Folio No:	3211-2024
Purchase Order:	PO AG2024/1/2: SCIN-33787
Contact:	Andy Godfrey Associates
Issue Date:	08.08.2024
Received Date:	25.07.2024

eDNA Report

Technical Report

SureScreen Scientifics

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eDNA Analysis

Summary

When aquatic organisms inhabit a waterbody such as a pond, lake or river they continuously release small amounts of their DNA into the environment. By collecting and analysing water samples, we can detect these small traces of environmental DNA (eDNA) to confirm the presence or absence of the target species within the waterbody.

Results

Lab ID	Site Name	OS Reference	Target Species	Sample Integrity Check	Result	Positive Replicates
FK2218	Semerwater - Sample 1	NY9287	Crayfish plague	Pass	Negative	0
			Signal crayfish	Pass	Negative	0
			White-clawed crayfish	Pass	Positive	1
FK2219	Semerwater - Sample 2	NY9287	Crayfish plague	Pass	Negative	0
			Signal crayfish	Pass	Negative	0
			White-clawed crayfish	Pass	Positive	1

Matters affecting result: none

Reported by:Lauryn Jewkes

Approved by: Chelsea Warner

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Methodology

Samples have been analyzed for the presence of target species eDNA following readily available and scientifically published eDNA assays and protocols.

The analysis is conducted in two phases. The sample first goes through an extraction process where the filter is incubated in order to obtain any DNA within the sample. The extracted sample is then tested via real-time PCR (also called q-PCR) for each of the selected target species. This process uses species-specific molecular markers (known as primers) to amplify a select part of the DNA, allowing it to be detected and measured in 'real time' as the analytical process develops. qPCR combines amplification and detection of target DNA into a single step. With qPCR, fluorescent dyes specific to the target sequence are used to label targeted PCR products during thermal cycling. The accumulation of fluorescent signals during this 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 each individual species. Separate primers are used for each of the species, ensuring no DNA from any other species present in the water is amplified. If target species DNA is present, the DNA is amplified up to a detectable level, resulting in positive species detection. If target DNA is not present then amplification does not occur, and a negative result is recorded.

Analysis of eDNA requires scrupulous attention to detail to prevent the risk of false positive and false negative results. True positive controls, negative controls, and spiked synthetic DNA are included in every analysis and these have to be correct before any result is declared. Stages of the analysis are also conducted in different buildings at our premises for added security. SureScreen Scientifics Ltd is ISO9001 accredited and participates in Natural England's proficiency testing scheme for GCN eDNA testing.

Interpretation of Results

Sample Integrity Check:

Laboratory Arrival:

When samples are received in the laboratory, they are inspected for any tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to inconclusive results. Any samples which fail this test are rejected and eliminated before analysis.

Degradation and Inhibition check:

Analysis of the spiked DNA marker to see if there has been degradation or inhibition of the kit or sample, between the date it was made to the date of analysis. Degradation of the spiked DNA marker may indicate a risk of false negative results. If inhibition is detected, samples are purified and re-analyzed. Inhibitors cannot always be removed, if the inhibition check fails, the sample should be re-collected.

Result:

Presence of eDNA (Positive/Negative/Inconclusive)

Positive: DNA was identified within the sample, indicative of species presence within the sampling location at the time the sample was taken or within the recent past.

Positive Replicates: Number of positive qPCR replicates out of a series of 12. If one or more of these are found to be positive the pond is declared positive for species presence. It may be assumed that small fractions of positive analyses suggest low level presence, but this cannot currently be used for population studies. Even a score as low as 1/12 is declared positive. 0/12 indicates negative species presence.

Negative: eDNA was not detected or is below the threshold detection level and the test result should be considered as evidence of species absence, however, does not exclude the potential for species presence below the limit of detection.

Inconclusive: Controls indicate inhibition or degradation of the sample, resulting in the inability to provide conclusive evidence for species presence or absence.