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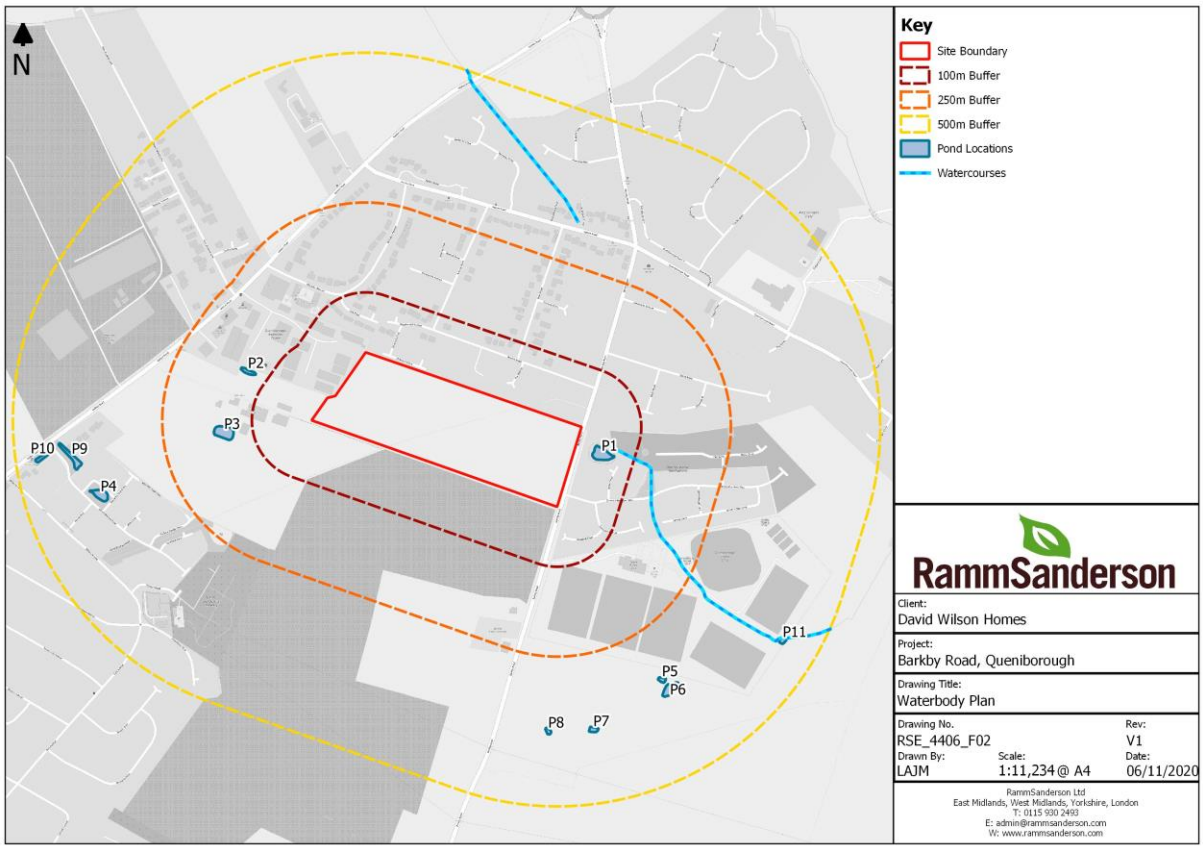
08 June 2021
Our Ref: RSE_4406_01_V1

Issued via email to: helen.bareford@dwh.co.uk

RE: Land at Barkby Road, Queniborough (GCN eDNA survey)

BACKGROUND INFORMATION

RammSanderson were commissioned by David Wilson Homes Ltd to carry out Great Crested Newt (GCN) eDNA surveys of three waterbodies within 250m of a site proposed for residential development at Barkby Road, Queniborough, Leicestershire. These surveys were requested by the Local Planning Authority ecologist, following submission of an Ecological Impact Appraisal (RammSanderson, 2020) to support a planning application. Previous presence/likely absence GCN surveys of P2 and P3 had been undertaken by RammSanderson in 2016, with GCN being confirmed as likely absent. A methodology for the eDNA assessment is provided at the end of this document.



RESULTS AND DISCUSSION

No ponds were located on site, however there are seven ponds within 500m of the site boundary. Four of these were located beyond barriers to dispersal in the form of the surrounding road network, or isolated within housing developments. The desk study showed that there were eight records of GCN within 2km of the site with the closest record 0.9km north-west of the site. Pond 1 was located approximately 190m from the site, and formed part of the sustainable urban drainage system (SUDs) for an adjacent housing development Pond 2 was a garden pond approximately 160m south west of the site, this pond has some areas of emergent vegetation and trees around its banks. This pond also contained large fish and there was a duck house present. Pond 3 was located 163m west of the site. This pond was very overgrown (and so heavily shaded) with limited emergent vegetation and poor water quality.

Figure 1: Pond 1

eDNA surveys of all three waterbodies was carried out by Abigail Marshall (GCN Class 1 (2019-40152-CLS-CLS)) and Aleah Maltby, on 24th May 2021, during optimal weather conditions. All three waterbodies returned a negative (likely absence) result, attached at the end of this document. As such the GCN are considered likely absent from the site and no further mitigation or surveys are required for this species. However, enhancements such as sympathetic design of any SUDs features within the proposals, should be considered, along with refugia provision with the site hedgerows and buffer habitats.

I hope this letter is satisfactory to your requirements, please do not hesitate to contact me if you require any further information.

Yours Sincerely

Amy Skuce

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GREAT CRESTED NEWT **DETECTION RESULTS**

Company:	RammSanderson Ecology
Order number:	102253
Project code:	RSE_4406b Barkby Road, Queniborough
Date of Report:	2 June 2021
Number of samples:	3

Thank you for sending your samples for analysis by NatureMetrics. Your samples have been processed in accordance with the protocol set out in Appendix 5 of Biggs et al. (2014).

Summary of the results

Results indicate GCN absence in 'P1', 'P2' and 'P3'.

The negative controls were blank, the extraction blank control was negative, and the positive controls and their replicates were standard.

Results are based on the samples as supplied by the client to the laboratory. Incorrect sampling methodology may affect the results. Note that a negative result does not preclude the presence of Great Crested Newts at a level below the limits of detection.

Methods

eDNA was precipitated via centrifugation at 14,000 x g and then extracted using Qiagen Blood and Tissue extraction kits. qPCR amplification was carried out in 12 replicates per sample, using GCN specific primers and probes described in Biggs et al. (2014), in the presence of positive controls, extraction controls, and template negative controls. A score is given for the number of positive replicates out of 12.

The qPCR method follows the recommendations set out by NatureMetrics for Natural England in the qPCR validation project and helps improve the reliability of the interpretation of the data. Results from the assay are considered to have a high rating of confidence according to our Validation Scale (Harper et al. 2021).

The quality control methods exceed the requirements outlined in Biggs et al. (2014) Appendix 5. These consist of the use of kit blanks, additional extraction blanks and template negative controls, and positive controls standards of known concentration in triplicate to generate limits of detection and give confidence to the low and late amplifications.

Kit ID	Pond ID	Arrived	Inhibition	Degradation	Score	Status
2342	'P1'	26-May	No	No	0	Negative
2344	'P2'	26-May	No	No	0	Negative
2341	'P3'	26-May	No	No	0	Negative

END OF REPORT

Report issued by: **Thomas Shannon**

Contact: **team@naturemetrics.co.uk**

Understanding your results

Positive Target DNA has been detected in this sample, meaning that at least 1 of the 12 qPCR replicates has amplified. This is not a quantitative test, so you should not interpret a high number of positive replicates (e.g. 12/12) as necessarily indicating a larger population of GCN than a low eDNA score (e.g. 1/12).

Negative No target DNA has been detected in this sample, and the internal and external controls worked as expected. This tells us that if there had been GCN DNA in the sample, we would have detected it, so we can be confident in its absence from the sample provided.

Inconclusive No GCN DNA was detected in the sample, but the internal controls failed to amplify as expected. This means that any GCN DNA in the sample might also have failed to amplify properly, so we cannot have confidence in this negative result. Inconclusive results can be caused by the degradation of the DNA (when the DNA marker contained in the ethanol in the kits fails to amplify) or by inhibition of the reaction (when the marker added in the lab fails to amplify) caused by certain chemicals or organic compounds that may be present in the water sample.

Validation Scale We have developed our own confidence assessment tool for qPCR eDNA assays that builds upon the Thalinger et al. (2021) validation scale and helps end-users to interpret the qPCR outputs but also contextualise these with the level of validation that the assay itself has gone through. Briefly, the level of confidence that can be assigned to results coming from an assay is derived from several validation steps:

- Basic analysis - can the assay work in principle on the computer?
- PCR protocol - has the protocol been optimised in the lab?
- Specificity analysis - has the assay been tested in the lab against other co-inhabiting and/or closely related species?
- How extensive has the assay been tested with natural samples?
- Have the theoretical limits of detection been established?
- Have detection probabilities been estimated with extensive site occupancy modelling?
- Have external factors affecting detectability been extensively tested (e.g. seasonality, spatial heterogeneity)?

- Low Results from these assays are difficult to interpret with confidence. It is impossible to conclusively tell if the target species is present or absent because of the limited amounts of *in silico*, *in vitro*, and *in vivo* testing.

- Medium Assays with this rating have been tested *in silico*, have optimised lab protocols, specificity and sensitivity tested in and out of the lab, but with no estimates of detection probabilities or extensive testing of external factors that may affect the detectability of the target. Positive results can be interpreted as meaning the target species DNA is present (assuming the correct sampling conditions), but negative results could mean that the target is absent or that external factors such as ecology, seasonality, spatial scales are influencing the detections.

- High High rating assays have everything that a Medium assay has, in addition to site occupancy modelling and extensive testing of external influencers such as ecological, temporal and spatial factors. Positive results can be conclusively interpreted, and negative results can be interpreted as meaning the target species DNA is absent (assuming the correct sampling conditions). In some instances, a probability of target species presence at a site and in a sample can be given.

Glossary

controls Controls are used to monitor both the performance of the assays but also any contamination. These samples are treated in the same way as a normal sample. This is particularly important given the sensitivity of these eDNA qPCR methods. Our full complement of controls enables us to fully monitor the whole GCN eDNA process from kits to data.

- kit blank Used to determine if the kits are contaminated but also to monitor the early stages of the pipelines - e.g. sample reception. These samples also act as uninhibited samples that can be used as a baseline to compare against. This is an additional control not specifically mentioned in the Biggs et al. 2014 protocol.

- EB Extraction blank. Used to monitor potential contamination during the DNA extraction process.

- TNC Template negative control. Used to monitor potential contamination during the qPCR setup process. For every qPCR reaction, we run we include more template negative controls than are prescribed in the Biggs et al. 2014 protocol.

- positive Used to determine whether the assay is working correctly. In addition to the 4 standard dilutions prescribed by the Biggs et al. 2014 protocol, we include an additional standard dilution and amplify all standards in triplicate. We can use this increased number of replicates and standards to generate standard curves that will allow us to calculate the limit of detection (LOD).

- LOD Limit of detection. The lowest concentration of positive control DNA that amplifies. LOD is determined for every single reaction performed. Target amplification below the LOD cannot automatically be considered as negative but should be further investigated as spurious amplifications are more prevalent at these low concentrations.

eDNA Short for 'environmental DNA'. Refers to DNA deposited in the environment through excretion, shedding, mucous secretions, saliva etc. This can be collected in environmental samples (e.g. water, sediment) and used to identify the organisms that it originated from. eDNA in water is broken down by environmental processes over a period of days to weeks. It can travel some distance from the point at which it was released from the organism, particularly

in running water. eDNA is sampled in low concentrations and can be degraded (i.e. broken into short fragments), which limits the analysis options.

inhibitors Naturally-occurring chemicals/compounds that cause DNA amplification to fail, potentially resulting in false-negative results. Common inhibitors include tannins, humic acids and other organic compounds. Inhibitors can be overcome by either diluting the DNA (and the inhibitors), but dilution carries the risk of reducing the DNA concentration below the limits of detection.

qPCR Stands for 'quantitative PCR', a PCR reaction incorporating a coloured dye that fluoresces during amplification, allowing a machine to track the progress of the reaction. Often used with species-specific primers where detection of amplification is used to infer the presence of the target species' DNA in the sample. If the species is not present in the sample, no fluorescence will be detected.

- primers Short sections of synthesised DNA that bind to either end of the DNA segment to be amplified by PCR.

- probe A short section of synthesised DNA that binds to a specific section of the target species' DNA within the section flanked by the primers. The probe is designed to be totally specific to that species. The probe is labelled such that it fluoresces during amplification, which is used to infer the presence of the target species' DNA in the sample.

References

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.

Harper KJ, Tang CQ, Bruce K, Ross-Gillespie A, Ross-Gillespie V, and Egeter B 2021. A framework for assessing confidence in environmental DNA qPCR assays and results. Natural England Report.

Thalinger B, Deiner K, Harper LR, Rees HC, Blackman RC, Sint D, Traugott M, Goldberg CS, and Bruce K (2021). A validation scale to determine the readiness of environmental DNA assays for routine species monitoring. bioRxiv 2020.04.27.063990; doi: https://doi.org/10.1101/2020.04.27.063990