Kronospan North Access Road

on behalf of Axis PED

Environmental Statement

Appendix 7.3: Great Crested Newt Presence or Absence (eDNA) Survey Report

avian ecology

Document Control				
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V1	16/08/2022	Draft for client comment	J. Stevens *BSc (Hons.)*	-
V2	26/09/2022	Final	J. Stevens *BSc (Hons.)*	N. Robinson *MSc BSc (Hons.) ACIEEM*
V3	05/12/2022	Updates following pre-app responses	J. Stevens *BSc (Hons.)*	N. Robinson *MSc BSc (Hons.) ACIEEM*

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ANNEXES

1 INTRODUCTION

1.1 Background

1.1.1 This appendix has been prepared to accompany Chapter 7: 'Biodiversity' of the Kronospan Lorry Park and 132kV Substation development (the 'Proposed Development') Environmental Statement.

1.1.2 It presents detailed methodologies and results of desk and field studies undertaken to establish the presence or likely absence of great crested newt (GCN) *Triturus cristatus* and inform the design and assessment of the Proposed Development.

1.2 Site Description

1.2.1 The Proposed Development Site is located to the north of the existing Kronospan manufacturing plant as shown in **Figure 7.1** in Volume 2 of the ES and comprises a series of grassland fields alongside the B5070 road. The Proposed Development Site is bounded by the B5070 road to the east, the existing Kronospan plant to the south, a sewage treatment plant and the Afon Bradley to west and the Afon Bradley to the north.

1.2.2 Within the wider surrounding landscape, habitats include agricultural fields, grasslands, hedgerows and woodland to the north and west. Residential housing is located to the south and east of the Kronospan manufacturing plant.

2 METHODOLOGY

2.1 Pond Identification

2.1.1 A review of Ordnance Survey mapping and publicly available aerial imagery (e.g., google maps) was undertaken to identify any ponds within 500m of the application site.

2.1.2 No waterbodies are present within or immediately adjacent to the Proposed Development Site.

2.1.3 Four constructed operational lagoons (P1-P4), which attenuate and treat effluent water, are located within 250m of the Proposed Development Site, north-west of the main Kronospan facility. Lagoons P1 and P2 are considered as one waterbody as they are connected by pipework and water flows through both lagoons in sequence as part of the treatment process.

2.1.4 Lagoons 1 and 3 (as shown on **Figure 7.6**) were accessed and eDNA survey sampling was undertaken to determine the presence or likely absence of GCN.

2.1.5 One additional pond is present between 250m and 500m of the Proposed Development, this being a pool within the former Chirk Golf course, however was excluded from survey due to the presence of the canal clear in and outflow reducing the suitability of this pond for GCN

2.1.6 No further waterbodies are located within 500m of the Proposed development. Waterbodies in the wider area were not accessed at the time of the great crested newt surveys.

2.2 eDNA

2.2.1 Environmental DNA (eDNA) is nuclear or mitochondrial DNA that is released from an organism into the environment. Sources of eDNA include secreted faeces, mucous, gametes, shed skin and carcasses. In aquatic environments, eDNA is diluted and distributed in the water where it persists for

7–21 days, depending on the conditions (Biggs *et al*., 2014[1]). The technique for determining presence/absence of GCN uses Polymerase Chain Reaction (PCR) laboratory techniques to detect the species eDNA within water samples.

2.2.2 Recent research by the Department for Environment Food and Rural Affairs (Defra) Project WC1067, concludes that the sampling of waterbodies collecting eDNA appears to be a highly effective method for determining whether great crested newts are present or absent during the breeding season, even where eDNA is present in very low concentrations (Biggs *et al.,* 2014).

2.2.3 Natural Resources Wales accepts the use of environmental DNA surveys as evidence of presence or absence of GCN[2], provided samples are taken when newts are likely to be present (this depends on location and conditions like the weather). Natural Resources Wales will only accept eDNA survey results undertaken between mid-April and 30th June, in strict accordance with the published technical advice note, by suitably trained, experienced and licensed GCN surveyors.

Field Sampling Technique

2.2.4 Lagoons were sampled on 24th June 2021. Samples were collected by a suitably experienced and ecologist, Z. Hinchcliffe *MRes* working as an accredited agent under the licence of A. Logan (Natural Resources Wales Licence Number: S088793/2)..

2.2.5 The protocol for sampling followed that outlined within the technical advice note for field and laboratory sampling of great crested newts (Biggs *et al*., 2014), which required the collection of 20 x 30ml subsamples from each waterbody, spaced as evenly as possible around the waterbody margin.

2.2.6 Each sample was then placed within a Whirl-Pak bag and shaken for 10 seconds, before a 15ml sample was pipetted from the bag and placed in a specimen tube for laboratory analysis. Following collection, samples were refrigerated prior to laboratory dispatch.

Laboratory Analysis

2.2.7 Laboratory analysis was undertaken by SureScreen Scientifics:

SureScreen Scientifics Division Ltd,
Morley Retreat,
Church Lane,
Morley,
Derbyshire,
DE7 6DE
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2.2.8 The laboratory follows the analysis methodology outlined within the Defra Project WC1067 (Biggs *et al*., 2014) using the q-PCR test conducted in two phases.

[1] Biggs J., Ewald N., Valentini A., Gaboriaud C, Griffiths R.A., Foster J., Wilkinson J., Arnett A., Williams P and Dunn F (2014). Analytical and methodological development for improved surveillance of the Great Crested Newt. Defra Project WC1067. Freshwater Habitats Trust: Oxford.

[2] https://naturalresources.wales/permits-and-permissions/species-licensing/list-of-protected-species/the-use-of-environmental-dna-test-for-great-crested-newt-licensing-purposes/?lang=en

2.2.9 The sample first goes through an extraction process to acquire as much eDNA as possible to produce a pooled sample. The pooled sample is then tested via 1-PCR.

2.2.10 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.

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

2.3 Limitations

2.3.1 Pond scoping was undertaken only from the land east of the Afon Bradley to be subject to new built development and excluded the area of grassland to the west of Afon Bradley Farm. This area will be subject to habitat enhancements only, with no discernible impacts to amphibians and therefore this isn't considered a significant limitation to the surveys.

3 RESULTS

3.1.1 Lagoons are described below. Lagoons 1 and 2 were observed to have through flow of water on a daily (i.e., flushed through approximately every 24hour) basis.

3.1.2 Lagoon 3 was understood to have a slower throughput of operational effluent but the water was observed to be black and odorous, with ammonia present. Pond photographs and descriptions are shown in **Table 7.3.1.**

Table 7.3.1: Pond descriptions and photographs

Pond	Description	Photo
Lagoon 1	Lagoon 1 and 2 were divided by a wall they were however surveyed as one waterbody as there was an obvious flow between the two treatment lagoons.	

Lagoon 3	Water remains longer in Lagoon 3 but the water was observed to be black in colour and odorous, with ammonia present.	

3.2 eDNA

3.2.1 Both lagoons returned a **negative** result (**Table 7.3.2**). Full laboratory reports are reproduced in **Annex 1**.

Table 7.3.2: eDNA survey results.

Waterbody	Sample Ref.	Inhibition Check	Degradation Check	Sample Integrity Score	Result
Lagoon 1	6531	Pass	Pass	Pass	Negative 0/12
Lagoon 3	6928	Pass	Pass	Pass	Negative 0/12

4 CONCLUSIONS

4.1.1 The eDNA sampling and analysis returned a negative result for both lagoons indicating that GCN are not present within these waterbodies. The lagoons provide sub-optimal conditions for GCN and other amphibian species.

Annex 1 – eDNA Laboratory Results

SureScreen Scientifics

Folio No: E11289
Report No: 1
Purchase Order: ae-21-151
Client: AVIAN ECOLOGY
Contact: Rachel Hughes

TECHNICAL REPORT

ANALYSIS OF ENVIRONMENTAL DNA IN POND WATER FOR THE DETECTION OF GREAT CRESTED NEWTS (TRITURUS CRISTATUS)

SUMMARY

When great crested newts (GCN), *Triturus cristatus*, inhabit a pond, 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 GCN habitation or establish GCN absence.

RESULTS

Date sample received at Laboratory: 28/06/2021
Date Reported: 07/07/2021
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
6531	Kronospon, pond 1		Pass	Pass	Pass	Negative	0
6928	Kronospon, pond 3		Pass	Pass	Pass	Negative	0

If you have any questions regarding results, please contact us: ForensicEcology@surescreen.com

Reported by: Chris Troth **Approved by:** Chris Troth

SureScreen Scientifics

METHODOLOGY

The samples detailed above have been analysed for the presence of GCN eDNA following the protocol stated in DEFRA WC1067 'Analytical and methodological development for improved surveillance of the Great Crested Newt, Appendix 5.' (Biggs et al. 2014). Each of the 6 sub-sample tubes are first centrifuged and pooled together into a single sample which then undergoes DNA extraction. The extracted sample is then analysed using real time PCR (qPCR), which uses species-specific molecular markers to amplify GCN DNA within a sample. These markers are unique to GCN DNA, meaning that there should be no detection of closely related species.

If GCN DNA is present, the DNA is amplified up to a detectable level, resulting in positive species detection. If GCN 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 risk of contamination. 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 and reported. Stages of the DNA analysis are also conducted in different buildings at our premises for added security.

SureScreen Scientifics Ltd is ISO9001 accredited and participate in Natural England's proficiency testing scheme for GCN eDNA testing. We also carry out regular inter-laboratory checks on accuracy of results as part of our quality control procedures.

INTERPRETATION OF RESULTS

SIC: **Sample Integrity Check** [Pass/Fail]
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.

DC: **Degradation Check** [Pass/Fail]
Analysis of the spiked DNA marker to see if there has been degradation of the kit or sample between the date it was made to the date of analysis. Degradation of the spiked DNA marker may lead indicate a risk of false negative results.

IC: **Inhibition Check** [Pass/Fail]
The presence of inhibitors within a sample are assessed using a DNA marker. If inhibition is detected, samples are purified and re-analysed. Inhibitors cannot always be removed, if the inhibition check fails, the sample should be re-collected.

Result: **Presence of GCN eDNA** [Positive/Negative/Inconclusive]
Positive: GCN DNA was identified within the sample, indicative of GCN presence within the sampling location at the time the sample was taken or within the recent past at the sampling location.
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 GCN presence. It may be assumed that small fractions of positive analyses suggest low level presence, but this cannot currently be used for population studies. In accordance with Natural England protocol, even a score of 1/12 is declared positive. 0/12 indicates negative GCN presence.
Negative: GCN eDNA was not detected or is below the threshold detection level and the test result should be considered as evidence of GCN absence, however, does not exclude the potential for GCN presence below the limit of detection.