Appendix 6.2 Great Crested Newts

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1. Introduction

1.1 Background

1. This appendix accompanies Chapter 6: Ecology of the Environmental Impact Assessment Report (EIAR). It provides detailed results of desk study and field survey (described in Appendix 6.1 Methodology) carried out to establish the baseline conditions within the zone of influence (ZoI) of the Proposed Development with respect of great crested newt *Triturus cristatus*.
2. Throughout this appendix, species are given their common and scientific names when first referred to and their common names only thereafter. All distances are cited as the shortest distance 'as the crow flies', unless otherwise specified. The area encompassed by the rEIA boundary shown on the accompanying figures is referred to throughout as the 'Site'.

2. Results

3. The desk study identified six waterbodies within 250 m of the original KINN Site. Two waterbodies, both located within the industrial yard north of the original KINN Site, were scoped out of further survey due to their being unsuitable; one was a large industrial tank at ground level with no means for a newt to access/egress, the other is a large artificial drainage area which rarely wets and contains sparse terrestrial vegetation. The location of ponds identified during desk study, and the subsequent results of eDNA survey, are portrayed on Figure 6.5: Important Species Survey Results.
4. A review of Appendix 6.3: ECU – LWUP XL Route Refurbishment Ecological Walkover DRAFT REPORT identified an additional three ponds within 250 m of the XL uprating and reconductoring works.

2.1 Habitat Suitability Index Assessment

KINN01

5. SUDS pond adjacent to A876 road with high cover of emergent vegetation dominated by common reed *Phragmites australis*.

Table 1. HSI results for Pond KINN01

Habitat parameter	Field Score	HSI Score
S1 Location	Zone B - Marginal	0.5
S2 Pond area	2600 m^2	Excluded
S3 Pond drying	Sometimes dries	0.5
S4 Water quality	Poor	0.33
S5 Shade	<60%	1
S6 Waterfowl	Absent	1
S7 Fish	Absent	1
S8 Pond count	2	0.82
S9 Terrestrial habitat	Poor	0.33
S10 Macrophyte cover	70%	1
	HSI Score	**0.68**
	Habitat suitability	**Average**

Image 1. Photographs of KINN01

KINN02

Larger SUDS pond adjacent to A876 road with high cover of emergent vegetation around the edges, dominated by common reed and transitioning into marsh with iris *Iris* sp., marsh marigold *Caltha palustris*, and meadowsweet *Filipendula ulmaria*.

Table 2. HSI results for Pond KINN02

Habitat parameter	Field Score	HSI Score
S1 Location	Zone B - Marginal	0.5
S2 Pond area	7000 m^2	Excluded
S3 Pond drying	Rarely dries	1
S4 Water quality	Poor	0.33
S5 Shade	<60%	1
S6 Waterfowl	Absent	1
S7 Fish	Absent	1
S8 Pond count	2	0.82
S9 Terrestrial habitat	Poor	0.33
S10 Macrophyte cover	50%	0.8
	HSI Score	**0.72**
	Habitat suitability	**Good**

Plate 2. Photographs of KINN02

KINN03

Pond surrounded by immature willow *Salix* sp. scrub with two distinct areas of open water. Vegetation around the edge of the southern area is dominated by common reed whilst the northern area is covered by bulrush *Typha latifoli*.

Table 3. HSI results for Pond KINN03

Habitat parameter	Field Score	HSI Score
S1 Location	Zone B - Marginal	0.5
S2 Pond area	10000 m^2	Excluded
S3 Pond drying	Rarely dries	1
S4 Water quality	Moderate	0.67
S5 Shade	<60%	1
S6 Waterfowl	Minor	0.67
S7 Fish	Possible	0.67
S8 Pond count	2	0.82
S9 Terrestrial habitat	Poor	0.33
S10 Macrophyte cover	70%	1
	HSI Score	**0.73**
	Habitat suitability	**Good**

Plate 3. Photograph of KINN03

Ponds 1, 2, and 8

6. Bowland Ecology Ltd identified and carried out HSI assessment of three ponds relevant to the XL uprating and reconductoring works. The results of HSI provided in Table 2 below are adapted from the Habitat Suitability Index scores for the ponds Table in Appendix 6.3: ECU – LWUP XL Route Refurbishment Ecological Walkover DRAFT REPORT.

Table 4. HSI results for Ponds 1, 2, and 3 obtained by Bowland Ecology Ltd

Pond number Distance from nearest tower	**Pond 1** 150 m from XL015	**Pond 2** 125 m from XL015	**Pond 8** 110 m from XL016
S1 Location	0.5	0.5	0.5
S2 Pond area	1	0.97	0.8
S3 Pond drying	0.5	0.5	0.09
S4 Water quality	0.33	0.33	0.67
S5 Shade	1	1	1
S6 Waterfowl	0.67	0.67	0.67
S7 Fish	0.67	0.67	0.67
S8 Pond count	0.85	0.85	0.85
S9 Terrestrial habitat	0.67	0.67	1
S10 Macrophyte cover	0.45	0.45	0.7
HSI Score	**0.63**	**0.63**	**0.60**
Habitat suitability	**Average**	**Average**	**Average**

2.2 eDNA Sampling

7. The following Technical Report produced by SureScreen Scientifics provides the results of eDNA analysis. The Site Name corresponds to a specific pond as follows:
   - Kincardine North 01 = KINN01
   - Kincardine North 02 = KINN02
   - Kincardine North 03 = KINN03

8. No eDNA analysis was carried out at ponds KINN04, Pond 1, Pond 2, or Pond 8. This limitation is discussed in Section 5 Limitations and Assumptions of Appendix 6.1: Methodology.

SP Energy Networks

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GCN Report

Technical Report

SureScreen Scientifics

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Issue Date:	22.04.2024

SureScreen Scientifics

GCN eDNA Analysis

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 analyzing water samples, we can detect these small traces of environmental DNA (eDNA) to confirm GCN habitation or establish GCN absence.

Results

Lab ID	Site Name	OS Reference	Degradation Check	Inhibition Check	Result	Positive Replicates
GCN1456	Kincardine North 03	NS9255489766	Pass	Pass	Negative	0/12
GCN1460	Kincardine North 01	NS9216789384	Pass	Pass	Negative	0/12
GCN1462	Kincardine North 02	NS9229289496	Pass	Pass	Negative	0/12

Matters affecting result: none

Reported by: Daisy Chambers

Approved by: Chelsea Warner

SureScreen Scientifics Ltd, Morley Retreat, Church Lane, Morley, Derbyshire, DE7 6DE, UK
+44 (0)1332 [illegible] | [illegible]

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Methodology

The samples detailed above have been analyzed 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 tube which then undergoes DNA extraction. The extracted sample is then analyzed 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 attention to detail to prevent the 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 analytical 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: 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 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.

Inhibition Check: **Pass/Fail.** The presence of inhibitors within a sample is assessed using a DNA marker. 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 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 the WC1067 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.
Inconclusive: Controls indicate inhibition or degradation of the sample, resulting in the inability to provide conclusive evidence for GCN presence or absence.

SureScreen Scientifics Ltd, Morley Retreat, Church Lane, Morley, Derbyshire, DE7 6DE, UK
+44 (0)1332 292003 | scientifics@surescreen.com | surescreenscientifics.com

2.3 KINN04

9. Land access issues curtailed the Site visit and resulted in the surveys being incomplete. A large proportion of the survey area, particularly that outside of the Site to the south, could not be accessed and this prevented access to pond KINN04. Subsequently, no HSI assessment or eDNA sampling was carried out on this pond.

10. The Site comprises arable fields which, due to farming practices, are unsuitable for foraging or dispersing great crested newts. The use of pesticides can further reduce terrestrial prey density in and around arable fields. Furthermore, KINN04 is approximately 125 m south of the Site, and the majority of adult great crested newt stay within around 250 m of a breeding pond, with the density of individuals gradually decreasing away from the pond (Ref 1). KINN04 is located towards the edge of broadleaved woodland which provides suitable habitat for sheltering newts. If present, newts are highly likely to remain within the broadleaved woodland in close proximity to the pond.

11. Mature trees as are found in the woodland are likely shade the pond, reducing its suitability for breeding great crested newts. Also, great crested newts live in a metapopulation structure which is made up of multiple small populations spread across a group of closely located ponds. Beyond the surveyed ponds, the closest waterbodies (as identified via desk study) are approximately 380 m east within a golf course and are separated by an active railway line as well as the A977 which act as barriers to movement. Considering great crested newt are absent from the ponds sampled for eDNA, there are no other known ponds within 250 m of KINN04[1], and the nearest identified ponds are separated by a railway line and main road, it is inconceivable that any single population would persist within one isolated pond.

12. Therefore, whilst the occupation status of KINN04 could not be confirmed during field survey due to access constraints, it is considered highly unlikely great crested newts are present.

2.4 Ponds 1, 2, and 8

13. In lieu of detailed survey information, Ponds 1, 2, and 8 are precautionarily assumed to support breeding populations of great crested newt.

3. References

Ref 1 Froglife (2001). Great Crested Newt Conservation Handbook. *Suffolk: Froglife, EN, CCW, SNH, JNCC, EA, SEPA, BHS, HCT, HGBI, The Pond Life Project, PCT, FWAG*. Available at: https://www.froglife.org/wp-content/uploads/2013/06/GCN-Conservation-Handbook_compressed.pdf. [Accessed: July 2024]

[1] Based on OS mapping and aerial imagery review.