

Technical Briefing Note

Ref: 1005176 TN04 eDNA dv1

Land at Stratfield Farm, Kidlington (1005176)

Great Crested Newt eDNA Survey Results

Date: August 2023

1. Introduction

- 1.1. Aspect Ecology has been commissioned by Manor Oak Homes in regard to the proposed development of land at Stratfield Farm, Kidlington, centred at OS grid reference SP 495 124 ('the site').
- 1.2. The site is the subject of an outline planning application (ref: 22/01611/OUT) for the construction of up to 118 no dwellings (all matters reserved except for access) with vehicular access from Oxford Road, which was submitted to Cherwell District Council in 2022. The application was informed by ecological survey and appraisal work, including as set out within the submitted Ecological Appraisal report (ref: 5176 EcoAp vf). In particular, the presence of suitable habitats for the fully protected amphibian species Great Crested Newt was noted within the previously submitted information, with specific survey work undertaken for this species in 2017 and again in 2020.
- 1.3. Subsequently, consultation comments received from Cherwell District Council's Ecology Officer (dated 24 May 2023) requested update survey work in regard to this species given the time that has now elapsed since the previous surveys. Accordingly, in order to update the position and further inform the application, further Great Crested Newt survey work has been undertaken at the site in 2023, the results of which are set out below, with reference to the previous conclusions.

2. Identification of Ponds

- 2.1. As set out within the previously submitted information, a total of two ponds (P1 and P2) and a single wet ditch (D1) were previously recorded to be present within the site (albeit P2 was recorded to remain dry for much of the original survey period). These features were subject to further inspection in June 2023 to confirm the current position.
- 2.2. During the updated inspection, P2 was confirmed to remain dry and therefore clearly does not provide potentially suitable breeding opportunities for Great Crested Newt and was scoped out of further surveys or consideration.
- 2.3. Pond P1 and Ditch D1 were recorded to support standing water at the time of surveys and accordingly, were subject to further survey, as detailed below.
- 2.4. A further three waterbodies were previously identified within 250m of the site based on available background information, (labelled P3 to P5 at Plan 5176/ECO5). On inspection, P3 was previously recorded to remain dry, such that it was not considered to provide potentially suitable breeding opportunities for Great Crested Newt at that time. Similarly the identified offsite waterbodies were subject to inspection in June 2023, during which time the position for each waterbody was recorded to remain broadly consistent with the previous information (i.e. P3 was not recorded to

support standing water and was therefore scoped out of further consideration, whilst P4 and P5 remained present and were therefore subject to further survey, as detailed below..

3. Survey Methodology

- 3.1. As set out above a total of two onsite waterbodies (P1 and D1), along with a further 2 offsite waterbodies located within 250m of the site (P4 and P5) continue to provide apparently potentially suitable breeding opportunities for Great Crested Newt.
- 3.2. Accordingly, ponds P1, P4 and P5, along with Ditch D1 were subject to further environmental DNA survey during 2023 in order to update the previous information and further confirm the presence or continued likely absence of this species.

Environmental DNA (eDNA) Sampling

- 3.3. As set out within the previously submitted information, an initial eDNA survey was carried out within pond P1 and ditch D1 during 2017, whilst further eDNA surveys of P1, P4 and D1 were undertaken in 2020.
- 3.4. Given the time that has elapsed since the previous surveys, in order to confirm the current position in regard to this species and update the previous information, further eDNA surveys were carried out to determine the presence/absence of Great Crested Newt within ponds P1, P4 and P5, along with ditch D1 in 2023.
- 3.5. Water samples were collected during June 2023, following the procedure outlined in the methods manual prepared for DEFRA by Biggs *et al.* (2014)¹. The survey fell within the acceptable seasonal window set out by Natural England (15th April to 30th June)². 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.

4. Results and Discussion

Previous Survey Results

- 4.1. As set out within the previously submitted information, specific eDNA survey work for Great Crested Newt was undertaken of ponds P1 and D1 during 2017, which returned a 'negative' result (GCN absent) from D1 and a 'positive' result for P1 (indicating Great Crested Newt as being likely present at that time). In order to provide updated information, further specific eDNA survey work for Great Crested Newt was subsequently undertaken of ponds P1, D1 and offsite P4 during 2020, all of which returned 'negative' results, indicating the likely absence of Great Crested Newt.
- 4.2. On this basis, it was concluded (Aspect Ecology, 2022) that either the previous (positive) eDNA sample from P1 in 2017 represented a 'false-positive' result (such that Great Crested Newt was in fact absent) or small numbers of this species were present during 2017, which had subsequently died out such that this species was no longer likely to be present at the time of the 2020 surveys.

¹ 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.

² 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.

Current Survey Results and Discussion

- 4.3. As set out above, pond P1 and ditch D1, along with offsite waterbodies P4 and P5 were subject to further specific presence/absence survey (eDNA sampling) during June 2023 in order to further update the position and confirm the current presence or likely absence of Great Crested Newt in relation to the site.
- 4.4. A summary of the Great Crested Newt survey results in relation to the identified waterbodies at the site and within 250m of the site boundary is set out in Table 4.1., below, with the associated laboratory analysis results appended to this report.

Table 4.1. Great Crested Newt survey results summary.

Waterbody Ref.	Approx. distance from site (m)	HSI Assessment		eDNA Survey Results			Conclusion/Summary
		HSI Score	Suitability	2017	2020	2023	
P1	0 (Onsite)	0.62	Average	Positive (GCN Present)	Negative (GCN Absent)	Negative (GCN Absent)	Great Crested Newt unlikely to be present – no further consideration required.
P2	0 (Onsite)	N/A – Previous HSI indicated poor suitability, whilst pond recorded to remain dry during 2023 such that it clearly does not provide potential suitability for use by breeding Great Crested Newt – scoped out of further survey work.					
D1	0 (Onsite)	0.61	Average	Negative (GCN Absent)	Negative (GCN Absent)	Negative (GCN Absent)	Great Crested Newt unlikely to be present – no further consideration required.
P3	N/A – Recorded to be dry during 2020 and 2023 inspections, such that it clearly does not provide potential suitability for use by breeding Great Crested Newt – scoped out of further survey work.						
P4	40	0.82	Excellent	N/A	Negative (GCN Absent)	Negative (GCN Absent)	Great Crested Newt unlikely to be present – no further consideration required.
P5	180	0.78	Good	N/A	N/A	Negative (GCN Absent)	Great Crested Newt unlikely to be present – no further consideration required.

- 4.5. As set out, the current eDNA surveys returned ‘negative’ results (i.e. likely absence of Great Crested Newt) for all of the four waterbodies sampled. This remains in line with the most recent previous (2020) survey results, such that there is no evidence for the presence of Great Crested Newt within the site or immediate surroundings (and further supporting the previous conclusions in regard to the historic (2017) survey results within pond P1).
- 4.6. Accordingly, the conclusions drawn in relation to amphibian species within the previously submitted Ecological Appraisal (Aspect Ecology, 2022) remain unchanged. In particular, the current survey results clearly support the previous conclusion in regard to the historic (2017) survey result indicating a positive result for P1, that either this sample produced a ‘false-positive’ result (such that Great Crested Newt was in fact absent at the time) or small numbers of this species were present during 2017, which have subsequently died out (prior to 2020) such that this species is no longer present
- 4.7. Great Crested Newt therefore appears likely to remain absent from the site and therefore does not appear to represent a particular/current constraint on the proposed development, nor require further specific consideration or mitigation measures (nor recourse to District Licensing).

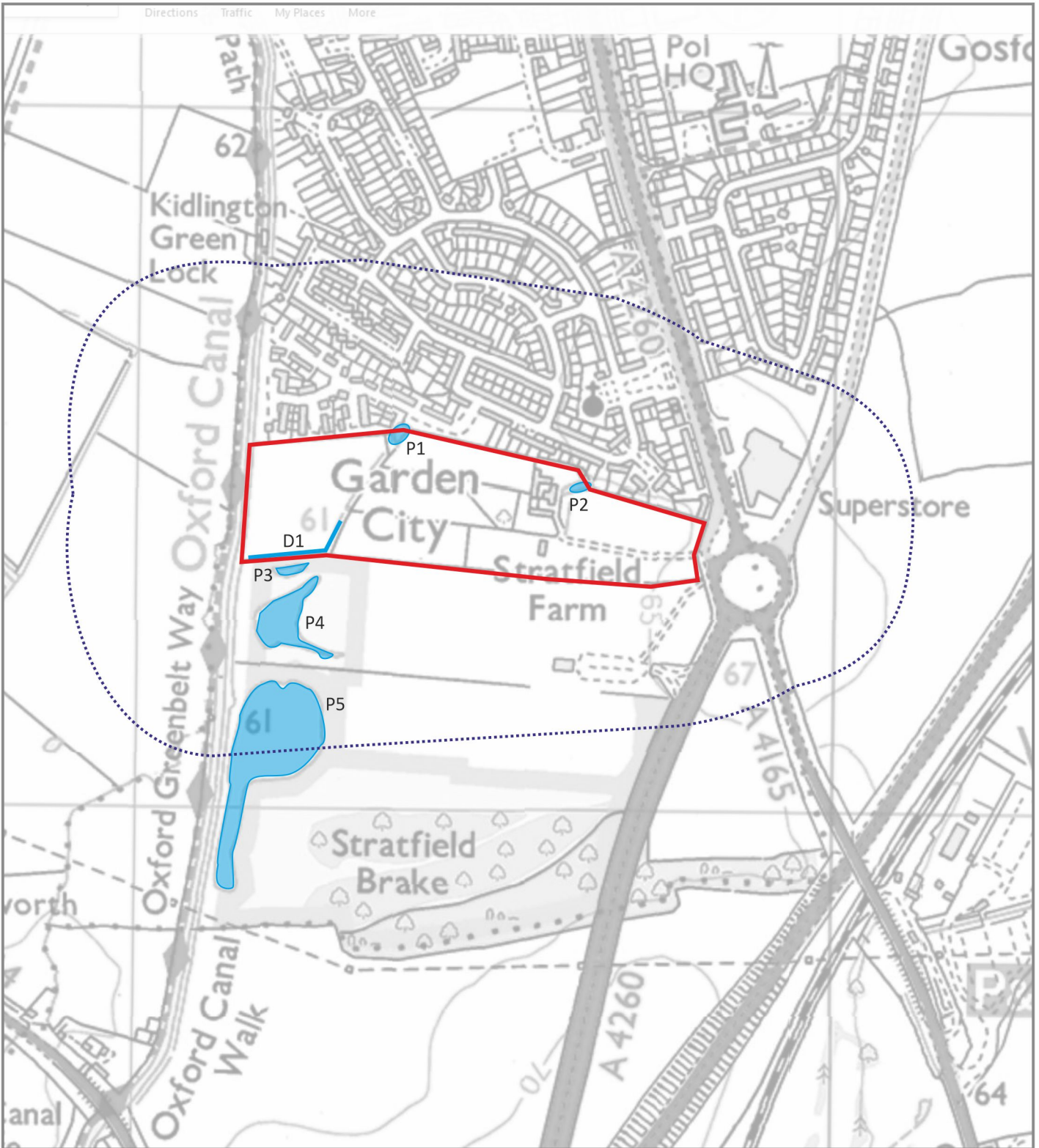
Nonetheless (in line with the previous considerations and standard guidelines), and particularly given the previous (historic) survey results should any further considerable delays ensue prior to any commencement of works at the site, it is recommended that the continued absence of this species be further confirmed at that time where appropriate.

5. Summary and Conclusions

- 5.1. This note presents the findings of specific Great Crested Newt (eDNA) surveys work undertaken in 2023, in order to update the previous surveys from 2017 and 2020 and confirm the current position in regard to the presence or likely absence of this species within identified ponds/waterbodies in the vicinity of the site and further inform the proposed development (including in order to address comments received from Cherwell DC Ecology Officer in this regard).
- 5.2. Pond P1 and ditch D1, along with offsite ponds P4 and P5 were subject to further eDNA survey work for Great Crested Newt (previous eDNA survey work having been undertaken in 2017 and 2020 to inform the proposals). No evidence was recorded for the presence of Great Crested Newt within any of the waterbodies present during 2023 (consistent with the most recent previous surveys in 2020).
- 5.3. Accordingly, the conclusions drawn in relation to amphibian species within the previously submitted Ecological Appraisal (Aspect Ecology, 2022) remain unchanged and Great Crested Newt appears to remain absent the site and is therefore does not appear to represent a particular constraint on the proposed development, nor require further specific consideration or mitigation measures. Nonetheless (in line with the previous considerations and standard guidelines), and particularly given the previous (historic) survey results should any further considerable delays ensue prior to any commencement of works at the site, it is recommended that the continued absence of this species be further confirmed at that time where appropriate.

Appended

- *Plan 5176/ECO5*
- *eDNA laboratory analysis results (Cellmark, July 2023)*



Key:



Site Boundary



Location of waterbody



Indicative 250m zone around site



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Stratfield Farm, Kidlington

PROJECT

GCN Pond Plan

TITLE

5176/EC05

DRAWING NO.

B/JP

REV.

April 2022

DATE



eDNA Technical Report



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Report Date	17 Jul 2023
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Dispatch Order Reference	P0000282						
Site Name	5176 - P1						
Site Location	Kidlington						
OS Reference	N/A						
Barcode	Received Date	Sampled Date	Sample Check	Degradation Check	Inhibition Check	Result	Positive Replicates
GCN003118	04/07/2023	28/06/2023	PASS	PASS	PASS	NEGATIVE	0 out of 12

Dispatch Order Reference	P0000282						
Site Name	5176 - P4						
Site Location	Kidlington						
OS Reference	N/A						
Barcode	Received Date	Sampled Date	Sample Check	Degradation Check	Inhibition Check	Result	Positive Replicates
GCN003056	04/07/2023	28/06/2023	PASS	PASS	PASS	NEGATIVE	0 out of 12

eDNA Technical Report



Dispatch Order Reference		P0000282					
Site Name		5176 - D1					
Site Location		Kidlington					
OS Reference		N/A					
Barcode	Received Date	Sampled Date	Sample Check	Degradation Check	Inhibition Check	Result	Positive Replicates
GCN003117	04/07/2023	28/06/2023	PASS	PASS	PASS	NEGATIVE	0 out of 12

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.

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 quantitative 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.

eDNA Technical Report



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Site Name	5176 - P5						
Site Location	Kidlington						
OS Reference	N/A						
Barcode	Received Date	Sampled Date	Sample Check	Degradation Check	Inhibition Check	Result	Positive Replicates
GCN003074	04/07/2023	28/06/2023	PASS	PASS	PASS	NEGATIVE	0 out of 12

eDNA Technical Report



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