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Star Farm
Colebatch
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Shropshire
SY9 5JY

Mr. R. Mills
Les Stephan Planning Limited
9 Sweetlake Business Village
Longden Road
Shrewsbury
SY3 9EW

Our Ref: LSP/1354/14.1

Your Ref:

26th May 2015

Re: Great Crested Newt eDNA Survey -
Pond 1, Pond 2 and Pond 6;
Land adjoining Crawforton, Hadnall, Shropshire, SY4 4AN.

On 7th May 2015 water samples were taken from Pond 1 and Pond 6.
Pond 2 is no longer in existence and, therefore, it was not possible for a water sample to be taken from Pond 2.

The water samples from Pond 1 and Pond 6 were analysed by the Fera eDNA testing service and results provided to Star Ecology on 22nd May 2015.
Water samples were collected and analysed in accordance with published protocols([1, 2]).

A copy of the Fera's report for the eDNA sampling of Pond 1 and Pond 6 is provided at the end of this letter.

The results of the eDNA analysis indicate that Great crested newt were not present within Pond 1 but were present in Pond 6, on 7th May 2015.

As detailed in Section 6 of Star Ecology report LSP/1282/14.1 ('Ecological Assessment of Land adjoining Crawforton', of 1st December 2014); Pond 6 is too far from the proposed development site to be of concern.
Therefore, it is not considered that further Great crested newt survey is required to inform the proposed development at Land adjoining Crawforton.
Furthermore, it is not considered that a European Protected Species Licence is required to allow the proposed development to lawfully proceed.

However, as Great crested newts have been recorded within the landscape surrounding the proposed development site; it is proposed that should the proposed development of Land adjoining Crawforton receive Planning Permission, Risk Avoidance Measures (RAMs) for Great crested newts should be adhered to.

A set of RAMs for Great crested newts, suitable for the proposed development, are provided in a separate document (Star Ecology reference LSP/1354/14.2).

Adoption of RAMs will negate any potential impact of the development on Great crested newts and other wildlife, including small mammals.

Dr. R. M. Jones MCIEEM

References:
([1]) Biggs J, Ewald N, Valentini A, Gaboriaud C, Griffiths RA, Foster J, Wilkinson J, Arnett A, Williams P and Dunn F 2014. Analytical and methodological development for improved surveillance of the Great Crested Newt. Appendix 5. Technical advice note for field and laboratory sampling of great crested newt (*Triturus cristatus*) environmental DNA. Freshwater Habitats Trust, Oxford.
([2]) Williams, P. (2013). How to collect a water sample to detect Great Crested Newt eDNA. GCN eDNA protocol, Freshwater Habitats Trust. August 2013.

DNA ANALYSIS REPORT Commercial in Confidence

Customer:	Star Ecology
Address:	Star Farm Colebatch Bishops Castle Shropshire SY9 5JY
Contact:	**Dr R M Jones**
Email:	info@starecology.co.uk
Tel:	07866440915
Report date:	22 May 2015
Order Number:	GCN087
Samples:	Pond Water
Analysis Requested:	Detection of Great Crested Newt eDNA from pond water.

Thank you for submitting your samples for analysis with the Fera eDNA testing service. The details of the analysis are as follows:

Method:
The method detects pond occupancy from great crested newts (GCN) using traces of DNA shed into the pond environment (eDNA). The detection of GCN eDNA is carried out using real time PCR to amplify part of the cytochrome 1 gene found in mitochondrial DNA. The method followed is detailed in 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.

The limits of this method are as follows: 1) the results are based on analyses of the samples supplied by the client and as received by the laboratory, 2) any variation between the characteristics of this sample and a batch will depend on the sampling procedure used. 3) the method is qualitative and therefore the levels given in the score are for information only, they do not constitute the quantification of GCN DNA against a calibration curve, 4) a 'not detected' result does not exclude presence at levels below the limit of detection.

The results are defined as follows:
Positive: DNA from the species was detected.
eDNA Score: Number of positive replicates from a series of twelve.
Negative: DNA from the species was not detected; in the case of negative samples the DNA extract is further tested for PCR inhibitors and degradation of the sample.
Inconclusive: Controls indicate degradation or inhibition of the sample, therefore the lack of detection of GCN DNA is not conclusive evidence for determining the absence of the species in the sample provided.

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Results:

Customer Reference	Fera Sample Ref.	GCN Detection	GCN Score	Inhibition	Degradation
~~Meadowlands 1~~	~~S15-050237~~	~~Negative~~	~~0~~	~~No~~	~~No~~
~~Meadowlands 2~~	~~S15-050238~~	~~Negative~~	~~0~~	~~No~~	~~No~~
~~Meadowlands 3~~	~~S15-050239~~	~~Negative~~	~~0~~	~~No~~	~~No~~
~~Meadowlands 4~~	~~S15-050240~~	~~Negative~~	~~0~~	~~No~~	~~No~~
Crawforton 1	S15-050241	Negative	0	No	No
Crawforton 6	**S15-050242**	**Positive**	**6**	n/a	n/a
~~Pear Tree Lane~~	~~S15-050243~~	~~Negative~~	~~0~~	~~No~~	~~No~~

The results indicate that eDNA for great crested newts was detected in one (S15-050242) of the seven samples submitted and in the remaining samples eDNA was not detected. Analysis was conducted in the presence of the following controls: 1) Extraction blank, 2) appropriate positive and negative PCR controls for each of the TaqMan assays (GCN, Inhibition, and Degradation). All controls performed as expected.

This test procedure was developed using research funded by the Department of Environment, Food and Rural Affairs, and was performed under the conditions of licensing arrangements with Applied Biosystems and patent rights owned by F. Hoffman-La Roche Ltd.

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