

Report: 19083-EBD-KP-1

Order number: EBD-19005-JO

# Great Crested Newt eDNA Results

**Company:** Ecology by Design Ltd  
**Contact:** Kate Philpot  
**Project code | Task code:** Ecology By Design | Oxpens - Oxpens - Pond 1  
**Date of Report:** 30 April 2019  
**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).

DNA 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 the primers and probe described by Biggs et al. (2014), in the presence of both positive and negative controls.

Results indicate GCN absence in 'Woodland Pond'. Inhibition was detected in 'Woodland Pond', which was overcome by diluting the DNA. All controls performed as expected and so the results are conclusive.

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.

Sample	Pond ID	Arrived	Inhibition	Degradation	Score	GCN status
701	'Woodland Pond'	26-Apr	No	No	0	Negative after dilution

End of report

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**Report issued by:** Dr. Cuong Tang

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## Understanding your results

- Positive:** GCN DNA has been detected in this sample, meaning that at least one of the 12 replicates has amplified. Remember that 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 GCN 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. Samples marked as 'Negative after dilution' are those where inhibition was detected (when the marker added in the lab fails to amplify) but overcome by diluting the DNA. Inhibition can be caused by certain chemicals or organic compounds that may be present in the water sample.
- 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 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.

