Land at Hillthorn Farm

on behalf of Rolton Kilbride Limited

Great crested Newt eDNA Survey Report

avian ecology

Document Control				
Project Name:		Land at Hillthorn Farm		
Project Number:		Pegas-075-785		
Report Title:		GCN eDNA Survey Report		
Issue	**Date**	**Notes**	**Prepared**	**Reviewed**
V1	14/07/2016	Draft for client review	C Baldock *MCIEEM*	H Fearn *MCIEEM*
V2	18/09/2017	Final	U Maginn *MCIEEM*	H Fearn *MCIEEM*

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SUMMARY

- Avian Ecology Ltd. was commissioned by Rolton Kilbride Limited to undertake a great crested newt (GCN) *Triturus cristatus* environmental DNA (eDNA) survey, on land at Hillthorn Farm in relation to the proposed Renewable Energy Centre Site.

- Two ponds were located within the Site boundary were subject to water sampling on the 16th June 2016, by suitably trained, competent and licenced surveyors. Laboratory analysis was conducted by SureScreen Scientifics.

- Results obtained indicate the **absence** of GCN from both ponds.

1 INTRODUCTION

1.1.1 Avian Ecology Ltd. was commissioned by Rolton Kilbride Limited to undertake a great crested newt (GCN) *Triturus cristatus* environmental DNA (eDNA) survey, on land at at Hillthorn Farm in relation to the proposed Renewable Energy Centre Site. The 'Site boundary' is illustrated in **Figure 1.**

1.1.2 Pond numbers referenced within this report correspond to the Landscape Masterplan 0114023_007_P01.

1.1.3 Ponds within the surrounding landscape were subject to detailed great crested newt survey during 2013[1] and 2014[2]. Both surveys found the species to be present, with a single pond (pond P5) was found to support a medium population in the 2013 survey and a small population was found in pond P6 during the 2014 survey. The nearest of these ponds is c. 190m north east of the Site boundary.

1.1.4 Survey effort was agreed in advance via correspondence with the Sunderland City Council Ecology Officer (Andrew Bewick). It was agreed that sampling would cover all ponds within the Site boundary (ponds P3 and P4).

1.1.5 This report evidences a detailed survey methodology and survey results.

2 METHODOLOGY

2.1.1 Ponds P3 and P4 were assessed for their suitability to support GCN using the HSI method as developed by Oldham *et al.* (2000[i]) and as detailed within ARG UK guidance (ARG UK, 2010[ii]) and were subject to eDNA survey sampling on the 16th June 2016. **Table 2.1** below provides brief pond descriptions. Full HSI scores are presented in **Table 3.1**.

Table 2.1: Pond Descriptions.

Pond	HSI Score	Suitability for GCN	Photographic Plate (Appendix 1)	Description and distance / orientation from Site boundary
P3	0.64	'Average'	Plate A	Area = c. 271m^2 A small pond, recently excavated on a clay substrate. The pond was devoid of macrophytes as vegetation had not yet had time to establish.
P4	0.70	'Good'	Plate B	Area = c. 299m^2 Recently excavated pond, on a clay substrate, quite shallow at the time of survey and with no macrophytes or marginal vegetation.

2.2 Habitat Suitability Index Assessment

2.2.1 The HSI assessment involves the measurement of ten different indices which, when combined, have been found to provide a good indication of the general suitability of ponds for great crested newts.

[1] Durham Wildlife Services (2013). Great Crested Newt Survey: Hillthorn Farm, Washington.

[2] AECOM (2014). A19 Enterprise Zone Great Crested Newt Survey Report 2014 (Report reference: 60287087_008_ENV_RT_0000_003).

Each of the indices is scored (between 0.01-1) using a series of graphs and figures within the guidance notes (ARG UK, 2010). These scores are then used to calculate an overall Habitat Suitability Score for each pond.

2.2.2 Final scores relate to pond suitability for great crested newt and range from 'poor' to 'excellent'.

2.3 eDNA

2.3.1 eDNA is nuclear or mitochondrial DNA that is released from an organism into the environment. Sources of eDNA include secreted faeces, mucous, gametes, shed skin, hair and carcasses. In aquatic environments, eDNA is diluted and distributed in the water where it persists for 7–21 days, depending on the conditions (Biggs *et al*., 2014a[iii]). The technique for determining presence/absence of great crested newt uses Polymerase Chain Reaction (PCR) laboratory techniques to detect the species eDNA within water samples.

2.3.2 Recent research by the Department for Environment Food and Rural Affairs (Defra) Project WC1067, concludes that the sampling of waterbodies collecting eDNA appears to be a highly effective method for determining whether GCN are present or absent during the breeding season, even where eDNA is present in very low concentrations (Biggs *et al.,* 2014).

2.3.3 Natural England, in their guidance, "Great crested newts: surveys and mitigation for development projects," outlined the acceptance of eDNA test results as survey evidence of presence or absence of GCN, providing samples are undertaken following the onset of suitable weather conditions between 15th April and 30th June, in strict accordance with the published technical advice note by a suitably trained, experienced and licenced GCN surveyor[3].

Field Sampling Technique

2.3.4 Two ponds (ponds P3 and P4) located within the Site boundary were sampled on 16th June 2016.

2.3.5 The ambient temperature was 14°C and weather conditions were cloudy with a light north easterly wind.

2.3.6 Samples were collected by suitably licenced, trained and experienced GCN surveyors Mr P. Antrobus (Licence No. CLS02498) and Miss C. Baldock (Licence No. 2016-19849-CLS-CLS)

2.3.7 The protocol for sampling followed that outlined within '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. (Biggs *et al*., 2014b[iv]), which required the collection of 20 x 30ml subsamples from each pond, spaced as evenly as possible around the pond margin.

2.3.8 Each sample was then placed within a Whirl-Pak bag and shaken, before a 15ml sample was pipetted from the bag and placed in a specimen tube for laboratory analysis. Following collections samples were refrigerated prior to laboratory dispatch.

2.3.9 This process was repeated for each sampled pond.

Laboratory Analysis

2.3.10 Laboratory analysis was undertaken by SureScreen Scientifics:

[3] Note: A survey licence is not required to take the water samples, but for licence applications NE require evidence and confirmation that experienced, licensed GCN surveyor/s collected the samples to support the proposals in the method statement.

SureScreen Scientifics Division Ltd,
Morley Retreat,
Church Lane,
Morley,
Derbyshire,
DE7 6DE
Tel: +44 (0)1332 292003
Email: scientifics@surescreen.com

2.3.11 The laboratory follows the analysis methodology outlined within the Defra Project WC1067 research note (Biggs *et* al., 2014) using the q-PCR test conducted in two phases.

2.3.12 The sample first goes through an extraction process to acquire as much eDNA as possible to produce a pooled sample. The pooled sample is then tested via 1-PCR.

2.3.13 Each pooled sample is replicated 12 times to ensure results are accurate. If one of the twelve replicates tests positive the sample is declared positive. The sample is only declared negative if no replicates show amplification. Inhibition and degradation checks are also carried out on each sample using a known DNA marker. Results of these quality control tests are recorded with each sample.

2.3.14 Samples are tested in a clean room and the different phases of testing are kept separate to reduce any risk of cross contamination.

3 RESULTS

3.1.1 HSI calculations were undertaken and these indicated that the ponds offered average to good habitat suitability for great crested newt. Both ponds had been recently excavated and did not contain any aquatic plant species or marginal plants which great crested newts could use for egg laying purposes. They were therefore considered unsuitable for breeding great crested newt at the time of survey; however the HSI results are considered to more closely reflect the level of pond suitability once vegetation has established. **Table 3.3** below presents the finding of the HSI assessment.

Table 3.1: Habitat Suitability Results

HSI	**P3**	**P4**
SI1 location	1.00	1.00
SI2 pond area	0.37	0.40
SI3 pond drying	0.50	1.00
SI4 water quality	0.67	0.67
SI5 shade	1.00	1.00
SI6 waterfowl	1.00	1.00
SI7 fish	1.00	1.00
SI8 no. of ponds	1.00	1.00
SI9 terrestrial habitat	0.33	0.33
SI10 macrophytes	0.30	0.30
HSI value	**0.64**	**0.70**
Pond Suitability	**Average**	**Good**

3.1.2 eDNA survey results are summarised in **Table 3.2**. Both ponds were identified as 'Negative' for great crested newt eDNA.

Table 3.2: eDNA survey results.

Pond	Sample Ref.	Result	Inhibition Check	Sample Integrity
P3	AEL-033	Negative 0/12	Acceptable	Acceptable
P4	AEL-032	Negative 0/12	Acceptable	Acceptable

4 CONCLUSIONS

4.1.1 eDNA sampling and analysis of Ponds P3 and P4 located within the Site boundary of the proposed Energy Centre did not identify the presence of great crested newts. In view of this result and it is considered that the species is absent from the ponds within the Site and no further action is normally required.

4.1.2 However, based on the recording of great crested newts within ponds c. 190m northeast of the Site (Durham Wildlife Services, 2013 and AECOM, 2014), it is recommended that 'Reasonable Avoidance Measures' (RAMs) are adopted as a precaution to further reduce any potential for harm to GCN or any other amphibian species.

REFERENCES

[i] 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(4), pp. 143-155.

[ii] ARG UK (2010) ARG UK Advice Note 5: Great Crested Newt Habitat Suitability Index. Amphibian and Reptile Groups of the United Kingdom.

[iii] 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. Defra Project WC1067. Freshwater Habitats Trust: Oxford.

[iv] 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.