

Denny to Wishaw Network Upgrade

Environmental Impact Assessment Report

Volume 3 Appendices

Appendix 8.4 – WB, CB, AA Great Crested Newt Results

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Appendix 8.4

1. Introduction

This appendix accompanies Volume 1, Chapter 8 Biodiversity of the EIA Report. It describes in detail the desk study and field survey carried out to establish baseline conditions in the Zone of Influence (Zoi) of the New Build Overhead Line, AA Route Undergrounding and CB Route Removal components of the Project with respect to great crested newt (GCN) *Triturus cristatus*¹.

This appendix is supported by the following figures located within Volume 2: Figures:

- Figure 8.8a: Great crested newt HSI assessment and eDNA survey results; and
- Figure 8.8b: Great crested newt population assessment survey results.

This appendix is also supported by the following annexes which are appended to the end of this document:

- Annex I: 2025 Habitat Suitability Index assessment detailed results
- Annex II: 2025 SureScreen laboratory certificates
- Annex III: 2025 Population assessment survey detailed results
- Annex IV: 2022 Great crested newt population assessment survey results

The areas within which the New Build Overhead Line, AA Route Undergrounding and CB Route Removal components are located are hereafter referred to together in this appendix as “the Sites”.

Relevant legislation and national planning policy pertaining to the protected or notable mammal species are discussed in Volume 1, Chapter 8 Biodiversity.

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. Locations are given as Ordnance Survey Grid References (OSGR).

2. Methodology

Desk study methods are as described in Volume 1, Chapter 8 Biodiversity.

As described in Volume 1, Chapter 8 Biodiversity, a mix of OS mapping and aerial imagery was reviewed to identify ponds potentially suitable for GCN within 250m from the Sites.

This was in line with Natural England guidance^{2,3} (as referenced by NatureScot⁴) which states that:

¹ The GCN methods and baseline results for other components of the project are detailed separately in Appendix 8.11 for the Upgraded Overhead Lines, Appendix 8.13 for Cumbernauld Substation and within the main chapter for the other components.

² Natural England (2022). *Great crested newts: advice for making planning decisions*. Available from: [Great crested newts: advice for making planning decisions - GOV.UK](https://www.gov.uk/government/publications/great-crested-newts-advice-for-making-planning-decisions)

³ English Nature (2001). *Great Crested Newt Mitigation Guidelines*. Available from: <https://webarchive.nationalarchives.gov.uk/ukgwa/20140605121141/http://publications.naturalengland.org.uk/publication/810429?category=30014>.

⁴ [Standing advice for planning consultations - Great Crested Newts | NatureScot](https://www.nature-scot.gov.uk/consultations/great-crested-newts)

“Surveys of water bodies within 250m of the development are usually sufficient. Developers may need to increase this to 500m if there are no obvious barriers to GCN dispersing into the wider environment.”

Additional studies have also found that GCN can use suitable terrestrial habitat up to 500m from a breeding pond but that there is a notable decrease in GCN abundance beyond 250m from a breeding pond^{5,6,7,8}.

Given the above and based on the landscape and topography of the Project and surrounding area (which often contains impediments to GCN dispersal such as major roads, large expanses of agricultural land, railways and rivers) it was considered that a buffer of 250m is appropriate when determining the potential presence of waterbodies and GCN populations which may be present.

Ponds were referenced based on the relevant components of the Project. Where ponds are relevant to multiple components, WB was used in preference to CB, and CB in preference to AA. Where ponds are lettered rather than numbered (e.g. WB0A) this is because they were identified in the field, after the desk study was completed.

Habitat Suitability Index assessment

HSI assessment on 20 and 21 April 2022 and May and June 2023 were carried out on waterbodies within 250m of the design as it was defined in 2022, (noting this varied to the final Site defined for this assessment). This survey was updated in 2025, with assessment conducted between 26 March 2025 and 19 May 2025 and covering all waterbodies within 250m of the Project, to establish their suitability for GCN and the likelihood that the species would be present. Only the results of the 2025 HSI assessment are present in Annex 1 as these results supersede the results of the 2022/23 assessment.

The results of the HSI assessment were factored in when determining what additional surveys would be carried out.

The HSI assessment was carried out in accordance with the standard methodology described in Oldham *et al*⁹ and included field-based observations of the habitat attributes described below, in addition to the use of Esri ArcGIS software to calculate the surface area of the waterbodies.

HSI for GCN is a numerical index between 0 (indicating unsuitable habitat) and 1 (representing optimal habitat). The assessment sought to establish the suitability of the ponds for GCN and thus the potential that they would be used by the species for breeding.

HSI assessment considers the following ten habitat attributes that influence the suitability of a pond for breeding GCN:

⁵ Franklin, P. (1993). *The migratory ecology and terrestrial habitat preferences of the great crested newt Triturus cristatus at Little Wittenham Nature Reserve*. M.Phil Thesis. De Montfort University. Dept. Applied Biology and Biotechnology.

⁶ Oldham, R.S. and Nicholson, M. (1986). *Status and Ecology of the Warty Newt Triturus cristatus*, Final Report. Report by Leicester Polytechnic under contract to Nature Conservancy Council, Contract No. HF 3/05/123 Year 3.

⁷ Jehle, R. (2000) *The terrestrial summer habitat of radio-tracked great crested newts (Triturus cristatus) and marbled newts (Triturus marmoratus)*. Herpetological Journal 10(4), pp 137-142.

⁸ Jehle, R. and Arntzen, J.W. (2000). *Post-breeding migrations of newts with contrasting ecological requirements*. Journal of Zoology, London 251, pp 297-306.

⁹ Oldham, R.S., Keeble, J., Swan M.J.S. and Jeffcote, M. (2000). *Evaluating the suitability of habitat for the Great Crested Newt (Triturus cristatus)*. Herpetological Journal 10(4), pp 143-155.

- Location – within a UK-wide context reflecting the differences in national distribution of this species;
- Area – waterbodies between 500-700m² in size are considered to represent the most suitable habitat for GCN;
- Drying – the number of years in which a pond dries over a ten year period. Occasional drying kills fish, which is beneficial for GCN, but the species predominantly favours ponds that do not dry out every year;
- Water quality – qualitative evidence-based assessment to infer good (diverse aquatic invertebrate assemblage), moderate (moderate invertebrate diversity), poor (low invertebrate diversity, few submerged plants) or bad (clearly polluted) water quality;
- Shade – percentage of pond perimeter shaded to at least 1m from the shore. GCN favour lightly shaded waterbodies;
- Waterfowl – qualitative evidence-based assessment of presence or absence and numbers is made. Large numbers of waterfowl can result in nutrient enrichment of the water and habitat damage, which is less favourable for GCN;
- Fish – qualitative evidence-based assessment of likely presence or absence is made. GCN favour breeding ponds that do not support fish because their open-water swimming larvae are vulnerable to fish predation;
- Number of waterbodies within 1km – GCN populations are typically best developed where they have access to a network of ponds, and therefore the species is more likely to be found where there are several ponds within 1km that are linked by suitable terrestrial habitat; and
- Macrophyte cover – percentage of pond surface area occupied by macrophyte cover. Female GCN require aquatic vegetation for egg-laying.

The ten field scores are converted into suitability index scores which are multiplied together then calculated to the power of 0.1, to give a HSI score between 0 and 1.

HSI scores are categorised in terms of pond suitability for GCN as below:

- <0.5 = Poor;
- 0.5 to 0.59 = Below average;
- 0.60 to 0.69 = Average;
- 0.7 to 0.79 = Good; and
- >0.8 = Excellent.

The system is not sufficiently precise to conclude that any particular waterbody with a high score will support GCN or that a waterbody with a low score will not support the species. Oldham *et al*⁹ reported that the lowest scoring pond supporting GCN in their study was 0.43.

However, low scoring ponds are in general less likely to support the species given that they likely have a combination of unsuitable features. Given that the GCN surveys for the Works covered all ponds with greater than Poor HSI rating (with a small number of exceptions – see Limitations) across the whole Site, over two seasons, and that positive eDNA results were confined to a single area, it is considered that sufficient data has been collected to confirm

likely absence of GCN outside of the one area of presence. This is supported by the desk study information.

Note that HSI scores were not available for ponds that were found to be dry as not all necessary criteria could be assessed.

Environmental DNA sampling

Environmental DNA (eDNA) is nuclear or mitochondrial DNA that is released from an organism into the environment¹⁰. In aquatic environments, eDNA is diluted and distributed in the water. Sources of eDNA in pond water derive from faeces, mucous, gametes, shed skin and carcasses. The eDNA sampling survey involves the collection of water samples for laboratory analysis for the DNA of species of interest, in this case GCN. The sampling procedure is prescribed in the published method and requires that the water sample be taken between mid-April and end of June.

In April 2022, eDNA sampling was carried out on waterbodies within 250m of the design as it was defined at the time (noting this varied to the final Site defined for this assessment) simultaneously with the HSI assessment (as this was done simultaneously, all ponds identified within the 2022 desk study (found to contain water) were subject to eDNA at this time, regardless of their HSI score). A further 12 waterbodies were identified in 2023, which were also subject to HSI and eDNA.

In 2025, eDNA sampling was carried between 17 April 2025 and 20 May 2025 out on ponds within 250m of the Sites determined to have a HSI score greater than 'Poor' (during the 2025 HSI survey) and which were not separated from the works by dispersal barriers or significantly isolated from other ponds via intensively managed agricultural land. In most cases this took place on the same visit as the HSI assessment.

GCN were considered likely absent from ponds with an HSI score of Poor.

Samples were collected from the waterbodies following approved field and laboratory protocols¹⁰. Waterbodies were not entered by surveyors during sample collection and new sterile equipment provided by the laboratory was used to collect each water sample to prevent contamination between waterbodies. A total of twenty sub-samples of 30ml each were collected from around the periphery of each waterbody and mixed together before transferring the samples to six laboratory-supplied test tubes, which were then shaken. Samples were collected by trained and experienced AECOM ecologists (in Scotland it is not necessary for those taking water samples for GCN eDNA analysis to hold a survey licence for this species).

Where ponds were very close in proximity, or where there was obvious water exchange, and laboratory protocols allowed (e.g. regarding total surface water area), a single eDNA sample was sometimes taken using water from more than one pond.

The collected samples were then transported to SureScreen (a laboratory which takes part in Natural England's eDNA proficiency testing scheme) for analysis to confirm the presence or likely absence of GCN eDNA.

¹⁰ 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.

Where a negative result was obtained from eDNA analysis, further surveys using traditional methods were not undertaken, as a negative result from this method provides a detection rate of 99%¹¹. Where a positive eDNA result was returned, ponds were subject to further surveys using the traditional methods described in this appendix. This is because eDNA can only be used in establishing presence or absence and does not provide a population estimate.

The results of eDNA survey and population assessment survey undertaken in 2022 were taken into account when determining which ponds would be subject to eDNA (and other) surveys in 2025. The ponds subject to survey in 2022 are therefore shown in Table 1.

The locations of all waterbodies subject to eDNA analysis are shown on Volume 2, Figure 8.8a

¹¹ <https://sciencesearch.defra.gov.uk/ProjectDetails?ProjectID=18650>

Table 1. Ponds subject to HSI assessment and eDNA sampling in 2022 and 2025

Pond	OSGR	2022 Subject to eDNA sampling	2022 Subject to population assessment	2025 Subject to HSI assessment	2025 Subject to eDNA sampling	2025 Subject to population assessment	Further details
WB01	NS 84632 79913	Yes	No	Yes	No	No	No eDNA sampling because HSI Score was Poor
WB02	NS 84813 79796	Yes	No	Yes	Yes	No	eDNA sampling undertaken before HSI Score confirmed to be Poor
WB03	NS 83439 78468	No	No	Yes	No	No	No eDNA sampling because HSI Score was Poor
WB04	NS 82859 78269	Yes	Yes	Yes	Yes	No	eDNA sampling because HSI Score greater than Poor
WB05	NS 82791 78253	Yes	No	Yes	Yes	No	eDNA sampling undertaken before HSI Score confirmed to be Poor
WB06	NS 82428 77901	Yes	No	Yes	Yes	No	eDNA sampling undertaken before HSI confirmed to be Poor
WB07	NS 82416 77893	Yes	Yes	Yes	No	Yes	No eDNA sampling because population assessment carried out ¹²
WB08	NS 82401 77901	Yes	Yes	Yes	No	Yes	No eDNA sampling because population assessment carried out ¹²
WB09	NS 82385 77776	Yes	No	Yes	Yes	No	eDNA sampling because HSI not Poor
WB10	NS 82416 77774	Yes	Yes	Yes	No	Yes	No eDNA sampling because population assessment carried out

¹² Note that for these ponds, population assessment provided the presence/likely absence result and thus eDNA was not considered necessary.

WB11	NS 82409 77751	Yes	No	Yes	No	Yes	No eDNA sampling because population assessment carried out ¹²
WB12	NS 82381 77684	Yes	Yes	Yes	No	Yes	No eDNA sampling because population assessment carried out ¹²
WB13	NS 82481 77665	Yes	No	Yes	Yes	No	eDNA sampling because HSI not Poor
WB14	NS 82506 77689	Yes	No	Yes	Yes	No	eDNA sampling because HSI not Poor
WB15	NS 82522 77688	Yes	No	Yes	Yes	No	eDNA sampling because HSI not Poor
WB16	NS 82542 77670	Yes	No	Yes	Yes	No	eDNA sampling because HSI not Poor
WB17	NS 82516 77661	Yes	No	Yes	Yes	No	eDNA sampling because HSI not Poor
WB18	NS 82531 77647	Yes	No	Yes	Yes	No	eDNA sampling because HSI not Poor
WB19	NS 82559 77646	Yes	No	Yes	Yes	No	eDNA sampling because HSI not Poor
WB20	NS 82281 77543	Yes	No	Yes	Yes	No	eDNA sampling undertaken before HSI confirmed to be Poor
WB21	NS 82427 77535	No	Yes	Yes	Yes	No	eDNA sampling because HSI not Poor
WB22	NS 82403 77508	Yes	Yes	Yes	No	No	No eDNA sampling because HSI Poor
WB23	NS 82435 77455	Yes	No	Yes	No	No	No eDNA sampling because HSI Poor

WB24	NS 82519 77543	No	Yes	Yes	Yes	No	eDNA sampling undertaken before HSI confirmed to be Poor
WB25	NS 82518 77507	No	Yes	Yes	Yes	No	eDNA sampling because HSI not Poor
WB26	NS 82584 77503	Yes	Yes	Yes	Yes	No	eDNA sampling because HSI not Poor
WB27	NS 82596 77484	n/a	n/a	n/a	n/a	No	No pond present
WB28	NS 82591 77460	n/a	n/a	n/a	n/a	No	No pond present
WB29	NS 82607 77442	Yes	Yes	Yes	No	Yes	No eDNA sampling because population assessment carried out ¹²
WB30	NS 82693 77462	Yes	No	Yes	Yes	No	eDNA sampling undertaken before HSI confirmed to be Poor
WB31	NS 82750 77508	n/a	n/a	n/a	n/a	No	No pond present
WB32	NS 82497 77248	Yes	No	n/a	No	No	No eDNA/HSI sampling because pond dry
WB33	NS 82109 77058	Yes	No	Yes	No	No	No eDNA sampling because HSI Poor
WB34	NS 82055 77036	Yes	No	Yes	No	No	No eDNA sampling because HSI Poor
WB35	NS 81832 76714	Yes	No	Yes	Yes	No	eDNA sampling undertaken before HSI confirmed to be Poor
WB36	NS 80990 76169	n/a	No	n/a	n/a	No	No pond present

WB37	NS 81350 76036	n/a	No	n/a	n/a	No	No pond present
WB38	NS 79038 74778	Yes	No	Yes	Yes	No	eDNA sampling because HSI not Poor
WB39	NS 78627 74357	Yes	No	Yes	Yes	No	eDNA sampling because HSI not Poor
WB40	NS 78497 74344	Yes	No	Yes	Yes	No	eDNA sampling because HSI not Poor
WB41	NS 78179 74304	Yes	No	Yes	Yes	No	eDNA sampling because HSI not Poor
WB42	NS 78157 74292	n/a	n/a	n/a	n/a	No	No pond present
WB43	NS 78619 73900	Yes	No	Yes	Yes	No	eDNA sampling because HSI not Poor
WB44	NS 77909 73851	No	No	Yes	No	No	No eDNA sampling because HSI Poor
WB45	NS 77749 73879	No	No	Yes	Yes	No	eDNA sampling because HSI not Poor
WB46	NS 78153 73532	Yes	No	Yes	Yes	No	eDNA sampling because HSI not Poor
WB47	NS 78112 73477	Yes	No	n/a	No	No	No eDNA/HSI sampling because pond dry
WB48	NS 78121 73476	Yes	No	n/a	No	No	No eDNA/HSI sampling because pond dry
WB49	NS 78037 73472	Yes	No	Yes	Yes	No	eDNA sampling because HSI not Poor

WB50	NS 78082 73448	Yes	No	Yes	Yes	No	eDNA sampling because HSI not Poor
WB51	NS 78138 73413	Yes	No	Yes	Yes	No	eDNA sampling because HSI not Poor
WB55	NS 78430 73080	No	No	Yes	Yes	No	eDNA sampling because HSI not Poor
WB57	NS 77583 70832	Yes	No	n/a	No	No	No eDNA/HSI sampling because pond dry
WB58	NS 75787 69038	Yes	No	n/a	No	No	No eDNA/HSI sampling because pond dry
WB59	NS 76143 68978	Yes	Yes	Yes	Yes	No	eDNA sampling because HSI not Poor
WB60	NS 76144 68902	No	No	n/a	No	No	No eDNA/HSI sampling because pond dry
WB61	NS 76143 68884	Yes	No	n/a	No	No	No eDNA/HSI sampling because pond dry
WB0A	NS 84936 80009	No	No	Yes	No	No	No eDNA sampling because HSI Poor
WB0B	NS 82457 77578	No	No	Yes	Yes	Yes	eDNA sampling undertaken before HSI confirmed to be Poor
WB0C	NS 82449 77511	No	No	Yes	Yes	Yes	eDNA sampling undertaken before HSI confirmed to be Poor
WB0D	NS 82613 77438	No	No	Yes	Yes	Yes	eDNA sampling because HSI not Poor
WB0E	NS 84815 78823	No	No	Yes	No	No	No eDNA sampling because HSI Poor

WB0F	NS 77349 70819	No	No	Yes	No	No	No eDNA sampling because pond dry
WB0G	NS 77326 70810	No	No	Yes	No	No	No eDNA sampling because pond dry
WB0H	NS 75790 69097	No	No	Yes	No	No	No eDNA sampling because HSI was Poor
WB0I	NS 78281 74440	No	No	Yes	Yes	No	eDNA sampling undertaken before HSI confirmed to be Poor
WB0J	NS 81322 76262	No	No	Yes	Yes	No	eDNA sampling because HSI not Poor
WB0K	NS 78198 71662	No	No	Yes	No	No	No eDNA sampling because HSI Poor
AA01	NS 84241 80089	No	No	Yes	No	No	No eDNA sampling or population assessment because not identified until November 2025
AA02	NS 84370 79517	No	No	Yes	No	No	No eDNA sampling or population assessment because not identified until November 2025
AA03	NS 84328 79445	No	No	Yes	No	No	No eDNA sampling or population assessment because not identified until November 2025
CB01	NS 83366 77466	No	No	Yes	No	No	No eDNA sampling or population assessment because not identified until November 2025

Ponds WB27, WB28, WB31, WB36, WB37 and WB42 were identified as possible ponds during the desk study but were found not to exist during the visits for HSI and eDNA survey. These locations are shown on Volume 2, Figure 8.8a but are not mentioned further in this appendix.

Ponds WB52, WB53, WB54 and WB56 were also identified as possible ponds during the desk study. These ponds are only within 250m of an existing access track (not suitable habitat for the species and unlikely to be accessed except rarely for brief periods), from which they are also separated by either Palacerigg Road or the short-mown grass of Cumbernauld Model Flying Club (both similarly unsuitable for the species, further reducing the likelihood they would occur on the access track) and as such any GCN which may be associated with these ponds or surrounding terrestrial habitat were not considered to be a risk of any impact from the Project, given the extreme unlikelihood of them occurring on the access track. These ponds have been scoped out of the assessment and were not subject to survey. For completeness these ponds are shown on Volume 2, Figure 8.8a but are not mentioned further in this appendix.

Population assessment survey

Population assessment survey is used to determine the size of the GCN population present. In line with Natural England¹³ survey methodology guidelines on the assessment of population class size, a minimum of four survey visits were conducted at these waterbodies, extending to six survey visits where GCN were recorded. Where possible, three field survey techniques were utilised during each survey visit (subject to Limitations, as detailed below).

Population assessment surveys were undertaken in 2022 and 2025.

Ponds which returned a positive eDNA result in 2022 were subject to population assessment surveys, with the exception of five, WB07, WB08, WB10, WB12 and WB29 which were identified late into the 2022 survey season. These were subject to population assessment surveys in 2025.

Four additional ponds – WB11, WB0B, WB0C and WB0D - also received population assessment surveys in 2025, as justified below.

WB11 returned a negative eDNA result in 2022. However, this pond is immediately adjacent to WB10 (which returned a positive eDNA result in 2022), and, as observed during the initial 2025 HSI survey, movement of newts between these two ponds seemed extremely likely given their similar character and thin strip of damp intervening habitat. WB11 was therefore also subject to population assessment survey, though bottle trapping of this pond did not commence until Survey visit 2.

WB0B, WB0C and WB0D were not surveyed in 2022 because they were not identified until the HSI assessment survey in 2025. Given this (and the close proximity of these to other ponds which were already planned to receive population assessment survey), these ponds were therefore added to the 2025 population assessment survey programme.

Note that, due to i) WB11 not being subject to trapping on the Survey visit 1 (see Limitations); and, ii) torching being curtailed by equipment failure during Survey visit 6a (see Limitations), a

¹³ Natural England (2015). *Great crested newts: surveys and mitigation for development projects*. Standing advice for local planning authorities who need to assess the impacts of development on great crested newts. Available from: <https://www.gov.uk/guidance/great-crested-newts-surveys-and-mitigation-for-development-projects>.

seventh survey visit (“Survey visit 6b”) was carried-out to ensure all techniques were carried out six times at each pond (as far as possible).

The population assessment survey visits in April 2022 and between 03 April 2025 and 07 May 2025, with visits 3, 4, 5, 6a and 6b being completed within the peak survey period between mid-April to mid-May.

Natural England suggests that presence or absence of GCN can be detected after four survey visits¹³. No GCN were detected in ponds WB0B, WB0C or WB0D after four survey visits. However, given that no trapping took place in WB0B or WB0C due to their shallow depth, and that torching was limited in all three by the presence of dense pondweed (where identified this was broad-leaved pondweed *Potamogeton natans*), the population assessment surveys for the full six visits to increase the likelihood that GCN would be detected (if present). In addition, these ponds were also subject to eDNA sampling.

The dates of the surveys, weather conditions, and notes on survey methodology are summarised in, the locations of ponds subject to population assessment are shown on Volume 2, Figure 8.8b

More detailed descriptions of each technique are provided under the sub-headings below.

Table 2. Great crested newt population assessment survey 2025 details

Survey visit	Date	Weather conditions on arrival	Maximum temperature previous day (°C)	Minimum overnight temperature (°C)	Sunset time	Time trap deployment completed	Time trap collection completed	Notes on survey methodology
1	03/04/2025	9°C, clear, dry, light breeze.	12	5	20:00	20:00	09:00	WB0B and WB0C, WB11, were not bottle trapped. WB29 and WB0D were only torched.
2	08/04/2025	15°C, 25% cloud, dry, moderate breeze.	13	5	20:08	20:08	09:34	
3	14/04/2025	11°C, intermittent cloud cover and rain, light breeze.	12	6	20:20	20:20	09:45	
4	28/04/2025	17°C, intermittent cloud cover, dry, light breeze.	17	5	20:47	20:47	09:29	WB0B and WB0C, WB11, were not bottle trapped. WB29 was only torched
5	01/05/2025	16°C, intermittent cloud cover, dry, light breeze.	17	6	20:54	20:54	09:09	

6a	07/05/2025	14°C, intermittent cloud cover, dry, calm breeze.	14	5	21:06	21:06	09:24	WB11, WB12, and WB0D were not subject to torching. WB0B, WB0C and WB29 were not surveyed.
6b	20/05/2025	19°C, intermittent cloud cover, dry, calm breeze.	21	11	21:31	21:31	08:45	WB07, WB08 and WB10 were not surveyed. WB12, WB29 and WB0D were only subjected to torching. WB0B and WB0C were not bottle trapped.

Bottle trapping

Bottle trapping was carried out in accordance with the methods described in English Nature³. In summary, two litre plastic drinks bottles were cut in half, with the top section inverted and inserted into the bottom section, and were secured to the substrate of the pond using bamboo canes. The entrance to the bottles was submerged, with a pocket of air left at the top of each trap.

The bottle traps were placed at approximately 2m intervals around the entire perimeter of the pond. They were set in the evening, shortly before sunset, and then checked and removed from the pond early the following morning. In line with English Nature³ guidance for traps with an air bubble, the traps were checked between 06:00 and 11:00 and were not left unchecked for more than 17 hours (all checks took place less than 14 hours after the traps were deployed).

The total number of bottle traps set per survey visit was noted to ensure the correct number were retrieved.

Ponds WB0B and WB0C were not of sufficient depth, and WB29 was considered unsafe to bottle trap (see Limitations).

Lower water levels following dry weather meant that fewer bottle traps were used on later visits at WB12 (see Limitations).

Torching

Torching surveys were carried out shortly after sunset following the setting of bottle traps, allowing time for the water to settle. A high-powered torch was used to scan the waterbody for adult GCN.

Torching was constrained by the presence of dense pondweed and turbidity at individual ponds (see Limitations).

Egg searching

Newts lay single eggs which are protected by folding pliable material, usually the leaves of aquatic plants, around them. Surveyors examined submerged marginal vegetation surrounding the pond for these typical folded leaves and for concealed GCN eggs within.

Egg searching was constrained by the presence of dense pondweed and bank characteristics conditions at individual ponds (see Limitations).

Population size class assessment

Using the results of the population assessment surveys, population size class was estimated, in line with guidelines published by Natural England¹³. Population size class was estimated by taking the peak counts of GCN identified by a single survey technique on a single survey visit and adding them together.

Male and female GCN can be readily distinguished from one another. Thus, the peak count has been given as combined peak counts of male and females. This avoids undercounting which would occur if, for example, a male newt and a female newt captured on consecutive visits were each counted as “one newt” when the true count is two individuals.

Population classes are expressed as:

- ‘Small’ for maximum counts of up to ten adults;
- ‘Medium’ for maximum counts of between 11 and 100 adults; and
- ‘Large’ for maximum counts of more than 100 adults.

Population size classes for ponds WB04, WB21, WB24, WB25 and WB26 were determined following 2022 population assessment surveys and, given that the habitat conditions have not significantly changed, these results are considered to provide a likely estimate of population size.

Identification of breeding ponds

Breeding ponds are usually identified by the presence of GCN eggs found during egg searching or juveniles captured during bottle trapping.

However, the identification of these features is thought to have been limited both by access to/abundance of pondweed and potentially by the timing of surveys, which were possibly too early in the year for this location (see Limitations).

A precautionary approach has therefore been taken whereby any ponds within which both male and female GCN were identified by torching and/or bottle trapping are considered to be potential breeding ponds.

Limitations

2022

Ponds WB25 and WB24 were identified after eDNA testing was completed. However, as both were then subject to population assessment surveys and GCN presence was confirmed, this has not impacted the results.

However, WB25 was only identified during the first trapping / torching survey visit on 19 May 2022 and as such was not subject to bottle trapping on this occasion (surveyors had not brought a sufficient number of traps to allow for trapping additional ponds). As such, WB25 was only subject to bottle trapping on a total of five survey visits rather than the recommended six for population assessment. However, WB25 did receive the recommended six torching surveys and these combined with the five trapping surveys show that the peak count of GCN is well below the threshold for the next population size class, and it can reliably be concluded that the population in the pond is Small.

No GCN were recorded during population assessment surveys in ponds WB59 and WB22 and thus the species is thought to be absent from these ponds, or at population levels which were detected by eDNA survey but not large enough to be detected by population assessment survey. Other possible reasons for positive eDNA results in the absence of GCN include contamination, which is likely in pond WB22 given its proximity and likely water exchange with WB21, within which the species was confirmed as being present.

Subsequent to the GCN population assessment surveys and during the course of other ecological field survey being carried out, an additional twelve ponds were identified within 250 m of the Scheme which had not been found by the review of aerial images or OS mapping (on aerial images the ponds were shaded by trees, and/or they are not shown on OS maps). These ponds were therefore not subject to any GCN survey in 2022. However, six of the nine are within 250 m of a pond confirmed to support GCN, and the remaining six are less than

360 m distant to such a pond. While no population assessment survey and population size class assessment were carried out, HSI assessment and eDNA surveys were conducted in end of May and beginning of June 2023 and since there have already been ponds identified to host a population of GCN, the absence of population assessment survey and a population size class assessment are not considered a significant limitation and do not misrepresent the results.

2025

It is possible that additional waterbodies may exist within 250m of the Sites which were not identified by the desk-based review of aerial images and OS mapping and which may be discovered by further survey, for example pre-construction survey. Further survey may be required to determine whether any as yet unknown ponds support GCN.

Ponds AA01, AA02, AA03 and CB01 were first identified in November 2025. AA01, AA03 and CB01 were found to have greater than Poor habitat suitability and as such required eDNA survey. However, given that they were found outside of the eDNA sample collection season, this could not be undertaken in 2025. This is a limitation given that further survey work would be required to determine the status of the species in these ponds and as such has been accounted for within the impact assessment, with a precautionary assumption made that GCN are present within these ponds.

An additional waterbody, St Helen's Loch, was also not identified until after the 2025 GCN eDNA and population assessment season was over. This waterbody is within 250m of access tracks but over 250m of the AA Route Undergrounding and CB Route Removal. No surveys were undertaken at St Helen's Loch, however this is not considered a significant limitation because the proposed access tracks within 250m follow existing roads or are within heavily managed fields, and GCN are extremely unlikely to occur in either of these areas. This waterbody is not considered further.

In 2025, ponds WB32, WB47, WB48, WB57, WB58, WB60, WB61, WB0F and WB0G were dry during the intended eDNA collection visit in 2025. This is not a significant limitation because ponds being dry during the eDNA sampling season (mid April – end of June) precludes their use by breeding GCN, meaning that the species can be assumed absent. Furthermore, ponds WB32, WB47, WB48, WB57, WB58 and WB61 were subject to eDNA sampling in 2022 (the ponds were not dry during sampling this year) and all returned negative results.

WB59 and WB22 returned positive eDNA results in 2022 but were not trapped in 2025. This is not a significant limitation because these are now thought to have been a false positive result, potentially caused by contamination. This would be consistent with the lack of GCN captured in both ponds during bottle trapping in 2022 and with the small number of replicates (1/12) found to be positive during lab analysis of the 2022 eDNA samples. Furthermore, eDNA sampling of WB59 in 2025 returned a negative result. WB22 itself is highly unsuitable for GCN given that it is stocked with perch, and the positive result in 2022 can be explained by it being fed with water from WB21, a pond within which GCN were confirmed to be present in 2022, and which is free from perch.

WB20 returned a positive eDNA result in 2025 but did not receive population assessment. The small number of replicates (1/12) suggest that this positive result was due to contamination, though this could not be confirmed. Regardless, it has been assumed that

GCN likely use all the land within and adjacent to Wester Drum Wildlife Site, South Drum Claypit Wildlife Site and South Drum Moss Wildlife Site, between Beam Road to the east and an unnamed road to the south (hereafter referred to as the “wider Drum Wildlife Site area”). Given the Poor habitat suitability and small size of this pond, and that other ponds in this area were generally of Small population size, it can be reliably assumed that pond WB20 would support a small population of the species, at most.

The high-powered torch required for torching survey failed during Survey visit 6a and prevented torching of ponds WB11, WB12, WB0B, WB0C and WB29. This is not a significant limitation given that an additional survey visit (Survey visit 6b) was carried out later in May, meaning that all ponds were torched six times.

Dense pondweed obscured the surfaces of ponds WB0B, WB0C and WB0D, making torching and egg-searching extremely difficult. Ponds WB0B and WB0C were also too shallow to bottle trap. These are not a significant limitation given that these ponds all returned negative eDNA results and were subject to torching and egg-searching on six nights, more than the recommended four nights required to determine absence.

Torching was occasionally constrained by the turbidity of the water, which was high in WB10, WB11, WB12, WB29 due in part to the peaty input from the surrounding bog. This is not a significant limitation because ponds WB10, WB11, WB12 were also successfully trapped, with similar results to the torching survey. Pond WB29 could not be trapped (see below) but the number of GCN identified by torching was well below the threshold for the next population size class, and it can reliably be concluded that the population in the pond is Small.

The water level in pond WB29 was significantly lower bank-top and this, combined with the steep bank and depth of the pond, meant it was considered unsafe to bottle trap. This is not a significant limitation given that this pond was able to be successfully torched, resulting in GCN being identified. The number of GCN identified was well below the threshold for the next population size class, and it can reliably be concluded that the population in the pond is Small.

Lower water levels at ponds WB08, WB10, WB11 and WB12 following dry weather meant that there was not always sufficient water to place bottle traps in certain areas and it was unsafe to walk any further into the ponds to reach deeper water due to the steep banks and floating vegetation. Thus, the number of traps used between survey visits varied, with generally lower numbers of bottle traps being used on later visits. This is not a significant limitation because the number of GCN trapped did not appear to correlate with the number of traps deployed, because bottle traps still covered a large proportion of the pond perimeter and because torching still allowed full coverage of the pond surfaces.

Egg searching was limited by the conditions at all ponds. Ponds WB07, WB08, WB10, WB11, WB12 and WB29 are extremely deep with a steep drop off from the edge. The majority of suitable egg-laying vegetation was located in the centre of these ponds which could not be easily accessed. Ponds WB0B, WB0C and WB0D were filled with dense pondweed, and it was impossible to examine every leaf. In addition, given that no GCN eggs or juvenile GCN (or eggs/juveniles of other newt species) were identified it is considered possible that the surveys, which, although within the time-frame recommended by Natural England, were too

early in the season for GCN populations in the Scottish lowlands. This, combined with the limitations related to the inaccessibility of egg laying vegetation, mean there is low confidence in the result of no observed GCN eggs. Furthermore, despite a lack of definitive evidence of breeding (GCN eggs or juveniles), courtship behaviour (a male GCN displaying to a female) was observed in pond WB08. However, the precautionary approach taken (see Identification of breeding ponds) is considered to compensate for these limitations, and they are not considered significant.

3. Baseline Conditions

Desk study

No international sites designated for GCN are present within 10km of the Sites, nor are there any nationally designated sites within 2km.

Species records were considered when from within 1km of the Sites and from the last 10 years (2015 onwards).

The Wildlife Information Centre returned one record listed as GCN from pond WB02 in 2020. However, this record was submitted to ARG UK/ARC Record Pool by a member of the public, and the record description does not allow the newt to be identified as a GCN. Given the resulting low confidence in this record, and that eDNA survey of WB02 in 2025 returned a negative result, this record can be discounted.

The NBN Atlas Scotland did not provide any records of GCN from within 1km of the Site.

The Amphibian and Reptile Groups of UK (ARGUK) did not return any records of GCN from within 1km of the Sites.

Habitat Suitability Index assessment and Environmental DNA results

Results of the HSI assessment, eDNA sampling and population assessment survey are summarised below in Table 3.

All ponds which returned positive eDNA results in 2025 were within the wider Drum Wildlife Site area.

A detailed breakdown of HSI results along with photographs is presented in Annex I.

Copies of the eDNA laboratory certificates are provided in Annex II.

Following HSI and eDNA survey, GCN were considered likely absent from all ponds with the exception of:

- Ponds AA01, AA03 and pond CB01. These ponds had greater than poor habitat suitability but were not subject to eDNA survey and as such the status of GCN within these ponds is unknown, but taking a precautionary approach presence is assumed; and,
- Ponds WB04 – WB31 and WB0B, WB0C and WB0D i.e. those within the wider Drum Wildlife Site area, where they were not of Poor habitat suitability or dry – these ponds were subject to population assessments

Table 3. 2022 and 2025 HSI, eDNA results and summary of population assessment results from 2022 and 2025

Pond name	2022 eDNA result	2022 Population assessment result	2025 HSI assessment result	2025 eDNA result	2025 Population assessment result
WB01	Negative	n/a	Poor	n/a	n/a
WB02	Negative	n/a	Poor	Negative	n/a
WB03	n/a	n/a	Poor	n/a	n/a
WB04	Positive	Present	Good	Positive	(Survey not updated in 2025 – assumed present)
WB05	Negative	n/a	Poor	Negative	n/a
WB06	Negative	n/a	Poor	Negative	n/a
WB07	Positive	n/a	Average	n/a*	Present
WB08	Positive	n/a	Average	n/a*	Present
WB09	Negative	n/a	Below average	Negative	n/a
WB10	Positive	n/a	Average	n/a*	Present
WB11	Negative	n/a	Average	n/a*	Present
WB12	Positive	n/a	Poor	n/a*	Present
WB13	Negative	n/a	Below average	Negative	n/a
WB14	Negative	n/a	Average	Negative	n/a
WB15	Negative	n/a	Good	Negative	n/a
WB16	Negative	n/a	Good	Negative	n/a
WB17	Negative	n/a	Good	Negative	n/a
WB18	Negative	n/a	Average	Negative	n/a
WB19	Negative	n/a	Below average	Negative	n/a
WB20	Negative	n/a	Poor	Positive	n/a
WB21	n/a	Present	Good	Negative	(Survey not updated in 2025 – assumed present)
WB22	Positive	Absent	Poor	n/a	(Survey not updated in 2025 – assumed absent)
WB23	Negative	n/a	Poor	n/a	n/a

WB24	n/a	Present	Poor	Positive	Present
WB25	n/a	Present	Below average	Positive	Present
WB26	Positive	Present	Average	Negative	(Survey not updated in 2025 – assumed present)
WB29	Positive	n/a	Poor	n/a*	Present
WB30	Negative	n/a	Poor	Negative	n/a
WB32	Negative	n/a	n/a	n/a	n/a
WB33	Negative	n/a	Poor	n/a	n/a
WB34	Negative	n/a	Poor	n/a	n/a
WB35	Negative	n/a	Poor	Negative	n/a
WB38	Negative	n/a	Average	Negative	n/a
WB39	Negative	n/a	Below average	Negative	n/a
WB40	Negative	n/a	Good	Negative	n/a
WB41	Negative	n/a	Below average	Negative	n/a
WB43	Negative	n/a	Excellent	Negative	n/a
WB44	n/a	n/a	Poor	n/a	n/a
WB45	n/a	n/a	Average	Negative	n/a
WB46	Negative	n/a	Excellent	Negative	n/a
WB47	Negative	n/a	Average	n/a	n/a
WB48	Negative	n/a	Average	n/a	n/a
WB49	Negative	n/a	Average	Negative	n/a
WB50	Negative	n/a	Good	Negative	n/a
WB51	Negative	n/a	Average	Negative	n/a
WB55	n/a	n/a	Average	Negative	n/a
WB57	Negative	n/a	n/a	n/a	n/a
WB58	Negative	n/a	n/a	n/a	n/a
WB59	Positive	Absent	Good	Negative	n/a
WB60	n/a	n/a	n/a	n/a	n/a
WB61	Negative	n/a	n/a	n/a	n/a
WB0A	n/a	n/a	Poor	n/a	n/a
WB0B	n/a	n/a	Poor	Negative*	Absent
WB0C	n/a	n/a	Poor	Negative*	Absent

WB0D	n/a	n/a	Average	Negative*	Absent
WB0E	n/a	n/a	Poor	n/a	n/a
WB0F	n/a	n/a	Below average	n/a	n/a
WB0G	n/a	n/a	Below average	n/a	n/a
WB0H	n/a	n/a	Poor	n/a	n/a
WB0I	n/a	n/a	Poor	Negative	n/a
WB0J	n/a	n/a	Below average	Negative	n/a
WB0K	n/a	n/a	Poor	n/a	n/a
AA01	n/a	n/a	Below average	n/a	n/a
AA02	n/a	n/a	Poor	n/a	n/a
AA03	n/a	n/a	Below average	n/a	n/a
CB01	n/a	n/a	Good	n/a	n/a – not subject to eDNA or population survey, so assumed Present

*an asterisk denotes pond which were subject to population assessment.

Population assessment survey, population size classes and breeding

The results of the 2025 population assessment surveys are summarised below in Table 4 and detailed in Annex III.

Adult GCN were recorded in six out of the nine ponds surveyed in 2025. One of these six ponds (WB08) was found to have a Medium sized population of GCN, and the other five (WB07, WB10, WB11, WB12, WB29) had small populations (see Table 4).

No GCN eggs or efts were found in any of the ponds in 2025, however this was possibly due to the suitable egg laying vegetation being mainly inaccessible, or so abundant that it could not be effectively searched, or due to the timing of the surveys (see Limitations). However, courtship behaviour comprising a male arching its back and waving its tail in front of a female was observed in pond WB08 indicating breeding is likely taking place and, given the above limitations it is considered that ponds WB07, WB08, WB10, WB12 have the potential to be used for breeding.

Population assessment surveys undertaken in 2022 identified a further five ponds as supporting populations of GCN. Evidence of breeding was observed in four of these, with one additional pond known to support both male and female GCN. The results of the 2022 surveys are also summarised below in Table 4.

Table 4. Summary of 2025 and 2022 population assessment results

Pond	Peak count of male GCN	Peak count of female GCN	Total peak count of GCN	Population size class	Evidence of breeding?
Population assessment undertaken in 2025					
WB07	5	1	6	Small	No (but both sexes present)
WB08	9	7	16	Medium	Yes (courtship behaviour)
WB10	4	3	7	Small	No (but both sexes present)
WB11	1	0	1	Small	No
WB12	1	1	2	Small	No (but both sexes present)
WB0B	0	0	0	No GCN detected	
WB0C	0	0	0	No GCN detected	
WB29	2	0	2	Small	No
WB0D	0	0	0	No GCN detected	
Population assessment undertaken in 2022					
WB04	n/a		5	Small	Yes (egg)
WB21	n/a		2	Small	No (but both sexes present)
WB22	n/a		0	No GCN detected	
WB24	n/a		1	Small	Yes (egg)
WB25	n/a		3	Small	Yes (eft)
WB26	n/a		3	Small	Yes (egg)
WB59	n/a		0	No GCN detected	

4. Summary and Conclusions

The survey methods adopted were complex, and the Methodology and Limitations sections should be considered in full when reviewing the below.

In summary, over the course of the 2025 and 2022 surveys and HSI assessment and eDNA sample collection, GCN were confirmed to be present by population assessment in the following 11 ponds (Table 6), which are all within 250m of the New Build Overhead Line and located within the wider Drum Wildlife Site area:

- WB04;
- WB07;

- WB08;
- WB10;
- WB11;
- WB12;
- WB21;
- WB24;
- WB25;
- WB26; and
- WB29.

All of the above ponds were found to have Small populations of GCN, with the exception of WB08 which falls just within the Medium category.

As shown in Table 6, the only potential evidence of breeding in recorded 2025 was courtship behaviour in pond WB08. In 2022 either eggs or efts were recorded in ponds WB04, WB24, WB25 and WB26. However, the 2025 surveys are thought to have been limited, possibly by the time in the season they were carried out (see Limitations). A precautionary approach has therefore been taken whereby all ponds within which both male and female GCN were identified are considered as potential breeding ponds. This excludes only WB11 and WB29.

Ponds within the wider Drum Wildlife Site area are separated by highly suitable terrestrial habitat and are arranged in clusters between which GCN could easily navigate. GCN associated with the above ponds likely use the entire area during their terrestrial phase. As such, a Negative eDNA result from a suitable pond in this area in one year does not preclude its use by the species in other years. For example, WB21 returned a Negative eDNA result in 2025, but GCN were trapped here during population assessment in 2022.

GCN are considered absent from the remainder of the New Build Overhead Line Site.

In relation to the CB Route, GCN are assumed to be present in pond CB01. Ponds AA01, AA02 and AA03 all scored below average or poor in the HSI and are generally isolated, it is likely that GCN are absent from these ponds.

Annex I 2025 Habitat Suitability Index assessment detailed results

Annexe 1. Habitat Suitability Index assessment results

Below are the full results of the Habitat Suitability Index assessment

Table 1. HSI results for waterbody AA01

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	50 m ²	0.10
S3	Pond drying	Dries annually	0.10
S4	Water quality	Poor	0.01
S5	Shade	90%	0.40
S6	Waterfowl	Absent	1.00
S7	Fish	Absent	1.00
S8	Pond count	4	0.70
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	0%	0.30
Score			0.29 (poor)

Photograph 1. Waterbody AA01



Table 2. HSI results for waterbody AA02

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	50 m ²	0.10
S3	Pond drying	Dries annually	0.10
S4	Water quality	Poor	0.01
S5	Shade	90%	0.40
S6	Waterfowl	Absent	1.00
S7	Fish	Absent	1.00
S8	Pond count	4	0.70
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	0%	0.30
Score			0.29 (poor)

Photograph 2. Waterbody AA02



Table 3. HSI results for waterbody AA03

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	50 m ²	0.10
S3	Pond drying	Dries annually	0.10
S4	Water quality	Poor	0.01
S5	Shade	90%	0.40
S6	Waterfowl	Absent	1.00
S7	Fish	Absent	1.00
S8	Pond count	4	0.70
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	0%	0.30
Score			0.29 (poor)

Photograph 3. Waterbody WBAA



Table 4. HSI results for waterbody CB01

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	50 m ²	0.10
S3	Pond drying	Dries annually	0.10
S4	Water quality	Poor	0.01
S5	Shade	90%	0.40
S6	Waterfowl	Absent	1.00
S7	Fish	Absent	1.00
S8	Pond count	4	0.70
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	0%	0.30
Score			0.29 (poor)

Table 5. HSI results for waterbody WB0A

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	50 m ²	0.10
S3	Pond drying	Dries annually	0.10
S4	Water quality	Poor	0.01
S5	Shade	90%	0.40
S6	Waterfowl	Absent	1.00
S7	Fish	Absent	1.00
S8	Pond count	4	0.70
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	0%	0.30
Score			0.29 (poor)

Photograph 4. Waterbody WB0A



Table 6. HSI results for waterbody WB0B

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	50 m ²	0.10
S3	Pond drying	Dries annually	0.10
S4	Water quality	Poor	0.01
S5	Shade	0-60%	1.00
S6	Waterfowl	Absent	1.00
S7	Fish	Absent	1.00
S8	Pond count	13+	1.00
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	70%	1.00
Score			0.37 (poor)

Photograph 5. Waterbody WB0B



Table 7. HSI results for waterbody WB0C

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	<50 m ²	0.05
S3	Pond drying	Dries annually	0.10
S4	Water quality	Poor	0.01
S5	Shade	65%	0.90
S6	Waterfowl	Absent	1.00
S7	Fish	Absent	1.00
S8	Pond count	13+	1.00
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	70%	1.00
Score			0.34 (poor)

Photograph 6. Waterbody WB0C



Table 8. HSI results for waterbody WB0D

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	<50 m ²	0.05
S3	Pond drying	Never dries	0.90
S4	Water quality	Moderate	0.67
S5	Shade	80%	0.60
S6	Waterfowl	Absent	1.00
S7	Fish	Absent	1.00
S8	Pond count	13+	1.00
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	70%	1.00
Score			0.62 (average)

Photograph 7. Waterbody WB0D



Table 9. HSI results for waterbody WB0E

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	50 m ²	0.10
S3	Pond drying	Dries annually	0.10
S4	Water quality	Moderate	0.67
S5	Shade	0-60%	1.00
S6	Waterfowl	Absent	1.00
S7	Fish	Absent	1.00
S8	Pond count	2	0.55
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	70%	1.00
Score			0.53 (below average)

Photograph 8 & 9. Waterbody WB0E



Table 10. HSI results for waterbody WB0F and WB0G

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	50 m ²	0.10
S3	Pond drying	Dries annually	0.10
S4	Water quality	Moderate	0.67
S5	Shade	0-60%	1.00
S6	Waterfowl	Absent	1.00
S7	Fish	Absent	1.00
S8	Pond count	2	0.55
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	70%	1.00
Score			0.53 (below average)

Ponds WB0F and WB0G received the same HSI scores

Photograph 10 & 11. Waterbody WB0F/0G



Table 11. HSI results for waterbody WB0H

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	50 m ²	0.10
S3	Pond drying	Dries annually	0.10
S4	Water quality	Bad	0.33
S5	Shade	0-60%	1.00
S6	Waterfowl	Minor	0.67
S7	Fish	Absent	1.00
S8	Pond count	7	0.85
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	10%	0.40
Score			0.45 (poor)

Photograph 12 & 13. Waterbody WB0H



Table 12. HSI results for waterbody WB01

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	<50 m ²	0.05
S3	Pond drying	Sometimes dries	0.50
S4	Water quality	Poor	0.01
S5	Shade	0-60%	1.00
S6	Waterfowl	Absent	1.00
S7	Fish	Absent	1.00
S8	Pond count	12	0.98
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	70%	1.00
Score			0.41 (poor)

Photograph 14 & 15. Waterbody WB01



Table 13. HSI results for waterbody WB0J

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	300 m ²	0.60
S3	Pond drying	Rarely dries	1.00
S4	Water quality	Poor	0.01
S5	Shade	0-60%	1.00
S6	Waterfowl	Absent	1.00
S7	Fish	Absent	1.00
S8	Pond count	3	0.66
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	30%	0.60
Score			0.51 (below average)

Photograph 16. Waterbody WB0J



Table 14. HSI results for waterbody WB0K

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	<50 m ²	0.05
S3	Pond drying	Sometimes dries	0.50
S4	Water quality	Poor	0.01
S5	Shade	0-60%	1.00
S6	Waterfowl	Absent	1.00
S7	Fish	Absent	1.00
S8	Pond count	0	0.01
S9	Terrestrial habitat	Moderate	0.67
S10	Macrophyte cover	0%	0.30
Score			0.22 (poor)

Photograph 17. Waterbody WB0K



Table 15. HSI results for waterbody WB01

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	500 m ²	1.00
S3	Pond drying	Dries annually	0.10
S4	Water quality	Poor	0.01
S5	Shade	100%	0.20
S6	Waterfowl	Minor	0.67
S7	Fish	Absent	1.00
S8	Pond count	4	0.70
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	70%	1.00
Score			0.37 (poor)

Photograph 18 & 19. Waterbody WB01



Table 16. HSI results for waterbody WB02

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	100 m ²	0.20
S3	Pond drying	Rarely dries	1.00
S4	Water quality	Poor	0.01
S5	Shade	80%	0.60
S6	Waterfowl	Major	0.01
S7	Fish	Absent	1.00
S8	Pond count	4	0.70
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	70%	1.00
Score			0.29 (poor)

Photograph 20 & 21. Waterbody WB02



Table 17. HSI results for waterbody WB03

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	400 m ²	0.80
S3	Pond drying	Dries annually	0.10
S4	Water quality	Poor	0.01
S5	Shade	0-60%	1.00
S6	Waterfowl	Major	0.01
S7	Fish	Absent	1.00
S8	Pond count	4	0.70
S9	Terrestrial habitat	Moderate	0.67
S10	Macrophyte cover	40%	0.70
Score			0.26 (poor)

Photograph 22 & 23. Waterbody WB03



Table 18. HSI results for waterbody WB04

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	100 m ²	0.20
S3	Pond drying	Never dries	0.90
S4	Water quality	Good	1.00
S5	Shade	0-60%	1.00
S6	Waterfowl	Absent	1.00
S7	Fish	Possible	0.67
S8	Pond count	13+	1.00
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	60%	0.90
Score			0.75 (good)

Photograph 24 & 25. Waterbody WB04.



Table 19. HSI results for waterbody WB05

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	> 2000 m ²	Exclude
S3	Pond drying	Never dries	0.90
S4	Water quality	Good	1.00
S5	Shade	0-60%	1.00
S6	Waterfowl	Minor	0.67
S7	Fish	Major	0.01
S8	Pond count	13+	1.00
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	10%	0.40
Score			0.47 (poor)

Photograph 26. Waterbody WB05.



Table 20. HSI results for waterbody WB06

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	50 m ²	0.10
S3	Pond drying	Never dries	0.90
S4	Water quality	Poor	0.01
S5	Shade	100%	0.20
S6	Waterfowl	Minor	0.67
S7	Fish	Absent	1.00
S8	Pond count	13+	1.00
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	5%	0.35
Score			0.34 (poor)

Photograph 27 & 28. Waterbody WB06



Table 21. HSI results for waterbody WB07

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	100 m ²	0.20
S3	Pond drying	Never dries	0.90
S4	Water quality	Moderate	0.67
S5	Shade	75%	0.70
S6	Waterfowl	Minor	0.67
S7	Fish	Absent	1.00
S8	Pond count	13+	1.00
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	5%	0.35
Score			0.63 (average)

Photograph 29. Waterbody WB07



Table 22. HSI results for waterbody WB08

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	100 m ²	0.20
S3	Pond drying	Never dries	0.90
S4	Water quality	Moderate	0.67
S5	Shade	0-60%	1.00
S6	Waterfowl	Minor	0.67
S7	Fish	Absent	1.00
S8	Pond count	13+	1.00
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	5%	0.35
Score			0.65 (average)

Photograph 30 & 31. Waterbody WB08



Table 23. HSI results for waterbody WB09

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	<50 m ²	0.05
S3	Pond drying	Never dries	0.90
S4	Water quality	Moderate	0.67
S5	Shade	0-60%	1.00
S6	Waterfowl	Absent	1.00
S7	Fish	Absent	1.00
S8	Pond count	13+	1.00
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	0%	0.30
Score			0.58 (below average)

Photograph 32 & 33. Waterbody WB09



Table 24. HSI results for waterbody WB10

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	50 m ²	0.10
S3	Pond drying	Never dries	0.90
S4	Water quality	Moderate	0.67
S5	Shade	0-60%	1.00
S6	Waterfowl	Minor	0.67
S7	Fish	Absent	1.00
S8	Pond count	13+	1.00
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	20%	0.50
Score			0.63 (average)

Photograph 34. Waterbody WB10



Table 25. HSI results for waterbody WB11

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	50 m ²	0.10
S3	Pond drying	Never dries	0.90
S4	Water quality	Moderate	0.67
S5	Shade	0-60%	1.00
S6	Waterfowl	Absent	1.00
S7	Fish	Absent	1.00
S8	Pond count	13+	1.00
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	30%	0.60
Score			0.67 (average)

Photograph 35 & 36. Waterbody WB11



Table 26. HSI results for waterbody WB12

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	50 m ²	0.10
S3	Pond drying	Never dries	0.90
S4	Water quality	Poor	0.01
S5	Shade	0-60%	1.00
S6	Waterfowl	Minor	0.67
S7	Fish	Absent	1.00
S8	Pond count	13+	1.00
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	30%	0.60
Score			0.42 (poor)

Photograph 37. Waterbody WB12



Table 27. HSI results for waterbody WB13

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	<50 m ²	0.05
S3	Pond drying	Never dries	0.90
S4	Water quality	Moderate	0.67
S5	Shade	0-60%	1.00
S6	Waterfowl	Minor	0.67
S7	Fish	Absent	1.00
S8	Pond count	13+	1.00
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	5%	0.35
Score			0.57 (below average)

Photograph 38. Waterbody WB13



Table 28. HSI results for waterbody WB14

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	100 m ²	0.20
S3	Pond drying	Never dries	0.90
S4	Water quality	Moderate	0.67
S5	Shade	0-60%	1.00
S6	Waterfowl	Minor	0.67
S7	Fish	Absent	1.00
S8	Pond count	13+	1.00
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	0%	0.30
Score			0.64 (average)

Photograph 39. Waterbody WB14



Table 29. HSI results for waterbody WB15

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	200 m ²	0.40
S3	Pond drying	Never dries	0.90
S4	Water quality	Moderate	0.67
S5	Shade	0-60%	1.00
S6	Waterfowl	Minor	0.67
S7	Fish	Absent	1.00
S8	Pond count	13+	1.00
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	5%	0.35
Score			0.7 (good)

Photograph 40. Waterbody WB15



Table 30: HSI results for waterbody WB16

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	250 m ²	0.50
S3	Pond drying	Never dries	0.90
S4	Water quality	Moderate	0.67
S5	Shade	0-60%	1.00
S6	Waterfowl	Minor	0.67
S7	Fish	Absent	1.00
S8	Pond count	13+	1.00
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	0%	0.30
Score			0.7 (good)

Photograph 41. Waterbody WB16



Table 31. HSI results for waterbody WB17

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	250 m ²	0.50
S3	Pond drying	Never dries	0.90
S4	Water quality	Moderate	0.67
S5	Shade	0-60%	1.00
S6	Waterfowl	Minor	0.67
S7	Fish	Absent	1.00
S8	Pond count	13+	1.00
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	5%	0.35
Score			0.72 (good)

Photograph 42 & 43. Waterbody WB17



Table 32. HSI results for waterbody WB18

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	150 m ²	0.30
S3	Pond drying	Never dries	0.90
S4	Water quality	Moderate	0.67
S5	Shade	0-60%	1.00
S6	Waterfowl	Minor	0.67
S7	Fish	Absent	1.00
S8	Pond count	13+	1.00
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	0%	0.30
Score			0.67 (average)

Photograph 44. Waterbody WB18



Table 33. HSI results for waterbody WB19

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	<50 m ²	0.05
S3	Pond drying	Never dries	0.90
S4	Water quality	Moderate	0.67
S5	Shade	0-60%	1.00
S6	Waterfowl	Minor	0.67
S7	Fish	Absent	1.00
S8	Pond count	13+	1.00
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	0%	0.30
Score			0.56 (below average)

Photograph 45. Waterbody WB19



Table 34. HSI results for waterbody WB20

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	50 m ²	0.10
S3	Pond drying	Never dries	0.90
S4	Water quality	Poor	0.01
S5	Shade	0-60%	1.00
S6	Waterfowl	Minor	0.67
S7	Fish	Absent	1.00
S8	Pond count	13+	1.00
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	70%	1.00
Score			0.44 (poor)

Photograph 46. Waterbody WB20



Table 35. HSI results for waterbody WB21

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	100 m ²	0.20
S3	Pond drying	Sometimes dries	0.50
S4	Water quality	Moderate	0.67
S5	Shade	65%	0.90
S6	Waterfowl	Absent	1.00
S7	Fish	Absent	1.00
S8	Pond count	13+	1.00
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	70%	1.00
Score			0.7 (good)

Photograph 47. Waterbody WB21



Table 36. HSI results for waterbody WB22

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	> 2000 m ²	Exclude
S3	Pond drying	Never dries	0.90
S4	Water quality	Good	1.00
S5	Shade	65%	0.90
S6	Waterfowl	Minor	0.67
S7	Fish	Major	0.01
S8	Pond count	13+	1.00
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	5%	0.35
Score			0.46 (poor)

Photograph 48. Waterbody WB22



Table 37. HSI results for waterbody WB23

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	> 2000 m ²	Exclude
S3	Pond drying	Never dries	0.90
S4	Water quality	Good	1.00
S5	Shade	65%	0.90
S6	Waterfowl	Minor	0.67
S7	Fish	Major	0.01
S8	Pond count	13+	1.00
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	5%	0.35
Score			0.46 (poor)

Photograph 49. Waterbody WB23



Table 38. HSI results for waterbody WB24

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	50 m ²	0.10
S3	Pond drying	Never dries	0.90
S4	Water quality	Poor	0.01
S5	Shade	0-60%	1.00
S6	Waterfowl	Minor	0.67
S7	Fish	Absent	1.00
S8	Pond count	13+	1.00
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	5%	0.35
Score			0.4 (poor)

Photograph 50. Waterbody WB24



Table 39. HSI results for waterbody WB25

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	<50 m ²	0.05
S3	Pond drying	Never dries	0.90
S4	Water quality	Moderate	0.67
S5	Shade	0-60%	1.00
S6	Waterfowl	Absent	1.00
S7	Fish	Absent	1.00
S8	Pond count	13+	1.00
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	0%	0.30
Score			0.58 (below average)

Photograph 51. Waterbody WB25



Table 40. HSI results for waterbody WB26

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	150 m ²	0.30
S3	Pond drying	Never dries	0.90
S4	Water quality	Moderate	0.67
S5	Shade	0-60%	1.00
S6	Waterfowl	Minor	0.67
S7	Fish	Absent	1.00
S8	Pond count	13+	1.00
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	5%	0.35
Score			0.68 (average)

Photograph 52. Waterbody WB26



Table 41. HSI results for waterbody WB29

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	<50 m ²	0.05
S3	Pond drying	Never dries	0.90
S4	Water quality	Poor	0.01
S5	Shade	0-60%	1.00
S6	Waterfowl	Absent	1.00
S7	Fish	Absent	1.00
S8	Pond count	13+	1.00
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	5%	0.35
Score			0.39 (poor)

Photograph 53. Waterbody WB29



Table 42. HSI results for waterbody WB30

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	150 m ²	0.30
S3	Pond drying	Sometimes dries	0.50
S4	Water quality	Poor	0.01
S5	Shade	0-60%	1.00
S6	Waterfowl	Minor	0.67
S7	Fish	Absent	1.00
S8	Pond count	13+	1.00
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	70%	1.00
Score			0.47 (poor)

Photograph 54. Waterbody WB30



Table 43. HSI results for waterbody WB33

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	1000 m ²	0.95
S3	Pond drying	Never dries	0.90
S4	Water quality	Bad	0.33
S5	Shade	0-60%	1.00
S6	Waterfowl	Major	0.01
S7	Fish	Possible	0.67
S8	Pond count	13+	1.00
S9	Terrestrial habitat	Poor	0.33
S10	Macrophyte cover	0%	0.30
Score			0.4 (poor)

Photograph 55 & 56. Waterbody WB33



Table 44. HSI results for waterbody WB34

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	1850 m ²	0.82
S3	Pond drying	Never dries	0.90
S4	Water quality	Bad	0.33
S5	Shade	0-60%	1.00
S6	Waterfowl	Major	0.01
S7	Fish	Possible	0.67
S8	Pond count	13+	1.00
S9	Terrestrial habitat	Poor	0.33
S10	Macrophyte cover	0%	0.30
Score			0.39 (poor)

Photograph 57 & 58. Waterbody WB34



Table 45. HSI results for waterbody WB35

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	500 m ²	1.00
S3	Pond drying	Never dries	0.90
S4	Water quality	Bad	0.33
S5	Shade	0-60%	1.00
S6	Waterfowl	Major	0.01
S7	Fish	Possible	0.67
S8	Pond count	7	0.85
S9	Terrestrial habitat	Moderate	0.67
S10	Macrophyte cover	0%	0.30
Score			0.42 (poor)

Photograph 59 & 60. Waterbody WB35



Table 46. HSI results for waterbody WB38

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	200 m ²	0.40
S3	Pond drying	Never dries	0.90
S4	Water quality	Moderate	0.67
S5	Shade	75%	0.70
S6	Waterfowl	Minor	0.67
S7	Fish	Possible	0.67
S8	Pond count	5	0.72
S9	Terrestrial habitat	Moderate	0.67
S10	Macrophyte cover	5%	0.35
Score			0.6 (average)

Photograph 61 & 62. Waterbody WB38



Table 47. HSI results for waterbody WB39

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	<50 m ²	0.05
S3	Pond drying	Never dries	0.90
S4	Water quality	Moderate	0.67
S5	Shade	0-60%	1.00
S6	Waterfowl	Absent	1.00
S7	Fish	Absent	1.00
S8	Pond count	9	0.91
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	0%	0.30
Score			0.58 (below average)

Photograph 63 & 64. Waterbody WB39



Table 48. HSI results for waterbody WB40

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	1000 m ²	0.95
S3	Pond drying	Never dries	0.90
S4	Water quality	Good	1.00
S5	Shade	0-60%	1.00
S6	Waterfowl	Minor	0.67
S7	Fish	Possible	0.67
S8	Pond count	13+	1.00
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	0%	0.30
Score			0.75 (good)

Photograph 65 & 66. Waterbody WB40



Table 49. HSI results for waterbody WB41

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	300 m ²	0.60
S3	Pond drying	Never dries	0.90
S4	Water quality	Moderate	0.67
S5	Shade	100%	0.20
S6	Waterfowl	Minor	0.67
S7	Fish	Possible	0.67
S8	Pond count	13+	1.00
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	0%	0.30
Score			0.59 (below average)

Photograph 67 & 68. Waterbody WB41



Table 50. HSI results for waterbody WB43

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	300 m ²	0.60
S3	Pond drying	Rarely dries	1.00
S4	Water quality	Good	1.00
S5	Shade	0-60%	1.00
S6	Waterfowl	Absent	1.00
S7	Fish	Absent	1.00
S8	Pond count	13+	1.00
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	70%	1.00
Score			0.89 (excellent)

Photograph 69 & 70. Waterbody WB43



Table 51. HSI results for waterbody WB44

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	<50 m ²	0.05
S3	Pond drying	Dries annually	0.10
S4	Water quality	Poor	0.01
S5	Shade	0-60%	1.00
S6	Waterfowl	Minor	0.67
S7	Fish	Absent	1.00
S8	Pond count	13+	1.00
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	5%	0.35
Score			0.3 (poor)

Photograph 71. Waterbody WB44



Table 52. HSI results for waterbody WB45

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	> 2000 m ²	Exclude
S3	Pond drying	Never dries	0.90
S4	Water quality	Moderate	0.67
S5	Shade	90%	0.40
S6	Waterfowl	Minor	0.67
S7	Fish	Possible	0.67
S8	Pond count	13+	1.00
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	5%	0.35
Score			0.64 (average)

Photograph 72 & 73. Waterbody WB45



Table 53. HSI results for waterbody WB46

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	300 m ²	0.60
S3	Pond drying	Never dries	0.90
S4	Water quality	Good	1.00
S5	Shade	0-60%	1.00
S6	Waterfowl	Absent	1.00
S7	Fish	Possible	0.67
S8	Pond count	13+	1.00
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	70%	1.00
Score			0.50

Photograph 74 & 75. Waterbody WB46



Table 54. HSI results for waterbody WB49

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	<50 m ²	0.05
S3	Pond drying	Rarely dries	1.00
S4	Water quality	Moderate	0.67
S5	Shade	0-60%	1.00
S6	Waterfowl	Absent	1.00
S7	Fish	Absent	1.00
S8	Pond count	13+	1.00
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	50%	0.80
Score			0.65 (average)

Photograph 76. Waterbody WB49



Table 55. HSI results for waterbody WB50

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	100 m ²	0.20
S3	Pond drying	Rarely dries	1.00
S4	Water quality	Moderate	0.67
S5	Shade	0-60%	1.00
S6	Waterfowl	Absent	1.00
S7	Fish	Absent	1.00
S8	Pond count	13+	1.00
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	15%	0.45
Score			0.7 (good)

Photograph 77 & 78. Waterbody WB50



Table 56. HSI results for waterbody WB51

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	50 m ²	0.10
S3	Pond drying	Never dries	0.90
S4	Water quality	Good	1.00
S5	Shade	0-60%	1.00
S6	Waterfowl	Absent	1.00
S7	Fish	Absent	1.00
S8	Pond count	13+	1.00
S9	Terrestrial habitat	Good	1.00
S10	Macrophyte cover	20%	0.50
Score			0.68 (average)

Photograph 79. Waterbody WB51



Table 57. HSI results for waterbody WB55

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	0.50
S2	Pond area	100 m ²	0.20
S3	Pond drying	Sometimes dries	0.50
S4	Water quality	Moderate	0.67
S5	Shade	0-60%	1.00
S6	Waterfowl	Absent	1.00
S7	Fish	Absent	1.00
S8	Pond count	10	0.94
S9	Terrestrial habitat	Moderate	0.67
S10	Macrophyte cover	70%	1.00
Score			0.68 (average)

Table 58. HSI results for waterbody WB59

Suitability index	Habitat parameter	Field Score	HSI score
S1	Location	Zone B	1.00
S2	Pond area	650 m ²	0.50
S3	Pond drying	Sometimes dries	0.67
S4	Water quality	Moderate	1.00
S5	Shade	0-60%	0.67
S6	Waterfowl	Minor	1.00
S7	Fish	Absent	1.00
S8	Pond count	6	1.00
S9	Terrestrial habitat	Good	0.80
S10	Macrophyte cover	100%	1.00
Score			0.79 (good)

Photograph 80. Waterbody WB59



Annex II 2025 Population assessment survey detailed results

Table 5. Trapping results for pond WB07.

Survey visit	Date	Bottle trapping			Torching			
		GCN M	GCN F	GCN J	GCN M	GCN F	GCN J	GCN?
1	03/04/2025	0	0	0	0	0	0	0
2	08/04/2025	0	0	0	0	0	0	0
3	14/04/2025	0	0	0	0	0	0	0
4	28/04/2025	0	1	0	5*	1*	0	0
5	01/05/2025	2*	0	0	3	0	0	0
6a	07/05/2025	0	1*	0	1	0	0	0

GCN = great crested newt; M = male; F = female; J = juvenile; ? = identification uncertain; * Indicates highest recorded numbers for pond.

Table 6. Trapping results for pond WB08

Survey visit	Date	Bottle trapping			Torching			
		GCN M	GCN F	GCN J	GCN M	GCN F	GCN J	GCN?
1	03/04/2025	0	0	0	9*	0	0	0
2	08/04/2025	1	7*	0	7	2	0	2
3	14/04/2025	4	4	0	2	3	0	2
4	28/04/2025	4*	5	0	9	4	0	0
5	01/05/2025	1	1	0	6	7	0	0
6a	07/05/2025	1	0	0	8	7*	0	0

GCN = great crested newt; M = male; F = female; J = juvenile; ? = identification uncertain; * Indicates highest recorded numbers for pond.

Table 7. Trapping results for pond WB10

Survey visit	Date	Bottle trapping			Torching			
		GCN M	GCN F	GCN J	GCN M	GCN F	GCN J	GCN?
1	03/04/2025	0	0	0	2	0	0	1
2	08/04/2025	1	0	0	1	1	0	0
3	14/04/2025	1	0	0	4	0	0	0
4	28/04/2025	2*	0	0	3	0	0	0

5	01/05/2025	0	0	0	4*	3*	0	0
6a	07/05/2025	0	0	0			N/A	
6b	20/05/2025		N/A		4	0	0	0

GCN = great crested newt; M = male; F = female; J = juvenile; ? = identification uncertain; * Indicates highest recorded numbers for pond.

Table 8. Trapping results for WB11

Survey visit	Date	Bottle trapping			Torching			
		GCN M	GCN F	GCN J	GC N M	GCN F	GCN J	GCN ?
1	03/04/2025	N/A			1	0	0	0
2	08/04/2025	0	0	0	0	0	0	0
3	14/04/2025	0	0	0	1	0	0	0
4	28/04/2025	1*	0	0	1*	0	0	0
5	01/05/2025	0	0	0	0	0	0	0
6a	07/05/2025	0	0	0			N/A	
6b	20/05/2025	0	0	0	0	0	0	0

GCN = great crested newt; M = male; F = female; J = juvenile; ? = identification uncertain; * Indicates highest recorded numbers for pond.

Table 9. Trapping results for WB12

Survey visit	Date	Bottle trapping			Torching			
		GCN M	GCN F	GCN J	GCN M	GCN F	GCN J	GCN?
1	03/04/2025	0	0	0	0	0	0	0
2	08/04/2025	0	0	0	0	0	0	0
3	14/04/2025	0	0	0	0	1	0	0
4	28/04/2025	0	0	0	0	1*	0	0
5	01/05/2025	0	0	0	0	0	0	0
6a	07/05/2025	1*	0	0			N/A	
6b	20/05/2025		N/A		0	0	0	0

GCN = great crested newt; M = male; F = female; J = juvenile; ? = identification uncertain; * Indicates highest recorded numbers for pond.

Table 10. Trapping results for pond WB0B

Survey visit	Date	Bottle trapping			Torching			
		GCN	GCN F	GCN J	GCN M	GCN F	GCN J	GCN?
1	03/04/2025	N/A			0	0	0	0

2	08/04/2025	N/A	0	0	0	0
3	14/04/2025	N/A	0	0	0	0
4	28/04/2025	N/A	0	0	0	0
5	01/05/2025	N/A	0	0	0	0
6a	07/05/2025	N/A			N/A	
6b	20/05/2025	N/A			N/A	

GCN = great crested newt; M = male; F = female; J = juvenile; ? = identification uncertain; * Indicates highest recorded numbers for pond.

Table 11. Trapping results for WB0C

Survey visit	Date	Bottle trapping			Torching			
		GCN	GCN F	GCN J	GCN M	GCN F	GCN J	GCN?
1	03/04/2025		N/A		0	0	0	0
2	08/04/2025		N/A		0	0	0	0
3	14/04/2025		N/A		0	0	0	0
4	28/04/2025		N/A		0	0	0	0
5	01/05/2025		N/A		0	0	0	0
6a	07/05/2025		N/A				N/A	
6b	20/05/2025		N/A				N/A	

GCN = great crested newt; M = male; F = female; J = juvenile; ? = identification uncertain; * Indicates highest recorded numbers for pond.

Table 12. Trapping results for WB29

Survey visit	Date	Bottle trapping			Torching			
		GCN	GCN F	GCN J	GCN M	GCN F	GCN J	GCN?
1	03/04/2025		N/A		0	0	0	0
2	08/04/2025		N/A		0	0	0	0
3	14/04/2025		N/A		0	0	0	0
4	28/04/2025		N/A		1	0	0	0
5	01/05/2025		N/A		1	0	0	0
6a	07/05/2025		N/A				N/A	
6b	20/05/2025		N/A		2*	0	0	0

GCN = great crested newt; M = male; F = female; J = juvenile; ? = identification uncertain; * Indicates highest recorded numbers for pond.

Table 13. Trapping results for pond WB0D

Survey visit	Date	Bottle trapping			Torching			
		GCN	GCN F	GCN J	GCN M	GCN F	GCN J	GCN?
1	03/04/2025	0	0	0	0	0	0	0
2	08/04/2025	0	0	0	0	0	0	0
3	14/04/2025	0	0	0	0	0	0	0
4	28/04/2025	0	0	0	0	0	0	0
5	01/05/2025	0	0	0	0	0	0	0
6a	07/05/2025	0	0	0			N/A	
6b	20/05/2025		N/A		0	0	0	0

GCN = great crested newt; M = male; F = female; J = juvenile; ? = identification uncertain; * Indicates highest recorded numbers for pond.

Annex III 2025 SureScreen laboratory certificates

Note that the referencing used when collecting eDNA samples varies slightly from the references used in this report:

BG01, BG02, BG03 etc were updated to WB01, WB02, WB03 etc (unique numbering has been retained)

BGAA, BGAB, BGAC etc were updated to WB0A, WB0B, WB0C etc (unique lettering has been retained)

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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
GCN25 1981	Denny Wishaw - BG20	NS 82281 77546	Pass	Pass	Positive	1/12
GCN25 1982	Denny Wishaw - BG24	NS 82523 77545	Pass	Pass	Positive	7/12
GCN25 1983	Denny Wishaw - BGAB	NS 82456 77578	Pass	Pass	Negative	0/12
GCN25 1984	Denny Wishaw - BG21	NS 82428 77538	Pass	Pass	Negative	0/12
GCN25 1985	Denny Wishaw - BGAB	NS 82449 77509	Pass	Pass	Negative	0/12
GCN25 1986	Denny Wishaw - BG25	NS 82516 77510	Pass	Pass	Positive	6/12

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GCN25 1987	Denny Wishaw - BG15	NS 82522 77690	Pass	Pass	Negative	0/12
GCN25 1988	Denny Wishaw - BG05	NS 82786 78249	Pass	Pass	Negative	0/12
GCN25 1989	Denny Wishaw - BG04	NS 82860 78270	Pass	Pass	Positive	1/12
GCN25 1990	Denny Wishaw - BG16	NS 82539 77670	Pass	Pass	Negative	0/12
GCN25 1991	Denny Wishaw - BG18	NS 82529 77646	Pass	Pass	Negative	0/12
GCN25 1992	Denny Wishaw - BG19	NS 82557 77647	Pass	Pass	Negative	0/12
GCN25 1993	Denny Wishaw - BG13	NS 82492 77668	Pass	Pass	Negative	0/12
GCN25 1994	Denny Wishaw - BG17	NS 82507 77660	Pass	Pass	Negative	0/12
GCN25 1996	Denny Wishaw - BG30	NS 82689 77464	Pass	Pass	Negative	0/12
GCN25 1997	Denny Wishaw - BG26	NS 82582 77503	Pass	Pass	Negative	0/12
GCN25 1999	Denny Wishaw - BGAD	NS 82613 77440	Pass	Pass	Negative	0/12

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GCN25 2000	Denny Wishaw - BG14	NS 82508 77687	Pass	Pass	Negative	0/12
GCN25 2001	Denny Wishaw - BG09		Pass	Pass	Negative	0/12
GCN25 2002	Denny Wishaw - BG06	NS 82431 77893	Pass	Pass	Negative	0/12

Matters affecting result: none

Reported by: Amy Bermudez

Approved by: Lauryn Jewkes

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.

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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.

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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
GCN25 0008	DWNO - BG50	NS 78086 73449	Pass	Pass	Negative	0/12

Matters affecting result: none

Reported by: Amy Bermudez

Approved by: Vanessa Hind

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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.



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Result:

Presence of GCN eDNA (Positive/Negative/Inconclusive)

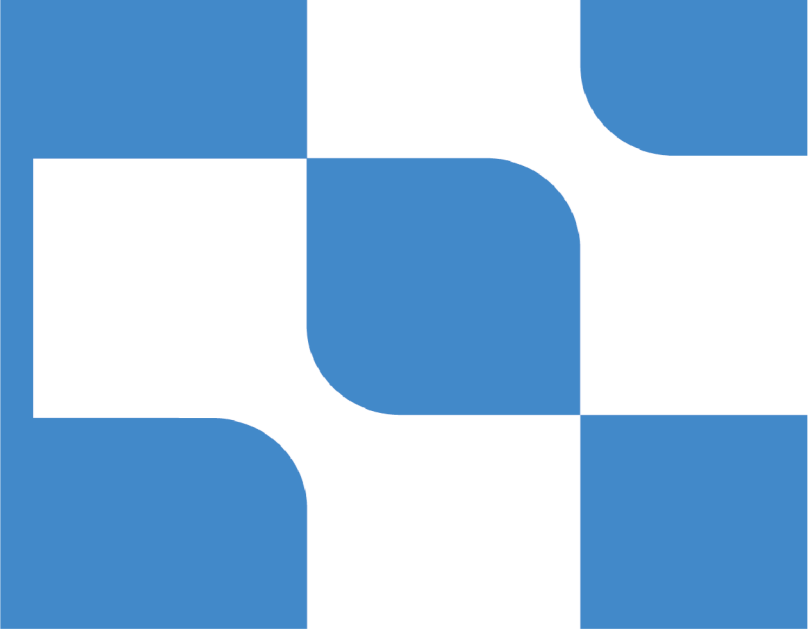
Positive: GCN DNA was identified within the sample, indicative of GCN presence within the sampling location at the time the sample was taken or within the recent past at the sampling location.

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.

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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
GCN25 1973	DWNO - BG46	NS 78154 73538	Pass	Pass	Negative	0/12
GCN25 1974	DWNO - BG35	NS 81851 76720	Pass	Pass	Negative	0/12
GCN25 1975	DWNO - BG49	NS 78040 73472	Pass	Pass	Negative	0/12
GCN25 1976	DWNO - BG39	NS 78626 74357	Pass	Pass	Negative	0/12
GCN25 1977	DWNO - BG02	NS 84837 79805	Pass	Pass	Negative	0/12
GCN25 1978	DWNO - BG45	NS 77762 73876	Pass	Pass	Negative	0/12
GCN25 1979	DWNO - BG51	NS 78140 73415	Pass	Pass	Negative	0/12



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GCN25 1980	DWNO - BG41	NS 78168 74321	Pass	Pass	Negative	0/12
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Matters affecting result: none

Reported by: Amy Bermudez

Approved by: Vanessa Hind

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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



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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.

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GCN Report

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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
GCN25 6254	DWNO - BG43	NS 78623 73899	Pass	Pass	Negative	0/12
GCN25 6255	DWNO - BG38	NS 79029 74792	Pass	Pass	Negative	0/12
GCN25 6256	DWNO - BGAI	NS 78279 74444	Pass	Pass	Negative	0/12
GCN25 6257	DWNO - BG59	NS 76131 68949	Pass	Pass	Negative	0/12
GCN25 6258	DWNO - BG55	NS 78432 73073	Pass	Pass	Negative	0/12
GCN25 6261	DWNO - BG40	NS 78539 74338	Pass	Pass	Negative	0/12

Matters affecting result: none

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Reported by: Amy Bermudez

Approved by: Vanessa Hind

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Received Date: 23.05.2025

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.



Folio no: 1801-2025
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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.

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Folio No: 3529-2025
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Contact: Aecom Infrastructure &
Environment Uk Ltd
Issue Date: 17.07.2025
Received Date: 03.07.2025

GCN Report

Technical Report



SureScreen Scientifics

Folio no: 3529-2025
Purchase Order: UK1720086
Contact: Aecom Infrastructure & Environment Uk Ltd
Issued Date: 17.07.2025
Received Date: 03.07.2025

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
GCN25 7301	DW - DN04	NS 82687 84458	Pass	Pass	Negative	0/12
GCN25 9691	DW - XX02b	NS 70907 67256	Pass	Pass	Negative	0/12
GCN25 9693	DW - XX19	NS 76069 67953	Pass	Pass	Negative	0/12
GCN25 9695	DW - XX02a	NS 70907 67258	Pass	Pass	Negative	0/12
GCN25 9697	DW - XR29	NS 83475 55037	Pass	Pass	Negative	0/12
GCN25 9699	DW New Line - BGAJ	NS 813 762	Pass	Pass	Negative	0/12

Folio no: 3529-2025
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Issued Date: 17.07.2025
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GCN25 9701	DW - XXR28	NS 83523 55123	Pass	Pass	Negative	0/12
GCN25 9702	DW - XR24	NS 84404 57987	Pass	Pass	Negative	0/12
GCN25 9703	DW - XR30	NS 83462 55025	Pass	Pass	Negative	0/12
GCN25 9706	DW - XR19	NS 80671 60095	Pass	Pass	Negative	0/12
GCN25 9709	XR36		Pass	Pass	Negative	0/12
GCN25 9710	XR38		Pass	Pass	Negative	0/12
GCN25 9711	XR33 (1)		Pass	Pass	Negative	0/12
GCN25 9716	XR44		Pass	Pass	Negative	0/12
GCN25 9717	XR33 (2)		Pass	Pass	Negative	0/12

Matters affecting result: none

Reported by: Amy Bermudez

Approved by: Consuela Sopronyi

Folio no: 3529-2025
Purchase Order: UK1720086
Contact: Aecom Infrastructure & Environment Uk Ltd
Issued Date: 17.07.2025
Received Date: 03.07.2025

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.

Folio no: 3529-2025
Purchase Order: UK1720086
Contact: Aecom Infrastructure & Environment Uk Ltd
Issued Date: 17.07.2025
Received Date: 03.07.2025

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.



Annex IV 2022 SureScreen laboratory certificates

References used when collecting eDNA samples in 2022 differ from those used in 2025, Table 14 provides the corresponding references used in both years.

Table 14. Ponds where eDNA samples were taken in 2022 and their corresponding 2025 references

2022 Reference	2025 Reference
N14	WB22
N29	WB01
N13	WB23
N18a	WB30
N09	WB35
N17	WB26
N19	WB19
N20	WB18
N21	WB17
N22	Not surveyed in 2025
N23	WB16
N24	WB13
N25	WB14
N26	WB15
N10	WB34
N28	WB02
N27	WB05
N27a	WB04
N04	WB51
N32	WB39
N06	WB49
N11	WB33
N12	WB32
N05	WB50
N07a + N07b	Not surveyed in 2025
N03a + N03b	WB57

N01a	WB61
N31	WB46
N08	WB40
N30	WB58
N02	WB59

Folio No: E13208
 Report No: 1
 Purchase Order: #1523045
 Client: AECOM INFRASTRUCTURE AND ENVIRONMENT
 Contact: Jenny Davidson

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: 04/05/2022

Date Reported: 11/05/2022

Matters Affecting Results: None

Lab Sample no.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive replicates
2356	N14 Central Upgrade	NS 82407 77523	Pass	Pass	Pass	Positive	1
2359	N29	NS 84650 79914	Pass	Pass	Pass	Negative	0
2360	N13	NS 82429	Pass	Pass	Pass	Negative	0
2361	N18a	NS 82686 77470	Pass	Pass	Pass	Negative	0
2362	N09	NS 81846 76711	Pass	Pass	Pass	Negative	0
2363	N17	NS 82588 77512	Pass	Pass	Pass	Positive	4
2365	N19-N26 Sample 3	NS 82492 77664	Pass	Pass	Pass	Negative	0



2366	N19-N26 Sample 1	NS 82492 77664	Pass	Pass	Pass	Negative	0
2367	N10	NS 82021 77055	Pass	Pass	Pass	Negative	0
2368	N28	NS 84838 79790	Pass	Pass	Pass	Negative	0
2369	N27	NS 82758 78226	Pass	Pass	Pass	Negative	0
2370	N27a	NS 82855 78252	Pass	Pass	Pass	Positive	3
2371	N04	NS 78148 73425	Pass	Pass	Pass	Negative	0
2372	N32	NS 78625 74361	Pass	Pass	Pass	Negative	0
2373	N19-N26 Sample 2	NS 82492 77664	Pass	Pass	Pass	Negative	0
2374	N06	NS 78043 73466	Pass	Pass	Pass	Negative	0
2375	N11	NS 82123 77080	Pass	Pass	Pass	Negative	0
2376	N12	NS 82485 77251	Pass	Pass	Pass	Negative	0
2377	N05	NS 78081 73434	Pass	Pass	Pass	Negative	0
2378	N07a+b	NS 78126 73467	Pass	Pass	Pass	Negative	0
2379	N03a+b	NS 77571 70818	Pass	Pass	Pass	Negative	0
2380	N01a	NS 76142 68884	Pass	Pass	Pass	Negative	0
2381	N31	NS 78159 73521	Pass	Pass	Pass	Negative	0
2382	N08	NS 76716 74178	Pass	Pass	Pass	Negative	0
2383	N30	NS 75792 69051	Pass	Pass	Pass	Negative	0
2384	N02	NS 76141 69004	Pass	Pass	Pass	Positive	1



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

Reported by: Esther Strafford Approved by: Chris Troth 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.



Folio No: E17908
 Report No: 1
 Purchase Order: PO 1596245
 Client: AECOM INFRASTRUCTURE AND ENVIRONMENT
 Contact: Natali Nikolova

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: 08/06/2023

Date Reported: 15/06/2023

Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
0626	Denny - Wishaw - N36	NS 82384 77777	Pass	Pass	Pass	Negative	0
0627	Denny - Wishaw - N35	NS 82426 77907	Pass	Pass	Pass	Negative	0
0628	Denny - Wishaw - N33	NS 82400 77904	Pass	Pass	Pass	Positive	4
0629	Denny - Wishaw - N40	NS 82283 77543	Pass	Pass	Pass	Negative	0
0630	Denny - Wishaw - N37	NS 82415 77770	Pass	Pass	Pass	Positive	4
0631	Denny - Wishaw - N41	NS 82606 77442	Pass	Pass	Pass	Positive	2
0632	Denny -	NS 82414	Pass	Pass	Pass	Positive	2



Wishaw - N34		77897					
0633	Denny - Wishaw - N39	NS 82426 77682	Pass	Pass	Pass	Positive	3
0634	Denny - Wishaw - N38	NS 82411 77753	Pass	Pass	Pass	Negative	0
R116	Denny - Wishaw - N42	NS 79019 74772	Pass	Pass	Pass	Negative	0
R117	Denny - Wishaw - N44	NS 78608 73892	Pass	Pass	Pass	Negative	0
R118	Denny - Wishaw - N43	NS 78182 74301	Pass	Pass	Pass	Negative	0

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

Reported by: Chelsea Warner Approved by: Jackson Young 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.



Annex VI 2022 Great crested newt population assessment survey detailed results

Table 15 Results of great crested newt population assessment surveys in pond WB59 (N02)

Survey visit	Survey date	Bottle Trapping		Torching		Egg searching	
		GCN	PN	GCN	PN	GCN	PN
1	19/05/2022	0	11	0	5	0	0
2	26/05/2022	0	13	0	4	0	0
3	31/05/2022	0	0	0	6	0	0
4	09/06/2022	0	2	0	1	0	0

GCN = Great crested newt; PN = Palmate Newt; * = Peak count of great crested newt within pond

Table 16 Results of great crested newt population assessment surveys in pond WB22 (N14)

Survey visit	Survey date	Bottle Trapping		Torching		Egg searching	
		GCN	PN	GCN	PN	GCN	PN
1	19/05/2022	0	4	0	0	0	0
2	26/05/2022	0	0	0	0	0	0
3	31/05/2022	0	0	0	0	0	0
4	09/06/2022	0	1	0	0	0	0
5	13/06/2022	0	0	0	0	0	0
6	20/06/2022	0	0	0	0	0	0

GCN = Great crested newt, PN = Palmate Newt, * = Peak count of great crested newt within pond

Table 17. Results of great crested newt population assessment surveys in pond WB21 (N14a)

Survey visit	Survey date	Bottle Trapping		Torching		Egg searching	
		GCN	PN	GCN	PN	GCN	PN
1	19/05/2022	0	0	0	0	0	0
2	26/05/2022	0	4	0	0	0	0
3	31/05/2022	1	1	0	0	0	0
4	09/06/2022	0	1	0	1	0	0

5	13/06/2022	2*	2	1	1	0	0
6	20/06/2022	1	0	0	0	0	0

GCN = Great crested newt, PN = Palmate Newt, * = Peak count of great crested newt within pond

Table 18. Results of great crested newt population assessment surveys in pond WB26 (N17)

Survey visit	Survey date	Bottle Trapping		Torching		Egg searching	
		GCN	PN	GCN	PN	GCN	PN
1	19/05/2022	3*	54	1	3	Present	0
2	26/05/2022	1	20	0	2	0	0
3	31/05/2022	1	8	3*	9	Present	0
4	09/06/2022	0	7	0	1	0	0
5	13/06/2022	0	12	0	10	0	0
6	20/06/2022	0	7	2	16	0	0

GCN = Great crested newt, PN = Palmate Newt, * = Peak count of great crested newt within pond

Table 19 Results of great crested newt population assessment surveys in pond WB25 (N17a)

Survey visit	Survey date	Bottle Trapping		Torching		Egg searching	
		GCN	PN	GCN	PN	GCN	PN
1	19/05/2022	N/A	1	1	0	0	1
2	26/05/2022	0	0	1	3	0	0
3	31/05/2022	0	3	1	2	0	0
4	09/06/2022	0	3	0	6	0	0
5	13/06/2022	2	0	1	3	0	0
6	20/06/2022	1	1	3*	14	0	0

GCN = Great crested newt, PN = Palmate Newt, * = Peak count of great crested newt within pond

Table 20. Results of great crested newt population assessment surveys in pond WB24 (N17b)

Survey visit	Survey date	Bottle Trapping		Torching		Egg searching	
		GCN	PN	GCN	PN	GCN	PN

1	19/05/2022	0	8	0	0	Present	0
2	26/05/2022	0	8	0	0	0	0
3	31/05/2022	0	3	0	3	Present	0
4	09/06/2022	1*	7	0	4	0	0
5	13/06/2022	0	2	0	0	0	0
6	20/06/2022	1*	5	0	4	0	0

GCN = Great crested newt, PN = Palmate Newt, * = Peak count of great crested newt within pond

Table 21. Results of great crested newt population assessment surveys in pond WB04 (N27a)

Survey visit	Survey date	Bottle Trapping		Torching		Egg searching	
		GCN	PN	GCN	PN	GCN	PN
1	19/05/2022	0	16	1	3	0	0
2	26/05/2022	0	16	5*	3	Present	0
3	31/05/2022	1	6	0	0	0	0
4	09/06/2022	1	5	1	2	0	0
5	13/06/2022	0	3	2	5	0	0
6	20/06/2022	1	3	1	10	0	0

