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Environmental Statement Volume III

Appendix 10.3: Phase 1 Habitat Survey & GCN eDNA Survey

Axis J9, Phase 3

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Appendix 10.3 – Data Search, Phase 1 Habitat Survey and GCN eDNA Survey

Data Search

1.18 The aim of the data search is to collate existing ecological records for the site and adjacent areas. Obtaining existing records is an important part of the assessment process as it provides information on issues that may not be apparent during a single survey, which by its nature provides only a 'snapshot' of the ecology of a given site.

1.19 This data search covered the study area using the distances defined in the previous section. It was completed in June 2021 with the following organisations and resources contacted and consulted:

- Thames Valley Environmental Records Centre (TVERC)
- Multi-Agency Geographic Information for the Countryside (MAGIC) Interactive Maps, for locations of statutory sites;
- Section 41 of the Natural Environment and Rural Communities (NERC) Act for Priority Species and habitats in England; and
- Cherwell District Council website for details of relevant local planning policies and supplementary planning guidance.

1.20 Information supplied by these organisations has where relevant, been incorporated into the following report.

Phase 1 Habitat Survey

1.21 An extended Phase I habitat survey of the site was undertaken on 16th June 2021. The habitat survey methodology was based on guidance set out in the 'Handbook for Phase 1 habitat survey' (JNCC, 2010) and entailed recording the main plant species and classifying and mapping habitat types with reference to the Habitat Definitions provided by the UK Habitat Classification Working Group (Butcher et al, 2020).

1.22 Note was taken of the more conspicuous fauna and any evidence of, or potential for the presence of protected or notable flora and fauna. Where access allowed, adjacent habitats were also considered in order to assess the site within the wider landscape and to provide information with which to assess possible impacts within the context of the site boundary.

Great Crested Newt Survey Methodology and Results

1.23 Great crested newts are known to be present within Cherwell and records for this species were returned within the data search.

1.24 Furthermore three waterbodies were identified in close proximity to the site and as such, an assessment of the site and adjacent habitats for their potential to support GCN was undertaken in 2021.

1.25 GCN are able to move up to 500m between breeding ponds although as a general guide only habitats within 250m of a breeding pond are most frequently used (English Nature, 2001). The potential survey area for GCN extended to include any waterbodies within 250m of the site (Pond P1), where habitat connectivity was available (see Plan 10706_P02). The presence of

ponds and other waterbodies within this area was established with the use of ordnance survey maps and aerial photographs.

Habitat Suitability Index Assessment

1.26 A Habitat Suitability Index (HSI) assessment was undertaken in pond P1 on 16th June 2021. The National Amphibian and Reptile Recording Scheme (NARRS) HSI guidance (based on the Oldham et al (2000) methods) was used, whereby a number of factors including pond location, water quality, macrophyte cover and shading were assessed. A score is given to each waterbody between 0 and 1, with scores closer to 0 having lower probability of GCN occurrence.

- The HSI scores are provided below:
- <0.5 Poor;
- 0.5 – 0.59 Below average;
- 0.6 – 0.69 Average;
- 0.7 – 0.79 Good; and
- >0.8 Excellent.

1.27 Although the HSI score cannot confirm the presence or likely absence of GCN, it can be used as a guide to assess the habitat in terms of its potential to support GCN.

Environmental DNA (eDNA) Surveys

1.28 Three waterbodies within 500m of the site were identified as having some potential to support GCN. It was possible to access Pond P1 to undertake an eDNA survey. Ponds P2 and P3 were not accessible for completion of an HSI or eDNA survey.

1.29 This pond was nonetheless assessed as being of good suitability for GCN and as such was surveyed using Environmental DNA (eDNA) methodology to confirm the presence or likely absence of GCN. Water samples were taken using a sterile kit provided by the Naturemetrics following the standard methodology to prevent contamination (Biggs et al, 2014). This approach followed the methodology which is approved by Natural England and provides a rapid means of establishing the presence / likely absence of GCN.

1.30 Water samples from this pond were collected on the 16th June 2021 by Anthony Hiscocks, an experienced GCN surveyor and Natural England licence holder (Licence number 2017-28614-CLS-CLS).. The analysis followed the standard methodology (Biggs et al, 2014) and a full copy of the results is provided below.

Limitations

1.31 Ponds P2 and P3 are situated outside of the landownership boundary of the applicant, and it was not possible to obtain safe access to this area to undertake eDNA surveys. This is not considered to present a significant limitation as the impact assessment and mitigation measures set out in the biodiversity chapter for GCN assume that GCN are potentially present in Ponds P2 and P3.

HSI Results – Pond P1

1.32 The results of the HSI surveys on the waterbodies within and close to the site are provided in Table A4.1.

Table A4.1: HSI scoring

Waterbody	**P1**
Distance from site	180m
Geographic location	1
Pond area	1
Pond permanence	0.9
Water quality	0.3
Shade	0.3
Waterfowl effect	0.01
Fish presence	0.67
Pond Density	0.55
Terrestrial habitat	0.67
Macrophyte cover	0.3
HSI Score	**0.38**
Pond Suitability	**Poor**

eDNA Survey Result – Pond P1

1.33 Pond P1 was subject to an eDNA survey with the results set out below.

GREAT CRESTED NEWT **DETECTION RESULTS**

Company:	Tyler Grange
Order number:	102439
Project code:	10706: Land of Middleton Stoney
Date of Report:	8 July 2021
Number of samples:	1

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

Summary of the results

Results indicate GCN presence in 'Pond 1'.

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
2879	'Pond 1'	24-Jun	NA	No	11	Positive

END OF REPORT

Report issued by: **Laura Balcells**

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