Appendix 12.3: eDNA Results

Technical Report
Confidential

Folio No	**D1824**
Report No:	**1**
Client:	**RPS Planning**
Order No:	
Attn:	**Nick Betson**
Date:	**14th June 2016**

TECHNICAL REPORT

EXAMINATION OF ENVIRONMENTAL DNA

IN POND WATER FOR THE DETECTION OF

GREAT CRESTED NEWTS

D.Hickman

Methodology

When Great Crested Newts (GCN) 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 traces to detect GCN inhabitation.

The laboratory testing is conducted in two phases. The sample first goes through an extraction process where all 6 tubes are pooled together to acquire as much eDNA as possible. The pooled sample is then tested via real time PCR (or q-PCR). This process amplifies select part of DNA allowing it to be detected and measured.

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 signal during the exponential phase of the reaction is measured for fast and objective data analysis.

The primers used in this process are specific to a part of mitochondrial DNA only found in GCN ensuring no other DNA is amplified.

Samples are tested in a clean room and the different phases of testing are kept separate to reduce any risk of cross contamination.

Each pooled sample is replicated 12 times to ensure results are accurate. If one of the twelve replicates tests positive the sample is declared positive. The sample is only declared negative if no replicates show amplification.

Inhibition and degradation checks are also carried out on each sample using a known DNA marker. Results of these quality control tests are recorded with each sample.

Results

Lab Ref	Sample	Co-Ordinates	Inhibition Check	Sample integrity	Result
22511	Wealden, Pond 2	-	Acceptable	Acceptable	**Positive**
22512	Wealden, Pond 1	-	Acceptable	Acceptable	**Positive**

Advice

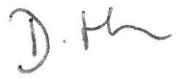
Negative results may not indicate the absence of GCN just the presence of eDNA below the detection limits of the method. However this method is extremely sensitive. It is still advised to survey a pond using traditional methods within 2km of a positive result or a known habitat for GCN.

Positive results may be true positives but also may be due to contamination of samples from another pond or improper sampling technique. Please ensure traditional surveys are performed on positive ponds and care is taken to avoid spreading GCN DNA.

Samples undergo integrity scores to check for degradation post sampling. Samples which are not acceptable should be re-sampled. Sample integrity scores are based on the amount of degradation of an artificial DNA marker placed in the kits and analysed by qPCR.

PCR inhibitors can cause false results. 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.

Analysed and reported By: **Derry Hickman**



Checked and approved:

Technical Report
Confidential

Folio No	**D1732**
Report No:	**1**
Client:	**RPS Planning**
Order No:	
Attn:	**Nick Betson**
Date:	**26th May 2016**

TECHNICAL REPORT

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

S.Humphrey

Methodology

When Great Crested Newts (GCN) 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 traces to detect GCN inhabitation.

The laboratory testing is conducted in two phases. The sample first goes through an extraction process where all 6 tubes are pooled together to acquire as much eDNA as possible. The pooled sample is then tested via real time PCR (or q-PCR). This process amplifies select part of DNA allowing it to be detected and measured.

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Each pooled sample is replicated 12 times to ensure results are accurate. If one of the twelve replicates tests positive the sample is declared positive. The sample is only declared negative if no replicates show amplification.

Inhibition and degradation checks are also carried out on each sample using a known DNA marker. Results of these quality control tests are recorded with each sample.

Results

Lab Ref	Sample	Co-Ordinates	Inhibition Check	Sample integrity	Result
22256	Wealden – Pond 3	-	Acceptable	Acceptable	**Negative**
22257	Wealden – Pond 4	-	Acceptable	Acceptable	**Negative**
22258	Wealden – Pond 5	-	Acceptable	Acceptable	**Negative**
22259	Wealden – Pond 6	-	Acceptable	Acceptable	**Negative**
22260	Wealden – Pond 7	-	Acceptable	Acceptable	**Negative**

Advice

Negative results may not indicate the absence of GCN just the presence of eDNA below the detection limits of the method. However this method is extremely sensitive. It is still advised to survey a pond using traditional methods within 2km of a positive result or a known habitat for GCN.

Positive results may be true positives but also may be due to contamination of samples from another pond or improper sampling technique. Please ensure traditional surveys are performed on positive ponds and care is taken to avoid spreading GCN DNA.

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PCR inhibitors can cause false results. 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.

Analysed and reported By: **Sam Humphrey**

Checked and approved: **Troy Whyte BSc**

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