the eDNA survey returned a positive result (confirming the presence of Great Crested Newt) within pond P5.

6.3. Pond P5 is located approximately 250m from the site boundary and over 500m from the closest proposed development under the proposals. None of the other ponds present within the site or surrounding area were recorded to support Great Crested Newt and accordingly, this species is unlikely to represent a constraint in relation to the proposed development, nor require further consideration or mitigation based on the current information.

Appended

- *Plan 6638/PP1*
- *eDNA laboratory analysis results (Cellmark, June 2023)*



Key:

- Site Boundary
- Wider Survey Area
- Pond
- Swimming Pool
- 250m Pond Buffer
- 250m Site Boundary Buffer

0 90 180 270 360 m

aspect ecology

Aspect Ecology Limited - West Court - Hardwick Business Park
Noral Way - Banbury - Oxfordshire - OX16 2AF
01295 279721 - info@aspect-ecology.com - www.aspect-ecology.com

Land South West of Huscote Farm & East of Daventry Road, Banbury

Pond Plan

PROJECT

TITLE

6638/PP1

DRAWING NO.

A/JP

REV

March 2023

DATE

P:\Project\Aspect Ecology Projects\ECO 6600\1006638\Graphics\GIS\6638 BNGA.gpr

eDNA Technical Report

Ms. Iona Anderson
Aspect Ecology Ltd
West Court
Hardwick Business Park
Noral Way
Banbury

OX16 2AF

T: 01295279721

E: edna@aspect-ecology.com

Report Reference	R0000406
Report Date	14 Jun 2023
Reported By	jshaw

Dispatch Order Reference	P0000282
Site Name	P3
Site Location	Oxfordshire
OS Reference	CN+S1

Barcode	Received Date	Sampled Date	Sample Check	Degradation Check	Inhibition Check	Result	Positve Replicates
GCN004984	08/06/2023	01/06/2023	PASS	PASS	PASS	NEGATIVE	0 out of 12

Dispatch Order Reference	P0000282
Site Name	P1
Site Location	Oxfordshire
OS Reference	CN + S1

Barcode	Received Date	Sampled Date	Sample Check	Degradation Check	Inhibition Check	Result	Positve Replicates
GCN004974	08/06/2023	01/06/2023	PASS	PASS	PASS	NEGATIVE	0 out of 12

16 Blacklands Way,
Abingdon Business Park
Abingdon, OX14 1DY

B1 Ordnance Road
Buckshaw Village
Chorley, PR7 7EL

T +44 (0)1235 528609
E edna@cellmark.co.uk
W www.greatcrestednewtedna.co.uk

eDNA Technical Report

Dispatch Order Reference	P0000282
Site Name	P7
Site Location	Oxfordshire
OS Reference	CN + S1

Barcode	Received Date	Sampled Date	Sample Check	Degradation Check	Inhibition Check	Result	Positve Replicates
GCN004959	08/06/2023	01/06/2023	PASS	PASS	PASS	NEGATIVE	0 out of 12

16 Blacklands Way,
Abingdon Business Park
Abingdon, OX14 1DY

B1 Ordnance Road
Buckshaw Village
Chorley, PR7 7EL

T +44 (0)1235 528609
E edna@cellmark.co.uk
W www.greatcrestednewtedna.co.uk

eDNA Technical Report

SUMMARY

The water samples listed in the tables above were submitted to Cellmark for environmental DNA (eDNA) testing for the presence of Great Crested Newt (GCN; *Triturus cristatus*) DNA. The laboratory testing was carried out in compliance with the guidelines described in WC1067: Analytical and methodological development for improved surveillance of The Great Crested Newt (version 1.1)

INTERPRETATION OF THE RESULTS

Barcode	Each kit is given a unique sample barcode. A kit and the six sample tubes contained within it are labelled with the same sample barcode. This allows Cellmark to track where each kit has been sent and to track the samples through the laboratory once they have been returned.
Site Name	The name of the sampling site.
OS Reference	Ordnance Survey grid reference: the location of the pond.
Sample Check	Upon receipt in the laboratory, the 6 sample tubes are scored for sample volume, leakage, damage and for the presence of sediment, algae and other debris within the sample tubes. They are scored as 'PASS' or 'FAIL'. Samples that fail at this stage may not be suitable for further processing.
Degradation Check	A control marker is spiked into the sample tubes during the kit manufacturing process. This marker is analysed for degradation and reported as 'DEGRADED' or 'PASS'.
Inhibition Check	Some substances (inhibitors) can cause the GCN assay to give a negative result despite the presence of GCN DNA. An assay is performed to determine whether inhibitors are present in the eDNA extract. If inhibition is detected, steps are taken to mitigate the effects on the GCN detection assay. The degradation assay is reported as 'INHIBITED' or 'PASS'.
Result	Results are reported as 'POSITIVE', 'NEGATIVE' or 'INCONCLUSIVE'. A positive result indicates that there is evidence that Great Crested Newts are present or have recently been present in the pond. If no GCN DNA is detected, a negative result is reported. The results are deemed inconclusive if we do not detect the presence of GCN DNA and there is an indication that something in the sample is interfering with the analysis (inhibition or degradation).
Positive Replicates	A single eDNA extract is produced for each pond. The extract is then analysed to detect the presence of GCN DNA. A total of 12 replicates of this analysis is performed per eDNA extract. If at least 1 of the replicates is positive for the presence of GCN DNA, the pond is declared positive for the presence of Great Crested Newts.

METHODOLOGY

Upon arrival in the laboratory, the 6 sample tubes are checked for sample volume, leakage and any other damage. The samples are also inspected for macroscopic debris. Based on the outcome of this inspection, the decision is made as to whether the sample is suitable for further processing. Samples that have passed this inspection step are centrifuged. The resulting pellets (containing the eDNA) from each tube are then combined. The eDNA is then isolated (extracted) from the combined pellet.

Inhibitors, more specifically PCR inhibitors, are substances in the eDNA sample which may be co-isolated with the DNA and which interfere with eDNA detection assays. All eDNA extracts are tested for the presence of inhibitors. When a sample has been shown to be inhibited and the results of the GCN detection assay are negative, we cannot be sure whether the sample is truly negative for GCN DNA or that the inhibitors have prevented the GCN assay from working correctly. In this scenario, the result is reported as inconclusive.

16 Blacklands Way,
Abingdon Business Park
Abingdon, OX14 1DY

B1 Ordnance Road
Buckshaw Village
Chorley, PR7 7EL

T +44 (0)1235 528609
E edna@cellmark.co.uk
W www.greatcrestednewtedna.co.uk

eDNA Technical Report

The ability to detect a control marker that has been spiked into the sample tubes during the kit manufacturing process is also tested. If this marker cannot be detected, it suggests that that DNA in the sample has been degraded. Some possible causes of degradation can be the conditions under which the sample has been stored (eg exposure to high temperatures or UV from excessive sunlight) or contamination with substances that destroy DNA. If the control DNA is not detected but the GCN detection assay is positive for GCN, then the sample is reported as positive for GCN DNA. However, if neither the control DNA nor GCN DNA is detected, the sample is reported as inconclusive because we cannot know whether there was any GCN DNA present in the sample but it was degraded prior to analysis.

The GCN detection assay targets a portion of the GCN mitochondrial DNA. This assay is detailed in WC1067 Analytical and Methodological Development for Improved Surveillance of The Great Crested Newt (version 1.1). This assay specifically detects GCN DNA. If GCN DNA is detected in at least 1 of the 12 replicate GCN detection assays, the sample is reported as positive for the presence of GCN. A technique called quantative PCR (qPCR) is used in the inhibition, degradation and GCN detection assays to detect specific regions of DNA. Positive and negative controls are used in each of the assays and these have to give the expected results in order for the sample to be declared positive or negative for GCN DNA.

Cellmark participates in the FERA proficiency testing scheme and achieved 100% in the 2021 test. Driven by quality, Cellmark has held international ISO quality certification since 1990. Cellmark provides a range of laboratory testing services accredited to ISO 17025 and although delivered to the same exacting quality standards, Cellmark's eDNA service is not yet included on the scope of its ISO 17025 accreditation. Cellmark is certified to ISO 9001, ISO 14001 and to ISO 27001.

16 Blacklands Way,
Abingdon Business Park
Abingdon, OX14 1DY

B1 Ordnance Road
Buckshaw Village
Chorley, PR7 7EL

T +44 (0)1235 528609
E edna@cellmark.co.uk
W www.greatcrestednewtedna.co.uk

PRIVATE & CONFIDENTIAL

eDNA Technical Report

Ms. Iona Anderson
Aspect Ecology Ltd
West Court
Hardwick Business Park
Noral Way
Banbury

OX16 2AF

T: 01295279721

E: edna@aspect-ecology.com

Report Reference	R0000427
Report Date	27 Jun 2023
Reported By	jshaw

Dispatch Order Reference	P0000282
Site Name	P5
Site Location	LE M40 Banbury 6638-01
OS Reference	CC+BH

Barcode	Received Date	Sampled Date	Sample Check	Degradation Check	Inhibition Check	Result	Positve Replicates
GCN004963	23/06/2023	13/06/2023	PASS	PASS	PASS	POSITIVE	12 out of 12

16 Blacklands Way,
Abingdon Business Park
Abingdon, OX14 1DY

B1 Ordnance Road
Buckshaw Village
Chorley, PR7 7EL

T +44 (0)1235 528609
E edna@cellmark.co.uk
W www.greatcrestednewtedna.co.uk

eDNA Technical Report

SUMMARY

The water samples listed in the tables above were submitted to Cellmark for environmental DNA (eDNA) testing for the presence of Great Crested Newt (GCN; *Triturus cristatus*) DNA. The laboratory testing was carried out in compliance with the guidelines described in WC1067: Analytical and methodological development for improved surveillance of The Great Crested Newt (version 1.1)

INTERPRETATION OF THE RESULTS

Barcode	Each kit is given a unique sample barcode. A kit and the six sample tubes contained within it are labelled with the same sample barcode. This allows Cellmark to track where each kit has been sent and to track the samples through the laboratory once they have been returned.
Site Name	The name of the sampling site.
OS Reference	Ordnance Survey grid reference: the location of the pond.
Sample Check	Upon receipt in the laboratory, the 6 sample tubes are scored for sample volume, leakage, damage and for the presence of sediment, algae and other debris within the sample tubes. They are scored as 'PASS' or 'FAIL'. Samples that fail at this stage may not be suitable for further processing.
Degradation Check	A control marker is spiked into the sample tubes during the kit manufacturing process. This marker is analysed for degradation and reported as 'DEGRADED' or 'PASS'.
Inhibition Check	Some substances (inhibitors) can cause the GCN assay to give a negative result despite the presence of GCN DNA. An assay is performed to determine whether inhibitors are present in the eDNA extract. If inhibition is detected, steps are taken to mitigate the effects on the GCN detection assay. The degradation assay is reported as 'INHIBITED' or 'PASS'.
Result	Results are reported as 'POSITIVE', 'NEGATIVE' or 'INCONCLUSIVE'. A positive result indicates that there is evidence that Great Crested Newts are present or have recently been present in the pond. If no GCN DNA is detected, a negative result is reported. The results are deemed inconclusive if we do not detect the presence of GCN DNA and there is an indication that something in the sample is interfering with the analysis (inhibition or degradation).
Positive Replicates	A single eDNA extract is produced for each pond. The extract is then analysed to detect the presence of GCN DNA. A total of 12 replicates of this analysis is performed per eDNA extract. If at least 1 of the replicates is positive for the presence of GCN DNA, the pond is declared positive for the presence of Great Crested Newts.

METHODOLOGY

Upon arrival in the laboratory, the 6 sample tubes are checked for sample volume, leakage and any other damage. The samples are also inspected for macroscopic debris. Based on the outcome of this inspection, the decision is made as to whether the sample is suitable for further processing. Samples that have passed this inspection step are centrifuged. The resulting pellets (containing the eDNA) from each tube are then combined. The eDNA is then isolated (extracted) from the combined pellet.

Inhibitors, more specifically PCR inhibitors, are substances in the eDNA sample which may be co-isolated with the DNA and which interfere with eDNA detection assays. All eDNA extracts are tested for the presence of inhibitors. When a sample has been shown to be inhibited and the results of the GCN detection assay are negative, we cannot be sure whether the sample is truly negative for GCN DNA or that the inhibitors have prevented the GCN assay from working correctly. In this scenario, the result is reported as inconclusive.

16 Blacklands Way,
Abingdon Business Park
Abingdon, OX14 1DY

B1 Ordnance Road
Buckshaw Village
Chorley, PR7 7EL

T +44 (0)1235 528609
E edna@cellmark.co.uk
W www.greatcrestednewtedna.co.uk

eDNA Technical Report

The ability to detect a control marker that has been spiked into the sample tubes during the kit manufacturing process is also tested. If this marker cannot be detected, it suggests that that DNA in the sample has been degraded. Some possible causes of degradation can be the conditions under which the sample has been stored (eg exposure to high temperatures or UV from excessive sunlight) or contamination with substances that destroy DNA. If the control DNA is not detected but the GCN detection assay is positive for GCN, then the sample is reported as positive for GCN DNA. However, if neither the control DNA nor GCN DNA is detected, the sample is reported as inconclusive because we cannot know whether there was any GCN DNA present in the sample but it was degraded prior to analysis.

The GCN detection assay targets a portion of the GCN mitochondrial DNA. This assay is detailed in WC1067 Analytical and Methodological Development for Improved Surveillance of The Great Crested Newt (version 1.1). This assay specifically detects GCN DNA. If GCN DNA is detected in at least 1 of the 12 replicate GCN detection assays, the sample is reported as positive for the presence of GCN. A technique called quantative PCR (qPCR) is used in the inhibition, degradation and GCN detection assays to detect specific regions of DNA. Positive and negative controls are used in each of the assays and these have to give the expected results in order for the sample to be declared positive or negative for GCN DNA.

Cellmark participates in the FERA proficiency testing scheme and achieved 100% in the 2021 test. Driven by quality, Cellmark has held international ISO quality certification since 1990. Cellmark provides a range of laboratory testing services accredited to ISO 17025 and although delivered to the same exacting quality standards, Cellmark's eDNA service is not yet included on the scope of its ISO 17025 accreditation. Cellmark is certified to ISO 9001, ISO 14001 and to ISO 27001.

16 Blacklands Way,
Abingdon Business Park
Abingdon, OX14 1DY

B1 Ordnance Road
Buckshaw Village
Chorley, PR7 7EL

T +44 (0)1235 528609
E edna@cellmark.co.uk
W www.greatcrestednewtedna.co.uk

PRIVATE & CONFIDENTIAL

eDNA Technical Report

Ms. Iona Anderson
Aspect Ecology Ltd
West Court
Hardwick Business Park
Noral Way
Banbury

OX16 2AF

T: 01295279721

E: edna@aspect-ecology.com

Report Reference	R0000452
Report Date	07 Jul 2023
Reported By	jshaw

Dispatch Order Reference	P0000282						
Site Name	6638-P6						
Site Location	M40 - Banbury						
Barcode	**Received Date**	**Sampled Date**	**Sample Check**	**Degradation Check**	**Inhibition Check**	**Result**	**Positve Replicates**
GCN004977	28/06/2023	23/06/2023	PASS	PASS	PASS	NEGATIVE	0 out of 12

16 Blacklands Way,
Abingdon Business Park
Abingdon, OX14 1DY

B1 Ordnance Road
Buckshaw Village
Chorley, PR7 7EL

T +44 (0)1235 528609
E edna@cellmark.co.uk
W www.greatcrestednewtedna.co.uk

eDNA Technical Report

SUMMARY

The water samples listed in the tables above were submitted to Cellmark for environmental DNA (eDNA) testing for the presence of Great Crested Newt (GCN; *Triturus cristatus*) DNA. The laboratory testing was carried out in compliance with the guidelines described in WC1067: Analytical and methodological development for improved surveillance of The Great Crested Newt (version 1.1)

INTERPRETATION OF THE RESULTS

Barcode	Each kit is given a unique sample barcode. A kit and the six sample tubes contained within it are labelled with the same sample barcode. This allows Cellmark to track where each kit has been sent and to track the samples through the laboratory once they have been returned.
Site Name	The name of the sampling site.
OS Reference	Ordnance Survey grid reference: the location of the pond.
Sample Check	Upon receipt in the laboratory, the 6 sample tubes are scored for sample volume, leakage, damage and for the presence of sediment, algae and other debris within the sample tubes. They are scored as 'PASS' or 'FAIL'. Samples that fail at this stage may not be suitable for further processing.
Degradation Check	A control marker is spiked into the sample tubes during the kit manufacturing process. This marker is analysed for degradation and reported as 'DEGRADED' or 'PASS'.
Inhibition Check	Some substances (inhibitors) can cause the GCN assay to give a negative result despite the presence of GCN DNA. An assay is performed to determine whether inhibitors are present in the eDNA extract. If inhibition is detected, steps are taken to mitigate the effects on the GCN detection assay. The degradation assay is reported as 'INHIBITED' or 'PASS'.
Result	Results are reported as 'POSITIVE', 'NEGATIVE' or 'INCONCLUSIVE'. A positive result indicates that there is evidence that Great Crested Newts are present or have recently been present in the pond. If no GCN DNA is detected, a negative result is reported. The results are deemed inconclusive if we do not detect the presence of GCN DNA and there is an indication that something in the sample is interfering with the analysis (inhibition or degradation).
Positive Replicates	A single eDNA extract is produced for each pond. The extract is then analysed to detect the presence of GCN DNA. A total of 12 replicates of this analysis is performed per eDNA extract. If at least 1 of the replicates is positive for the presence of GCN DNA, the pond is declared positive for the presence of Great Crested Newts.

METHODOLOGY

Upon arrival in the laboratory, the 6 sample tubes are checked for sample volume, leakage and any other damage. The samples are also inspected for macroscopic debris. Based on the outcome of this inspection, the decision is made as to whether the sample is suitable for further processing. Samples that have passed this inspection step are centrifuged. The resulting pellets (containing the eDNA) from each tube are then combined. The eDNA is then isolated (extracted) from the combined pellet.

Inhibitors, more specifically PCR inhibitors, are substances in the eDNA sample which may be co-isolated with the DNA and which interfere with eDNA detection assays. All eDNA extracts are tested for the presence of inhibitors. When a sample has been shown to be inhibited and the results of the GCN detection assay are negative, we cannot be sure whether the sample is truly negative for GCN DNA or that the inhibitors have prevented the GCN assay from working correctly. In this scenario, the result is reported as inconclusive.

16 Blacklands Way,
Abingdon Business Park
Abingdon, OX14 1DY

B1 Ordnance Road
Buckshaw Village
Chorley, PR7 7EL

T +44 (0)1235 528609
E edna@cellmark.co.uk
W www.greatcrestednewtedna.co.uk

eDNA Technical Report

The ability to detect a control marker that has been spiked into the sample tubes during the kit manufacturing process is also tested. If this marker cannot be detected, it suggests that that DNA in the sample has been degraded. Some possible causes of degradation can be the conditions under which the sample has been stored (eg exposure to high temperatures or UV from excessive sunlight) or contamination with substances that destroy DNA. If the control DNA is not detected but the GCN detection assay is positive for GCN, then the sample is reported as positive for GCN DNA. However, if neither the control DNA nor GCN DNA is detected, the sample is reported as inconclusive because we cannot know whether there was any GCN DNA present in the sample but it was degraded prior to analysis.

The GCN detection assay targets a portion of the GCN mitochondrial DNA. This assay is detailed in WC1067 Analytical and Methodological Development for Improved Surveillance of The Great Crested Newt (version 1.1). This assay specifically detects GCN DNA. If GCN DNA is detected in at least 1 of the 12 replicate GCN detection assays, the sample is reported as positive for the presence of GCN. A technique called quantative PCR (qPCR) is used in the inhibition, degradation and GCN detection assays to detect specific regions of DNA. Positive and negative controls are used in each of the assays and these have to give the expected results in order for the sample to be declared positive or negative for GCN DNA.

Cellmark participates in the FERA proficiency testing scheme and achieved 100% in the 2021 test. Driven by quality, Cellmark has held international ISO quality certification since 1990. Cellmark provides a range of laboratory testing services accredited to ISO 17025 and although delivered to the same exacting quality standards, Cellmark's eDNA service is not yet included on the scope of its ISO 17025 accreditation. Cellmark is certified to ISO 9001, ISO 14001 and to ISO 27001.

16 Blacklands Way,
Abingdon Business Park
Abingdon, OX14 1DY

B1 Ordnance Road
Buckshaw Village
Chorley, PR7 7EL

T +44 (0)1235 528609
E edna@cellmark.co.uk
W www.greatcrestednewtedna.co.uk

eDNA Technical Report

Ms. Iona Anderson
Aspect Ecology Ltd
West Court
Hardwick Business Park
Noral Way
Banbury

OX16 2AF

T: 01295279721

E: edna@aspect-ecology.com

Report Reference	R0000409
Report Date	15 Jun 2023
Reported By	akarlsson

Dispatch Order Reference	P0000282
Site Name	P10
Site Location	Oxfordshire
OS Reference	CN + SN

Barcode	Received Date	Sampled Date	Sample Check	Degradation Check	Inhibition Check	Result	Positve Replicates
GCN004975	08/06/2023	01/06/2023	PASS	PASS	PASS	NEGATIVE	0 out of 12

Dispatch Order Reference	P0000282
Site Name	P6
Site Location	Oxfordshire
OS Reference	CN + S1

Barcode	Received Date	Sampled Date	Sample Check	Degradation Check	Inhibition Check	Result	Positve Replicates
GCN004962	08/06/2023	01/06/2023	PASS	DEGRADED	PASS	INCONCLUSIVE	0 out of 12

16 Blacklands Way,
Abingdon Business Park
Abingdon, OX14 1DY

B1 Ordnance Road
Buckshaw Village
Chorley, PR7 7EL

T +44 (0)1235 528609
E edna@cellmark.co.uk
W www.greatcrestednewtedna.co.uk

eDNA Technical Report

SUMMARY

The water samples listed in the tables above were submitted to Cellmark for environmental DNA (eDNA) testing for the presence of Great Crested Newt (GCN; *Triturus cristatus*) DNA. The laboratory testing was carried out in compliance with the guidelines described in WC1067: Analytical and methodological development for improved surveillance of The Great Crested Newt (version 1.1)

INTERPRETATION OF THE RESULTS

Barcode	Each kit is given a unique sample barcode. A kit and the six sample tubes contained within it are labelled with the same sample barcode. This allows Cellmark to track where each kit has been sent and to track the samples through the laboratory once they have been returned.
Site Name	The name of the sampling site.
OS Reference	Ordnance Survey grid reference: the location of the pond.
Sample Check	Upon receipt in the laboratory, the 6 sample tubes are scored for sample volume, leakage, damage and for the presence of sediment, algae and other debris within the sample tubes. They are scored as 'PASS' or 'FAIL'. Samples that fail at this stage may not be suitable for further processing.
Degradation Check	A control marker is spiked into the sample tubes during the kit manufacturing process. This marker is analysed for degradation and reported as 'DEGRADED' or 'PASS'.
Inhibition Check	Some substances (inhibitors) can cause the GCN assay to give a negative result despite the presence of GCN DNA. An assay is performed to determine whether inhibitors are present in the eDNA extract. If inhibition is detected, steps are taken to mitigate the effects on the GCN detection assay. The degradation assay is reported as 'INHIBITED' or 'PASS'.
Result	Results are reported as 'POSITIVE', 'NEGATIVE' or 'INCONCLUSIVE'. A positive result indicates that there is evidence that Great Crested Newts are present or have recently been present in the pond. If no GCN DNA is detected, a negative result is reported. The results are deemed inconclusive if we do not detect the presence of GCN DNA and there is an indication that something in the sample is interfering with the analysis (inhibition or degradation).
Positive Replicates	A single eDNA extract is produced for each pond. The extract is then analysed to detect the presence of GCN DNA. A total of 12 replicates of this analysis is performed per eDNA extract. If at least 1 of the replicates is positive for the presence of GCN DNA, the pond is declared positive for the presence of Great Crested Newts.

METHODOLOGY

Upon arrival in the laboratory, the 6 sample tubes are checked for sample volume, leakage and any other damage. The samples are also inspected for macroscopic debris. Based on the outcome of this inspection, the decision is made as to whether the sample is suitable for further processing. Samples that have passed this inspection step are centrifuged. The resulting pellets (containing the eDNA) from each tube are then combined. The eDNA is then isolated (extracted) from the combined pellet.

Inhibitors, more specifically PCR inhibitors, are substances in the eDNA sample which may be co-isolated with the DNA and which interfere with eDNA detection assays. All eDNA extracts are tested for the presence of inhibitors. When a sample has been shown to be inhibited and the results of the GCN detection assay are negative, we cannot be sure whether the sample is truly negative for GCN DNA or that the inhibitors have prevented the GCN assay from working correctly. In this scenario, the result is reported as inconclusive.

16 Blacklands Way,
Abingdon Business Park
Abingdon, OX14 1DY

B1 Ordnance Road
Buckshaw Village
Chorley, PR7 7EL

T +44 (0)1235 528609
E edna@cellmark.co.uk
W www.greatcrestednewtedna.co.uk

eDNA Technical Report

The ability to detect a control marker that has been spiked into the sample tubes during the kit manufacturing process is also tested. If this marker cannot be detected, it suggests that that DNA in the sample has been degraded. Some possible causes of degradation can be the conditions under which the sample has been stored (eg exposure to high temperatures or UV from excessive sunlight) or contamination with substances that destroy DNA. If the control DNA is not detected but the GCN detection assay is positive for GCN, then the sample is reported as positive for GCN DNA. However, if neither the control DNA nor GCN DNA is detected, the sample is reported as inconclusive because we cannot know whether there was any GCN DNA present in the sample but it was degraded prior to analysis.

The GCN detection assay targets a portion of the GCN mitochondrial DNA. This assay is detailed in WC1067 Analytical and Methodological Development for Improved Surveillance of The Great Crested Newt (version 1.1). This assay specifically detects GCN DNA. If GCN DNA is detected in at least 1 of the 12 replicate GCN detection assays, the sample is reported as positive for the presence of GCN. A technique called quantative PCR (qPCR) is used in the inhibition, degradation and GCN detection assays to detect specific regions of DNA. Positive and negative controls are used in each of the assays and these have to give the expected results in order for the sample to be declared positive or negative for GCN DNA.

Cellmark participates in the FERA proficiency testing scheme and achieved 100% in the 2021 test. Driven by quality, Cellmark has held international ISO quality certification since 1990. Cellmark provides a range of laboratory testing services accredited to ISO 17025 and although delivered to the same exacting quality standards, Cellmark's eDNA service is not yet included on the scope of its ISO 17025 accreditation. Cellmark is certified to ISO 9001, ISO 14001 and to ISO 27001.

16 Blacklands Way,
Abingdon Business Park
Abingdon, OX14 1DY

B1 Ordnance Road
Buckshaw Village
Chorley, PR7 7EL

T +44 (0)1235 528609
E edna@cellmark.co.uk
W www.greatcrestednewtedna.co.uk

Copyright

Legal Guidance

The information set out within this report in no way constitutes a legal opinion on the relevant legislation (refer to the original legislation). The opinion of a legal professional should be sought if further advice is required.

Liability

This report has been prepared for the exclusive use of the commissioning client and unless otherwise agreed in writing by Aspect Ecology, no other party may use, or rely on the contents of the report. No liability is accepted by Aspect Ecology for any use of this report, other than for the purposes for which it was originally prepared and provided. No warranty, express or implied, is made as to the advice in this report.

ecology • landscape planning • arboriculture

aspect

Aspect Ecology Ltd
West Court
Hardwick Business Park
Noral Way
Banbury
Oxfordshire OX16 2AF

T: 01295 279721
E: info@aspect-ecology.com
W: www.aspect-ecology.com