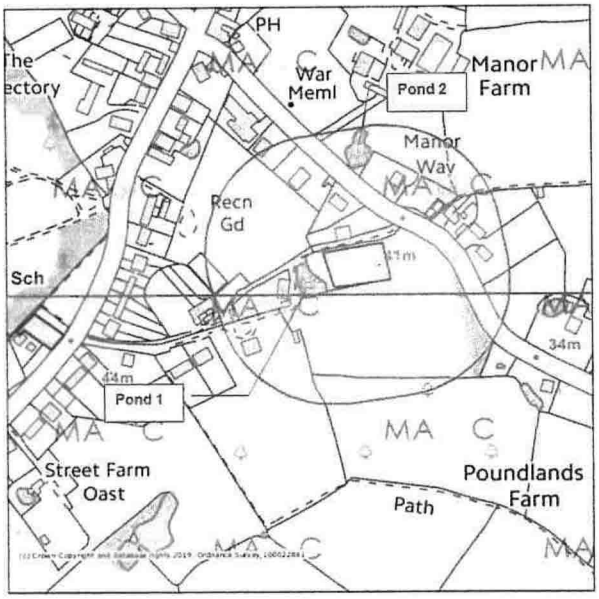
KB Ecology
A Kent Based Ecological Consultancy

3rd May 2019
Our reference: 2019/02/02

Pound Hill Field, Biddenden Road, Frittenden TN17 2EL

Following a 'Preliminary Ecological Appraisal' which identified the risk of great crested newts being present, KB Ecology Ltd has been commissioned to undertake a great crested newt survey of two ponds near Pound Hill Field, Biddenden Road, Frittenden TN17 2EL Kent, in support of a planning application for the erection of a new dwelling (and removal of mobile home).

Water samples of Pond 1 and Pond 2 (the only ponds present within 100m of the proposed development) were taken on 15th April 2019, following the strict methodology provided[1],[2], and sent to an accredited laboratory for eDNA analysis.



The result of the eDNA analysis came back as negative for Pond 1 and positive for Pond 2, indicating that great crested newts were present in Pond 2 in the 7-21 days prior to the sampling. The full details are present in Appendix A.

[1] Surveys and water sampling were undertaken by Megan Austin, a professional ecologist with over 10 years of experience (under Class Survey Licences Registration Number 2015-8717-CLS-CLS)
[2] Further information can be found here http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&Completed=0&ProjectID=18650

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Like nearly all amphibians, the great crested newt is dependent on water-bodies for breeding but usually spends most of its life on land. Optimal terrestrial habitats include woodland, scrub, ditches, hedgerows, taller/rougher grassland. The Great Crested Newt Conservation Handbook, 2001 states that 'very short pasture is easily traversed by newts, and provides night time foraging, but little in the way of shelter' (Great Crested Newt Conservation Handbook, 2001). This means that great crested newts are likely to be present on site during their terrestrial phase of life and are likely to be impacted by the proposed works.

As the proposed works would result in small scale permanent habitat destruction, the works should only be undertaken once a licence is in place for the project, which can be done in two ways:

1. A European Protected Species Mitigation licence (or EPSM licence) could be sought from Natural England to permit the proposed works. An application would need to be prepared and submitted to Natural England for determination, once full planning permission has been granted. A decision on the application would be made by Natural England within 30 days of receipt (although it has taken Natural England considerably more time in the last two years). The licence application would need to include full details of the proposed ecological mitigation / compensation and a program for these works.

2. Alternatively, it may be that the site can be part of the District licensing. The Licence permits acts, subject to licence conditions, including killing, injury, disturbance, capture and transport of GCN, as well as damage and destruction of their breeding sites and resting places. Impacts of development progressing under the Licence are being fully compensated for by off-site habitat provision that is being paid for by the developer and for this reason the Licence does not specifically require any on-site avoidance or mitigation measures to be undertaken. However, where desirable, reasonable measures can be undertaken to minimise suffering to any GCN which may be present within or immediately adjacent to the development footprint.

Should Option 1 be pursued, the mitigation strategy would entail fencing and trapping prior to ground work starting, to minimise risk to animals and creation of habitat piles.

Natural England requires objective evidence that the proposed activity fits the purpose set out in Regulation 44(2)(e) - "Preserving public health or public safety or other imperative reasons of overriding public interest including those of a social or economic nature and beneficial consequences of primary importance for the environment". If neither public health nor public safety grounds can be met, then Natural England must consider whether other imperative reasons of overriding public interest can be demonstrated. The word "imperative" means that there must be a high degree of "need" for the action concerned. The reason must also be of some significant substance or weight because it has to be judged to be of such public interest that it should override nature conservation interests.

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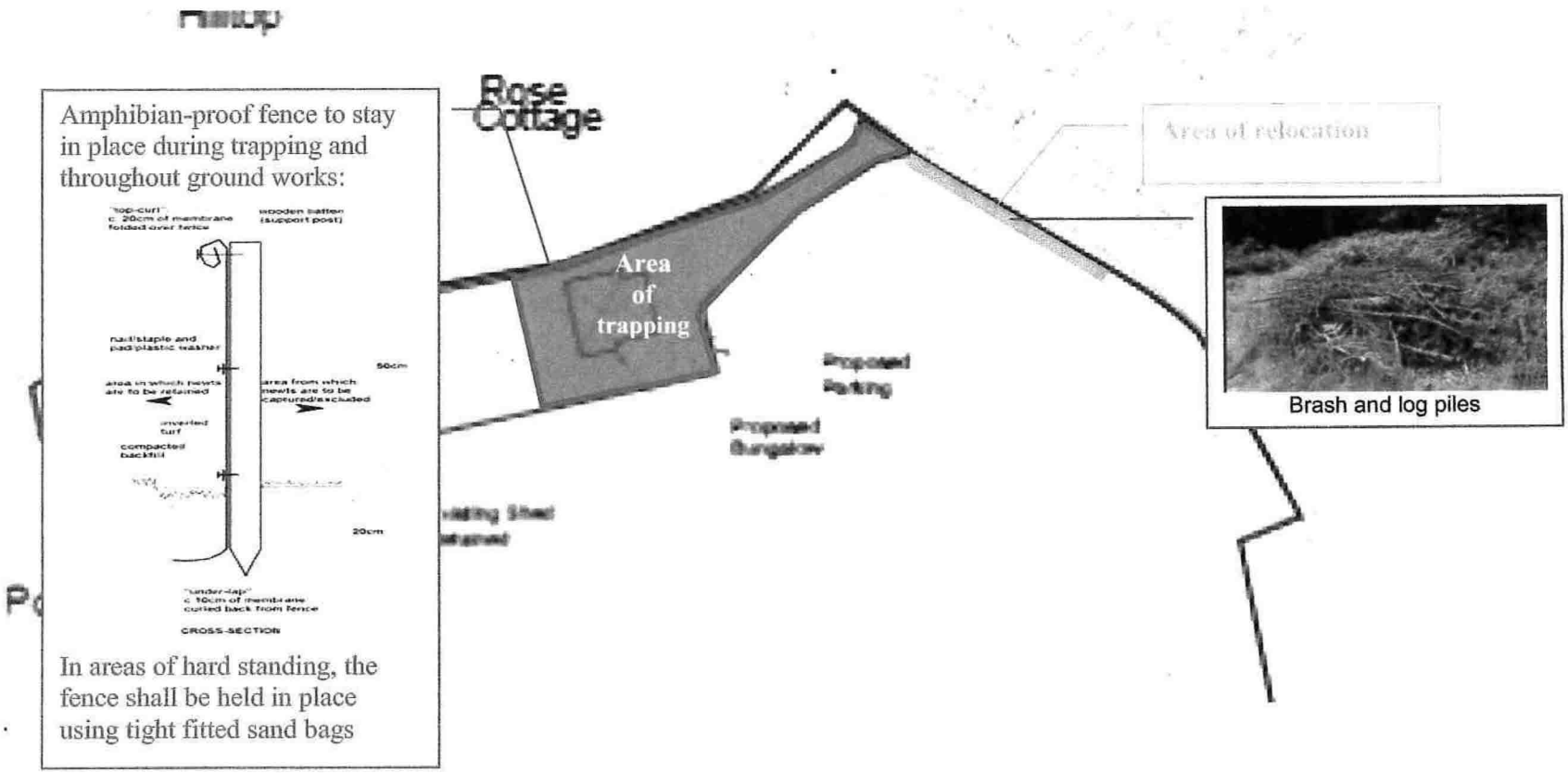

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The legislation also requires Natural England to be satisfied that there is "no satisfactory alternative" to the activity proposed. More information is present here: https://www.gov.uk/government/collections/great-crested-newt-licences

Should planning permission be granted, it is suggested to include a condition worded as such '*Development to proceed in accordance with the scheme subject to any variation required by Natural England under any licence issued'.*

Should Option 2 be pursued, the applicant would need to contact Natural England to request a costing to enter the scheme (the enquiry is itself charged). The details are present here: https://www.gov.uk/government/publications/great-crested-newts-district-level-licensing-schemes

For information, Natural England has identified 'Risk Zones' throughout Kent for its District Licensing scheme[3] and the site is within an 'Amber zone', i.e. which 'contains main population centres for GCN and comprises important connecting habitat that aids natural dispersal'.

[3] This dataset identifies areas where the distribution of great crested newts (GCN) has been categorised into zones relating to GCN occurrence and the level of impact development is likely to have on this species. Red zones contain key populations of GCN, which are important on a regional, national or international scale and include designated Sites of Special Scientific Interest for GCN. Amber zones contain main population centres for GCN and comprise important connecting habitat that aids natural dispersal. Green zones contain sparsely distributed GCN and are less likely to contain important pathways of connecting habitat for this species. White zones contain no GCN. However, as most of England forms the natural range of GCN, white zones are rare and will only be used when it is certain that there are no GCN. Full metadata can be viewed on data.gov.uk
https://naturalengland-defra.opendata.arcgis.com/datasets/c830aa87797c45a397baba125460e77f_0

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SureScreen Scientifics

Folio No: E4580
Report No: 1
Order No: 2019/02/02
Client: KB Ecology
Contact: Katia Bresso
Contact Details: katia.bresso@kbecology.co.uk
Date: 01/05/2019

TECHNICAL REPORT

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

Date sample received at Laboratory: 18/04/2019
Date Reported: 01/05/2019
Matters Affecting Results: None

RESULTS

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
0228	Pond 1 2019/02/02, Pound Hill Field, Biddenden Road	TQ 81614 41010	Pass	Pass	Pass	Negative	0
0229	Pond 2 2019/02/02, Pound Hill Field, Biddenden Road	TQ 81655 41118	Pass	Pass	Pass	Positive	12

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 security.

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: Derry Hickman **Approved by:** Sarah Evans

End Of Report