



NATURE
METRICS
DNA-BASED MONITORING

Environmental DNA Report

Great Crested Newt



Order number	SO01747
Prepared for	RammSanderson Ecology
Project	RSE_6764a Inglenook Farm Barwell
Number of samples	2
Sampling dates	2023/06/01
Report ID	GCN-XKT710
Date of report	2023-06-15



Thank you for choosing NatureMetrics

Welcome to your report. We are the leading provider of powerful, scalable biodiversity data delivered using environmental DNA.

Your sample(s) have been processed in accordance with the protocol set out in Appendix 5 of Biggs et al. (2014). 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 GCN at a level below the [limits of detection](#).

A results interpretation guide and a glossary of terms highlighted throughout this report can be found at the end of the report.

GCN Detection Results

Pond ID	Inhibition	Degradation	GCN Score	Result
RSE_6764_P1	No	No	0	Negative
RSE_6764_P2	No	No	0	Negative

Sample Information

Pond ID	Kit ID	Sampling Date	Received Date
RSE_6764_P1	GCN-23-00884	2023/06/01	2023/06/06
RSE_6764_P2	GCN-23-00469	2023/06/01	2023/06/06

Methods

eDNA was precipitated via centrifugation at 14,000 x g and then extracted using Qiagen DNeasy Blood and Tissue extraction kits. **qPCR** amplification was carried out in 12 replicates per sample, using GCN specific **primers** and **probe** (developed by Thomsen et al. (2012) and adopted by Biggs et al. (2014)), in the presence of **extraction negative controls**, **qPCR positive controls**, and **qPCR 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 GCN assay are considered to have a **high** confidence rating according to our **Validation Scale** (Harper et al. 2021).

The quality control methods exceed the requirements outlined in Appendix 5 of Biggs et al. (2014). These consist of the use of **kit blanks**, additional **extraction negative controls**, **qPCR negative controls**,



and **qPCR positive controls**. Using these controls ensures assay performance is as expected and increases confidence in any weak or late amplifications.

The extraction and qPCR negative controls analysed alongside your samples showed no target amplification and the triplicate positive controls performed as expected.

END OF REPORT

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



Result Interpretation Guide

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 eDNA score (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, but 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 (as indicated by delayed or no amplification of the DNA marker contained in the ethanol in the kits) or by inhibition of the reaction (as indicated by delayed or no amplification of the DNA marker added in the lab) 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 extensively 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, and 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 effects 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. Controls are treated in the same way as a normal sample. This is particularly important given the sensitivity of 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.

Extraction negative

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

qPCR negative

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

qPCR positive

Used to determine whether the qPCR run performed as expected. 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. The increased number of standard dilutions and replicates allow us to generate standard curves to assess run performance and assay sensitivity.

Limit of detection

The lowest standard positive control concentration at which 95 % of technical replicates amplify. 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 excretions and secretions, such as mucus, skin cells, saliva, faeces, urine 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

Chemicals/compounds that reduce or prevent DNA amplification, 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 DNA amplification, allowing a machine to track the progress of the reaction. Often used with species-specific primers and probe where 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 qPCR.

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. *Environmental DNA*, 3, 823–836.
- Thomsen PF, Kielgast JOS, Iversen LL, Wiuf C, Rasmussen M, Gilbert MTP, Orlando L and Willerslev E (2012). Monitoring endangered freshwater biodiversity using environmental DNA. *Molecular Ecology*, 21, 2565-2573.