the ecology partnership

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Wakako Hirose
Rapleys LLP
By email only

25/07/2018

Dear Wakako,

Great Crested Newt eDNA Testing – Land around Pump and Bloors Farm, Lower Rainham, Kent

Thank you for instructing The Ecology Partnership to carry out GCN eDNA surveys on water bodies on and surrounding your site at Pump and Bloors Farm.

Background

The site comprises two parcels on either side of Pump Lane, in Lower Rainham, Kent (TQ809674). The land is just less than 250m south of the Medway Estuary and Marshes Special Protection Area. A railway line borders the land to the southwest with the dense suburban area of Twydall just beyond. Further agricultural land is situated to the northwest, and Bloors Lane Community Woodland, allotments and low-density buildings to the southeast.

A Preliminary Ecological Appraisal was undertaken by EPR in 2017. The site was identified as having potential to support a number of protected species and various phase 2 surveys have been carried out by The Ecology Partnership in 2018.

Using OS Maps, two ponds were identified within 250m of the site, with an additional three ponds were identified off site within 500m of the site. The closest waterbody is within the curtilage of a private residential property and the additional three were located within the Riverside Country Park to the north of the site. The locations of the water bodies are depicted in figure 1 overleaf.

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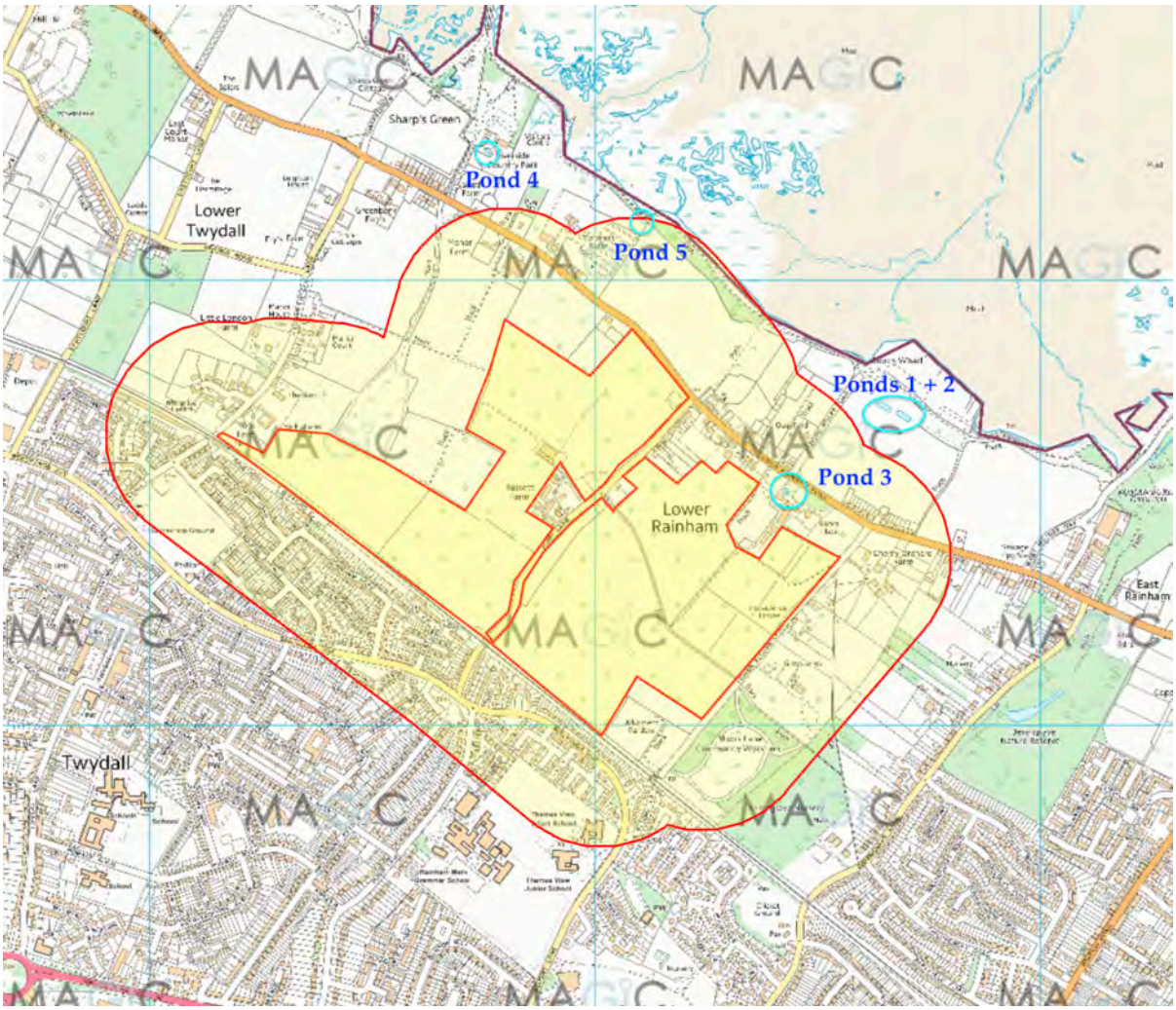

Figure 1. Waterbodies within 250m in relation to the site boundary

Results and Discussion

The water bodies within the Country Park were all assessed for their suitability for supporting GCN using the Habitat Suitability Index (HSI) in 2018. The results of which are detailed below in table 1.

Ponds 1 and 2 were accessible within the fields towards to the eastern end of the Country Park and permission was granted to survey these on the 28th June 2018. These two ponds were virtually identical in shape, size and vegetation and are 347m north of the edge of the site.

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Pond 3 was inaccessible and located on private land so could not be surveyed. The pond was noted to be surrounded by mown grassland and hardstanding. This pond is 70m from the site boundary.

Pond 4 within the grounds arounds around the visitors centre at the Country Park 370m from the site boundary, with the Lower Rainham Road as a barrier between the development and the pond.

Pond 5 is 250m from the site boundary but could be seen due to 7ft wooden fencing along the footpath. This pond was therefore not able to be surveyed.

Figure 2: Ponds 1 and 2

Figure 3: Pond 4

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Table 1: HSI Data for ponds 1, 2 and 4.

Suitability Indices No.	Feature	Pond 1 Score	Pond 2 Score	Pond 4 Score
1	Location	1	1	1
2	Area	0.75	0.75	0.2
3	Permanence	0.5	0.5	0.5
4	Water quality	0.67	0.67	0.67
5	Shading	1	1	0.8
6	Presence of waterfowl	0.67	0.67	0.67
7	Presence of fish	1	1	0.67
8	Pond density	0.6	0.6	0.5
9	Suitable newt habitat within 500m	0.33	0.33	0.33
10	Macrophyte content	0.5	0.5	0.7
10th Root		0.66	0.66	0.56
Pond Suitability		**Average**	**Average**	**Below Average**

eDNA sampling was undertaken on ponds 1 and 2. The water samples were taken on 28th June 2018 by Jade Brennan BSc (Hons) MSc Grad CIEEM (GCN Licence Ref – 2017 – 31295 – CLS - CLS), with Emma Bagguley BSc (Hons) MSc MCIEEM (GCN Licence Ref – 2016-23003-CLS-CLS). All samples were analysed by SureScreen Scientifics and were submitted for eDNA analysis to the protocol stated in DEFRA WC1067 (latest amendments). Full results and methods can be found in appendix 1.

Results from the samples taken from water bodies 1 and 2 were **negative for GCN presence**. Water samples were not taken from Ponds 3-5 due to inaccessibility.

Table 2. Summary of HSI and eDNA Results

Waterbody	Suitability to Support GCN	eDNA Result
Pond 1	Average	Negative
Pond 2	Average	Negative
Pond 4	Below Average	-

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The waterbodies within the Riverside Countryside Park are all considered to be of a significant distance from the proposed development at Pump and Bloors Farm, all being over 300m away, and the Lower Rainham Road between the ponds and site is considered to a significant barrier to dispersal for any amphibians onto the site.

eDNA surveys confirmed no GCN presence within ponds 1 and 2. Pond 4 is considered highly unlikely to contain any GCN due to the isolation from other suitable habitat and ponds, the presence of waterfowl and below average HSI score. The Lower Rainham Road is considered to pose a significant barrier between ponds 3 and 4 and the proposed development site over 300m south.

Pond 3 is the closest to the site boundary, 70m, and on the southern side of the Lower Rainham Road. This pond is considered to be isolated from other such waterbodies however. The closest waterbody on the same side of the main road is located within Berengrave Local Nature Reserve 690m east, for which there are no records of GCN presence, only common amphibians. Ponds 1 and 2 are 277m north of pond 4 and were negative for GCN presence. It is therefore considered unlikely that a population of GCN could persist within this waterbody.

The terrestrial habitat within the redline boundary is dominated by short managed grassland between the rows of apple trees within the orchard. This habitat is not considered to be suitable for GCN due to the lack of structure and cover. The site is bordered by mature treelines and hedgerows however which could provide dispersal opportunities for amphibians and small mammals around the edge of the site. Theses boundaries are understood to be retained within the scheme however, therefore there is to be no loss of suitable terrestrial habitat.

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Conclusions

The results from the water sample analysis found no GCN presence within ponds 1 and 2 in the Riverside Country Park 347m north of the proposed development boundary.

Given the isolation of additional waterbodies closer to the site boundary, it is considered highly unlikely that GCN are present in pond 3. The habitats within the site boundary are considered to be suboptimal for GCN during their terrestrial phase and it is unlikely that GCN would move onto the site from this waterbody.

No further surveys or species-specific mitigation is considered necessary for the presence of Great Crested Newts with regards to the proposed development at Pump and Bloors Farm.

The use of eDNA is considered sufficient to inform a planning application and as such this letter of report can be submitted to support the application.

If you have any questions or queries then please do not hesitate to get in touch. I look forward to hearing from you.

Alexia Tamblyn MA (Oxon) MSc CEnv MCIEEM FRGS
Managing Director & Principle Ecologist
The Ecology Partnership Ltd

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Appendix 1. Full eDNA Results and Methods

SureScreen Scientifics

Folio No: E3666
Report No: 1
Order No: KEN5491
Client: THE ECOLOGY PARTNERSHIP
Contact: Jade Brennan
Contact Details: jade@ecologypartnership.com
Date: 17/07/2018

TECHNICAL REPORT

ANALYSIS OF ENVIRONMENTAL DNA IN POND WATER FOR THE DETECTION OF GREAT CRESTED NEWTS

Date sample received at Laboratory: 03/07/2018
Date Reported: 17/07/2018
Matters Affecting Results: None

RESULTS

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
3762	Rainham Pond 2	TQ8169767690	Pass	Pass	Pass	Negative	0
3764	Rainham Pond 1	TQ8163867724	Pass	Pass	Pass	Negative	0

SUMMARY

When Great Crested Newts (GCN); Triturus cristatus inhabit a pond, they deposit traces of their DNA in the water as evidence of their presence. By sampling the water, we can analyse these small environmental DNA (eDNA) traces to confirm GCN habitation, or establish GCN absence.

The water samples detailed below were submitted for eDNA analysis to the protocol stated in DEFRA WC1067 (Latest Amendments). Details on the sample submission form were used as the unique sample identity.

RESULTS INTERPRETATION

Lab Sample No.- When a kit is made it is given a unique sample number. When the pond samples have been taken and the kit has been received back in to the laboratory, this sample number is tracked throughout the laboratory.

Site Name- Information on the pond.

O/S Reference – Location/co-ordinates of pond.

SIC- Sample Integrity Check. Refers to quality of packaging, absence of tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to results errors. Inspection upon receipt of sample at the laboratory. To check if the Sample is of adequate integrity when received. Pass or Fail.

DC- Degradation Check. Analysis of the spiked DNA marker to see if there has been degradation of the kit since made in the laboratory to sampling to analysis. Pass or Fail.

IC- Inhibition Check- PCR inhibitors can cause false results. Inhibitors are analysed to check the quality of the result. Every effort is made to clean the sample pre-analysis however some inhibitors cannot be extracted. An unacceptable inhibition check will cause an indeterminate sample and must be sampled again.

Result- NEGATIVE means that GCN eDNA was not detected or is below the threshold detection level and the test result should be considered as no evidence of GCN presence. POSITIVE means that GCN eDNA was found at or above the threshold level and the presence of GCN at this location at the time of sampling or in the recent past is confirmed. Positive or Negative.

Positive Replicates- To generate the results all of the tubes from each pond are combined to produce one eDNA extract. Then twelve separate analyses are undertaken. If one or more of these analyses are positive the pond is declared positive for the presence of GCN. It may be assumed that small fractions of positive analyses suggest low level presence but this cannot currently be used for population studies. In accordance with Natural England protocol, even a score of 1/12 is declared positive.

METHODOLOGY

The laboratory testing adheres to strict guidelines laid down in WC1067 Analytical and Methodological Development for Improved Surveillance of The Great Crested Newt, Version 1.1

The analysis is conducted in two phases. The sample first goes through an extraction process where all six tubes are pooled together to acquire as much eDNA as possible. The pooled sample is then tested via real time PCR (also called q-PCR). This process amplifies select part of DNA allowing it to be detected and measured in ‘real time’ as the analytical process develops. qPCR combines PCR amplification and detection into a single step. This eliminates the need to detect products using gel electrophoresis. With qPCR, fluorescent dyes specific to the target sequence are used to label PCR products during thermal cycling. The accumulation of fluorescent signals during the exponential phase of the reaction is measured for fast and objective data analysis. The point at which amplification begins (the Ct value) is an indicator of the quality of the sample. True positive controls, negatives and blanks as well as spiked synthetic DNA are included in every analysis and these have to be correct before any result is declared so they act as additional quality control measures.

The primers used in this process are specific to a part of mitochondrial DNA only found in GCN ensuring no DNA from other species present in the water is amplified. The unique sequence appropriate for GCN analysis is quoted in DEFRA WC 1067 and means there should be no detection of closely related species. We have tested our system exhaustively to ensure this is the case in our laboratory. We can offer eDNA analysis for most other species including other newts.

Analysis of eDNA requires scrupulous attention to detail to prevent risk of contamination. Kits are manufactured by SureScreen

Scientifics to strict quality procedures in a separate building and with separate staff, adopting best practice from WC1067 and WC1067 Appendix 5. Kits contain a ‘spiked’ DNA marker used as a quality control tracer (SureScreen patent pending) to ensure any DNA contained in the sampled water has not deteriorated in transit. Stages of the DNA analysis are also conducted in different buildings at our premises for added

SureScreen Scientifics Ltd also participate in Natural England’s proficiency testing scheme and we also carry out inter-laboratory checks on accuracy of results as part of our quality procedures.

Reported by: Sam Humphrey **Approved by:** Derry Hickman

End Of Report