3 June 2021

Francis Gooddy
Far Horizons
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Bampton
OX18 2AA

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Dear Francis,

Far Horizons, Bampton – Great Crested Newt eDNA Survey

I write to you in regard to the great crested newt environmental DNA survey conducted of one pond on site and two ponds within 500m of Far Horizons, Bampton on 20th May 2021.

Methods

Francis Gooddy commissioned Ecology by Design to conduct an environmental DNA survey of one pond on site and two ponds within 500m of Far Horizons, Mount Owen Road, Lew, Bampton, OX18 2AA (approximate central grid reference SP 32467 04725).

The survey was conducted by Ben Gardner (Natural England Level 1 - 2015–183136-CLS-CLS) and Olyvia Hall in dry, sunny conditions on 20th May 2021. The survey involved taking a number of water samples from the ponds and mixing them with sterile preservative. The samples were sent to an approved laboratory for analysis for great crested newt DNA. Water samples can only be taken between 15th April and 30th June and the recommended Natural England protocol was followed.

Results

The following results were returned. Please see attached map for pond locations and appended report from the laboratory

Pond 1 (Far Horizons) – Negative Result

Pond 2 – Negative Result

Pond 3 – Positive Result

Potential Impacts and Recommendations

Pond 3 is 150m north of the site within a residential setting, surrounded by agricultural fields. The pond is separated from Far Horizons by a garden, a road and a section of paddock. Using the Natural England risk calculator[1], the risk of harming a great crested newt is identified as being "offence highly unlikely" as shown in Figure 1 below.

Figure 1 – Natural England – Rapid Risk Assessment

Component	**Likely effect** (select one for each component; select the most harmful option if more than one is likely; lists are in order of harm, top to bottom)	Notional offence probability score
Great crested newt breeding pond(s)	No effect	0
Land within 100m of any breeding pond(s)	No effect	0
Land 100-250m from any breeding pond(s)	0.1 - 0.5 ha lost or damaged	0.1
Land >250m from any breeding pond(s)	No effect	0
Individual great crested newts	No effect	0
	Maximum:	0.1
Rapid risk assessment result:	GREEN: OFFENCE HIGHLY UNLIKELY	

There are no waterbodies to the south or east of the site therefore it is unlikely that great crested newts would commute across the site from Pond 3. Ponds 2 is 430m north-east of the site and returned a negative result for great crested newt DNA. The pond on site also tested negative, therefore it is considered that great crested newts will not pose a constraint to the proposed development. Following the recommendations below will further reduce any potential impact on great crested newts.

- Any trenches will be covered over night or ramps placed inside so that any animals that may get trapped inside can escape; and
- Any building materials will be kept on raised pallets and checked before being used to ensure nothing is sheltering beneath.

Yours sincerely,

[redacted]

Beth England BSc (Hons), MSc
Ecologist

Appended:

- Ponds Map
- Laboratory Report

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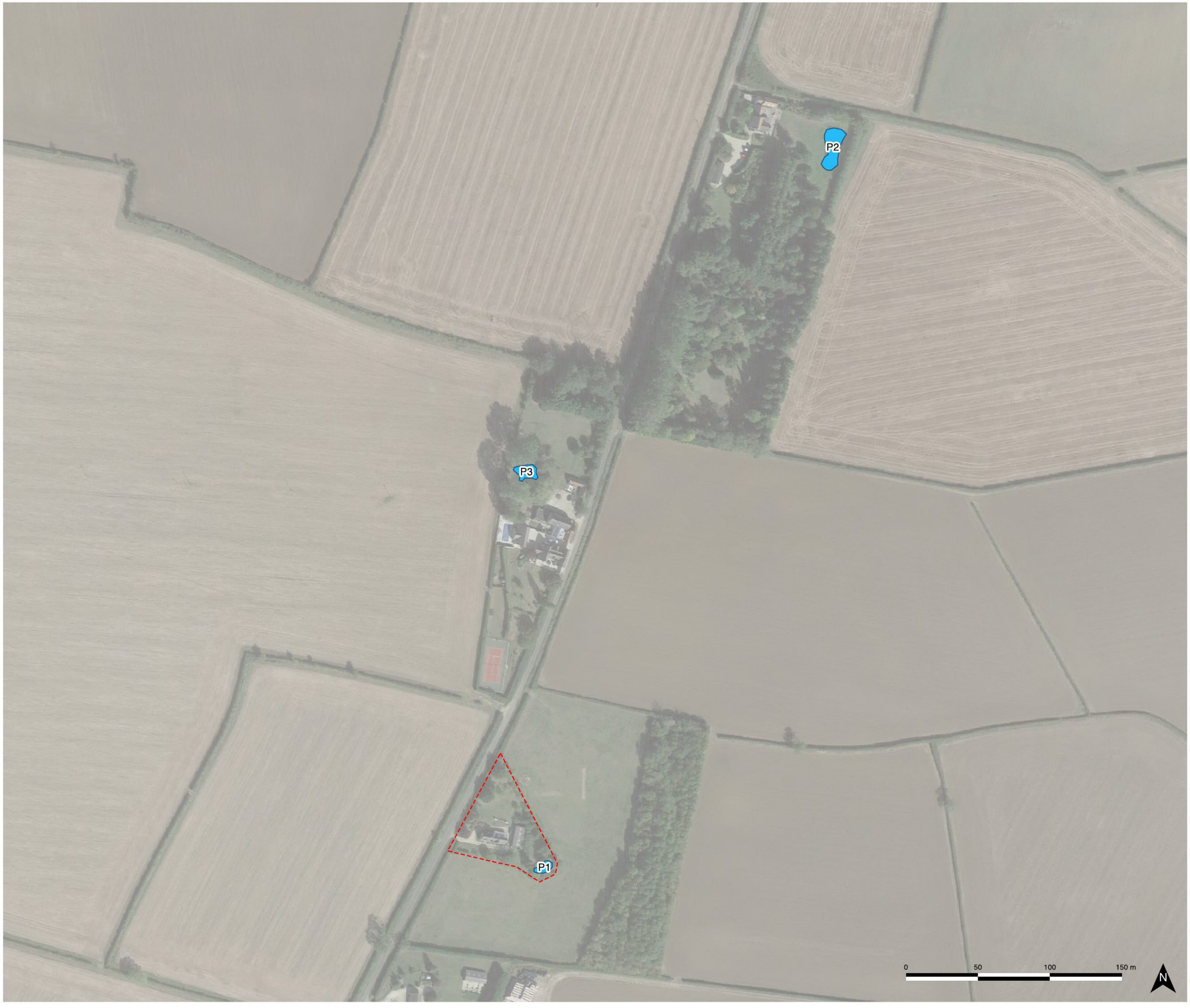
[1] Natural England (2019). *GCN Method Statement WML-A14-2* (Version March 2019) the Conservation of Habitats and Species Regulations 2017 (as amended) Method Statement to support application for licence under Regulation 55(2)(e) in respect of great crested newts *Triturus cristatus*. Natural England, Worcester.

Appendix 1: Pond Map

Next page.

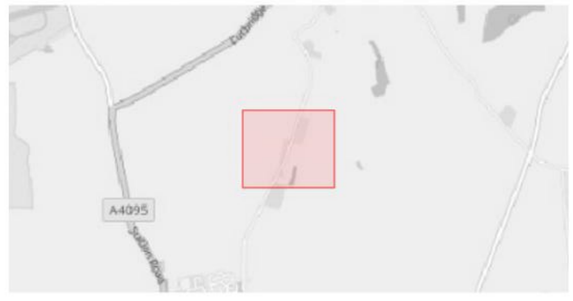


LEGEND

Site Boundary

Ponds

Location (1:75,000):



Project:

Far Horizons, Bampton

Client:

Francis Gooddy

Drawing Title:

Pond Map

Drawing No.: EBD_1912_DR001

Scale (@A3): 1:2500

Central Eastings, Northings: 432525, 204961

Date Drawn: 02/06/2021

Drawn by: BE

Approved by: BG

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Appendix 2: Laboratory Report

Next page.

PRIVATE & CONFIDENTIAL

eDNA Technical Report

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Report Reference	R0000057
Report Date	25 May 2021
Reported By	eackroyd

Site Name	Far Horizon						
Site Location	Pond 1						
Barcode	**Received Date**	**Sampled Date**	**Sample Check**	**Degradation Check**	**Inhibition Check**	**Result**	**Positve Replicates**
GCN000947	20/05/2021	20/05/2021	PASS	PASS	PASS	NEGATIVE	0 out of 12

Site Name	Far Horizon						
Site Location	Lews						
Barcode	**Received Date**	**Sampled Date**	**Sample Check**	**Degradation Check**	**Inhibition Check**	**Result**	**Positve Replicates**
GCN000928	20/05/2021	20/05/2021	PASS	PASS	PASS	NEGATIVE	0 out of 12

Site Name	Far Horizon						
Site Location	Tive House						
Barcode	**Received Date**	**Sampled Date**	**Sample Check**	**Degradation Check**	**Inhibition Check**	**Result**	**Positve Replicates**
GCN000872	20/05/2021	20/05/2021	PASS	PASS	PASS	POSITIVE	4 out of 12

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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.

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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.

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