Eastern Green Link 4:

Scottish Onshore Scheme

Volume 4: Appendices
Appendix 7.5: Great Crested Newt

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Prepared for:

SP Energy Networks
320 St Vincent St
Glasgow, G2 5AD

Prepared by:

AECOM Limited
1 Tanfield
Edinburgh EH3 5DA
United Kingdom

Table of Contents

01.
Introduction

Appendix 7.5 – Great Crested Newts

1. Introduction

1.1 Background

This appendix accompanies **Chapter 7: Ecology and Nature Conservation (Volume 2 Main Report)** of the Environmental Impact Assessment Report (EIAR). It describes in detail the desk study and field survey carried out to establish the baseline conditions within the zone of influence (ZoI) of the Scottish Onshore Scheme with respect to great crested newt *Triturus cristatus*.

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 shorted distance 'as the crow flies', unless otherwise specified. The area encompassed by the red line boundary shown on the accompanying figures is referred to throughout as the 'Site'.

This appendix is supported by **Figure 7.11 GCN Survey Results (Chapter 7: Ecology and Nature Conservation (Volume 2 Main Report))**

1.2 Quality Assurance

This appendix, and the desk study and field survey described within it, has been completed in accordance with the AECOM Integrated Management System (IMS). AECOM's IMS places emphasis on professionalism, technical excellence, quality, as well as covering health, safety, environment and sustainability management. All AECOM staff members are committed to maintaining this accreditation to those parts of BS EN ISO 9001:2015 and 14001:2015, as well as BS OHSAS 18001:2007 that are relevant to a consultancy service.

The field survey for great crested newt was carried out by suitably experienced AECOM consultants. All are members of the Chartered Institute of Ecology and Environmental Management (CIEEM) at the appropriate grade and adhered to their strict Code of Professional Conduct.

1.3 Legislation

Great crested newt is listed on Schedule 2 of the *Conservation (Natural Habitats, &c.) Regulations 1994* (as amended) (the 'Habitats Regulations'). This affords strict protection whereby (in summary) it is an offence to deliberately or recklessly:

- capture, kill or injure a great crested newt or its eggs;
- disturb a great crested newt while using any structure or place for shelter or protection (e.g. a breeding pond or a hibernation site or other terrestrial refuge);
- obstruct access to a breeding site or resting place, or otherwise deny use of such a site; and

- disturb a great crested newt in a manner or circumstance likely to significantly affect local distribution or abundance, or to impair its ability to survive, breed or reproduce.

It is also an offence to damage or destroy a breeding site or resting place used by a great crested newt, whether this is done deliberately or recklessly.

Under the Nature Conservation (Scotland) Act 2004, public bodies in Scotland have a duty to further the conservation of biodiversity. The Scottish Biodiversity List (SBL) is a list of habitats, plants and animals that Scottish Ministers consider to be of principal importance for biodiversity conservation in Scotland. The purpose of the SBL is to identify habitats and species that are of highest priority for biodiversity conservation, thereby helping public bodies to carry out their biodiversity duty. The great crested newt is listed on the SBL, and consequently public bodies should have regard to this species when implementing their biodiversity duty under the Nature Conservation (Scotland) Act 2004.

Since great crested newt is listed on Annex II of *Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora* (the 'Habitats Directive'), and is therefore considered of European importance, there are designated Special Areas of Conservation (SACs) for this species the Scotland and the wider UK. SACs are strictly protected via the Habitats Regulations.

Great crested newt is also listed as a priority freshwater and wetland species in Fife Local Biodiversity Action Plan (LBAP). Though not legislative, Fife LBAP sets out Fife Council's strategy for improving biodiversity within the local authority area, which contributes to the overall Scottish Biodiversity Strategy. The proposed Natural Environment (Scotland) Bill may in future introduce statutory nature recovery targets, to which LBAPs could contribute.

2. Methods

2.1 Desk Study

A desk study was carried out to identify nature conservation designations for which great crested newt is a qualifying or notified species and to search for records of great crested newt in proximity to the Scottish Onshore Scheme. The desk study sought to identify:

- Special Areas of Conservation (SACs) designated for great crested newt within at least 10 km of the Scottish Onshore Scheme (or further where a hydrological or other ecological connection may exist);
- SSSIs designated for great crested newt within 2 km of the Scottish Onshore Scheme;
- locally designated sites within 2 km of the Scottish Onshore Scheme for which for great crested newt are an identified reason for designation or, where no designation information is available, for which for great crested newt are likely to be part of the reason for site selection; and

- Records of for great crested newt within 1 km of the of the Scoping Boundary[1], made in the last 10 years.

The NatureScot SiteLink website (NatureScot, 2025) was used to identify nature conservation designations. Commercially available records of great crested newt were obtained from the Amphibian and Reptile Groups of the UK (ARG UK) Record Pool Service (ARG UK, 2025), the NBN Atlas Scotland (NBN, 2025), and from Fife Nature Records Centre. The JNCC UK conservation assessments of great crested newt was reviewed for information about this species range in Scotland (JNCC, 2019).

Aerial imagery and Ordnance Survey (OS) mapping was reviewed and waterbodies within 250 m of the Site were identified. This process cannot fully guarantee to identify all waterbodies, being limited by the data available. The area within which ponds were searched for is shown on **Figure 7.11 GCN Survey Results (Chapter 7: Ecology and Nature Conservation (Volume 2 Main Report)**).

2.2 Field Survey

A desk-based search for ponds within 250 m of the Site was carried using aerial imagery and OS mapping. Initially, 25 ponds potentially suitable for great crested newt were identified, as shown on **Figure 7.11 GCN Survey Results (Chapter 7: Ecology and Nature Conservation (Volume 2 Main Report))**[2]. Following design changes, one more pond was identified within 250 of the updated Site boundary (EGL20) and one previously identified pond was no longer within the ZoI of the Scottish Onshore Scheme (EGL19).

An additional two groups of waterbodies were identified that are unsuitable for great crested newt or irrelevant to the Scottish Onshore Scheme (EGL01 and EGL14). One comprised a group of waterbodies associated with a former quarry site and current energy recovery facility at Westfield. These are separated from the Site by the B9097 which acts as a barrier to movement for newts. The other comprises 3 apparent water treatment facility basins off the B926. The turbidity of water in these tanks is inhospitable for great crested newts. Subsequently, these waterbodies were scoped out from further survey.

Habitat Suitability Index Assessment

Habitat Suitability Index assessment (HSI assessment) was carried out on all ponds within the habitat survey area (a 50 m buffer of the site as it was understood at the time of survey) following industry-standard guidance (ARGUK, 2010) between 16 September and 23 October 2024. HSI assessment was carried out on any ponds which weren't assessed in 2024 (or where pond conditions had changed) simultaneously with eDNA sampling (see below).

The HSI assessment included field-based observations of the habitat attributes described below, in addition to the use of Esri ArcPro software to calculate the surface area of the waterbodies.

HSI for great crested newt is a numerical index between 0 (indicating unsuitable habitat) and 1 (representing optimal habitat). The assessment sought to establish the suitability of the

[1] A record search was carried out to inform the scoping report (AECOM, 2024) before the Site boundary was defined.
[2] Where ponds are in close proximity, they are referred to by one reference number with sub letters (a-c) as appropriate.

ponds for great crested newt 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 great crested newts:

- Location – within a UK-wide context reflecting the differences in national distribution of this species.
- Area – waterbodies between 100-300 m^2 in size are considered to represent the most suitable habitat for great crested new.
- Drying – the number of years in which a pond dries over a ten-year period. Occasional drying kills fish, which is beneficial for great crested newt, 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 1 m from the shore. Great crested newt favours 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 great crested newt.
- Fish – qualitative evidence-based assessment of likely presence or absence is made. Great crested newt favour breeding ponds that do not support fish because their open-water swimming larvae are vulnerable to fish predation.
- Number of waterbodies within 1 km – great crested newt 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 1 km that are linked by suitable terrestrial habitat.
- Macrophyte cover – percentage of pond surface area occupied by macrophyte cover. Female great crested newts 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 great crested newt as below:

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

The system is not sufficiently precise to conclude that any particular waterbody with a high score will support great crested newt or that a waterbody with a low score will not support the

species. Oldham *et al* (2000) reported that the lowest scoring pond supporting great crested newt in their study was 0.43, and was therefore considered to have Poor suitability.

Environmental DNA sampling

Environmental DNA (eDNA) is nuclear or mitochondrial DNA that is released from an organism into the environment (Biggs *et al*, 2014). 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 survey involves the collection of water samples for laboratory analysis for the DNA of species of interest, in this case great crested newt. Sample kits supplied by SureScreen (a laboratory which takes part in Natural England's eDNA proficiency testing scheme) were used to collect water samples. The sampling procedure is prescribed in the published method and requires that the water sample be taken between mid-April and end of June.

Ponds were returned to between 20 – 22 May 2025 and eDNA sampling was carried out following approved field and laboratory protocols (Biggs *et al*, 2014). 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 cross-contamination between waterbodies. A total of twenty sub-samples of 30 ml 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.

The collected samples were then transported to SureScreen for analysis to confirm the presence or likely absence of great crested newt eDNA.

2.3 Limitations and Assumptions

The aim of a desk study is to help characterise the baseline context of the Scottish Onshore Scheme and provide valuable background information that may not be captured by field survey alone. Information obtained during a desk study is dependent upon people and organisations having made and submitted records for the area of interest. As such, a lack of great crested newt records does not necessarily mean that they do not occur in the Study Area. Likewise, the presence of great crested newt records does not mean that these still occur within the area of interest or are relevant to the Scottish Onshore Scheme.

The desk study and field surveys were thorough; however, it cannot be fully guaranteed that they located all ponds within the relevant area. This is because OS mapping and aerial imagery both have limitations and might not show all ponds (especially very small or new ponds), and because small ponds not found by the desk study could also evade incidental detection during field surveys if they are well-hidden or located beyond the extent of those surveys. It is therefore possible, although fairly unlikely, that additional undetected waterbodies may exist within the search area, which might be discovered during pre-construction surveys. In the relatively unlikely event that such additional ponds are subsequently found, further survey may be required to avoid potential offences.

eDNA samples could not be collected from waterbodies that were dry at the time of survey. It is possible that these waterbodies may be inhabited by great crested newt if they become wet in some years. Similarly, owing to design changes and land access constraints, the waterbody at Grange Farm was not surveyed for great crested newt. However, considering no

positive result was obtained from any surveyed ponds, it is considered highly unlikely that great crested newt would become present in any seasonally wet depressions or non-surveyed waterbodies, and great crested newts are assumed to be absent from the Site.

3. Results

3.1 Desk Study

A single great crested newt record of two individuals associated with Auchterderan quarry pool (approximately 950 m east of the Site) was identified during the desk study, provided by Fife Nature Records Centre. The closest record available via RecordPool is located within OS grid square NT18; the edge of which is approximately 2.5 km west of the Site.

3.2 Field Survey

A summary of the results of the HSI assessment and eDNA sampling for the ponds are provided in Table 1. Full details of the HSI assessment for each waterbody is provided in **Annex A HSI Assessment Results** and laboratory analysis results are provided in **Annex B eDNA Sampling Laboratory Analysis**.

Table 1. Great crested newt HSI assessment and eDNA survey results

Pond reference	Distance from Site boundary	HSI Score	Suitability	eDNA analysis result
EGL01	150 m	Not surveyed	Not surveyed	Not sampled
EGL02	5 m	0.76 / 0.66	Good / Average	Negative
EGL03	40 m	0.34	Poor	Negative
EGL04	160 m	0.42	Poor	Negative
EGL05	50 m	0.64	Average	Not sampled
EGL06a	180 m	0.46	Poor	Negative
EGL06b	110 m	0.46	Poor	Negative
EGL06c	150 m	0.46	Poor	Negative
EGL07	225 m	0.67	Average	Negative
EGL08	220 m	0.46	Poor	Negative

Pond reference	Distance from Site boundary	HSI Score	Suitability	eDNA analysis result
EGL09	Within the Site	0.56	Below average	Not sampled
EGL10	210 m	0.43	Poor	Negative
EGL11	100 m	0.60	Below average	Not sampled
EGL12	40 m	0.35	Poor	Negative
EGL13a&b	30 m	0.69	Average	Negative
EGL13c	Within the Site	Not surveyed	Not surveyed	Not sampled
EGL14	160 m	Not surveyed	Not surveyed	Not sampled
EGL15	95 m	0.45	Poor	Negative
EGL16	25 m	0.69	Average	Negative
EGL17	170 m	0.69	Average	Not sampled
EGL18	20 m	0.46	Poor	Negative
EGL19	340 m	0.38	Poor	Negative
EGL20	10 m	Not surveyed	Not surveyed	Not sampled

4. Summary

In total, 26 waterbodies were identified within 250 m of the Site. Due to the close proximity/hydrological connectivity between some of these waterbodies, some were grouped into clusters when given a reference ID.

Of these, four waterbodies were scoped out of further assessment at the desk study stage since they are sufficiently isolated from the Scottish Onshore Scheme or unequivocally uninhabitable for great crested newts. Great crested newts are considered absent from a further five waterbodies (without eDNA analysis) as they were dry at the time of survey or considered sufficiently unsuitable when viewed in the field. Additionally, one pond (EGL20) was not accessed to carry out HSI or eDNA surveys owing to land access constraints.

HSI assessment and eDNA analysis was carried out on the remaining sixteen ponds. Suitability of ponds to support great crested newt varied from Poor to Average. The only pond

which scored Good from initial HSI assessment was downgraded to Average in subsequent surveys when pond quality had visibly reduced. No sampled ponds returned a positive eDNA result for great crested newts.

With cognisance to survey limitations, it is concluded that **great crested newts are absent from the ZoI of the Site**.

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Annex A HSI Assessment Results

A.1 EGL01

Large artificial waterbody formed from historic quarry activity. No emergent vegetation suitable for egg laying and separated from the Site by the B9097 which acts as a barrier to movement. Scoped out from further survey.

Table A1. HSI results for Pond EGL01

Habitat parameter	Field Score	HSI Score
SI1 Location	N/A	N/A
SI2 Pond area	N/A	N/A
SI3 Pond drying	N/A	N/A
SI4 Water quality	N/A	N/A
SI5 Shade	N/A	N/A
SI6 Waterfowl	N/A	N/A
SI7 Fish	N/A	N/A
SI8 Pond count	N/A	N/A
SI9 Terrestrial habitat	N/A	N/A
SI10 Macrophyte cover	N/A	N/A
	HSI Score	**N/A**
	Habitat suitability	**N/A**

A.2 EGL02

Pond in clearing at edge of conifer woodland, adjacent to cow pasture. During initial surveys, floating / emergent vegetation included pondweed *Potamogeton* Sp. and localised great reedmace *Typha latifolia* (first photo). Banks were lined primarily with soft rush *Juncus effusus* and there was evidence of historic poaching, particularly along the northern bank, but a reasonably new fence blocked cows accessing the pond.

When the pond was returned to for eDNA sampling, the waterbody appeared significantly different; floating / emergent vegetation had largely died off and an oily film was evident across the surface of the water (second photo).

Plate 1. Photograph of EGL02

Table 2. HSI results for Pond EGL02

Habitat parameter	Field Score	Initial HSI Score	Subsequent HSI Score
S1 Location	Zone B - Marginal	0.50	0.50
S2 Pond area	350 m^2	0.70	0.70
S3 Pond drying	Never dries	0.90	0.90
S4 Water quality	Good / Poor	1.00	0.33
S5 Shade	0-60%	1.00	1.00
S6 Waterfowl	Absent	1.00	1.00
S7 Fish	Possible	0.67	0.67
S8 Pond count	3	0.66	0.66
S9 Terrestrial habitat	Moderate	0.67	0.67
S10 Macrophyte cover	55% / 20%	0.88	0.50
Habitat suitability	**Good / Average**	**HSI Score**	**0.78 / 0.66**

A.3 EGL03

Large, flooded area within coniferous woodland with no emergent vegetation. Substrate is presumed to be mud with high coverage of needles.

Plate 2. Photograph of EGL03

Table 3. HSI results for Pond EGL03

Habitat parameter	Field Score	HSI Score
S1 Location	Zone B - Marginal	0.50
S2 Pond area	1200 m2	0.92
S3 Pond drying	Sometimes dries	0.50
S4 Water quality	Poor	0.33
S5 Shade	100%	0.20
S6 Waterfowl	Major	0.01
S7 Fish	Absent	1.00
S8 Pond count	3	0.66
S9 Terrestrial habitat	Moderate	0.67
S10 Macrophyte cover	0%	0.30
Habitat suitability	**Poor**	**HSI Score** **0.34**

A.4 EGL04

Deep pond surrounded by willow *Salix* sp. scrub and reed canary grass *Phalaris arundinacea*. Transitional habitat around the perimeter of the pond is dominated by great reedmace and localised large sedge *Carex* sp.. Waterfowl were flushed from the pond.

Plate 3. Photograph of EGL04

Table 4. HSI results for Pond EGL04

Habitat parameter	Field Score	HSI Score
S1 Location	Zone B - Marginal	0.50
S2 Pond area	400 m^2	0.80
S3 Pond drying	Rarely dries	1.00
S4 Water quality	Moderate	0.67
S5 Shade	95%	0.30
S6 Waterfowl	Major	0.01
S7 Fish	Absent	1.00
S8 Pond count	2	0.55
S9 Terrestrial habitat	Moderate	0.67
S10 Macrophyte cover	30%	0.60
Habitat suitability	**Poor**	**HSI Score** **0.42**

A.5 EGL05

Dried out swamp dominated by amphibious bistort *Persicaria amphibia* with frequent iris *Iris* sp. and great reedmace. No water during initial or subsequent survey but aquatic vegetation indicates the area may become seasonally inundated or land changes have recently drained it. Lack of water during breeding season renders this pond unsuitable for great crested newt despite average suitability indicated by HSI.

Plate 4. Photograph of EGL05

Table 5. HSI results for Pond EGL05

Habitat parameter	Field Score	HSI Score
S1 Location	Zone B - Marginal	0.50
S2 Pond area	800 m^2	0.98
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	3	0.66
S9 Terrestrial habitat	Moderate	0.67
S10 Macrophyte cover	100%	0.80
Habitat suitability	**Average**	**HSI Score** **0.64**

A.6 EGL06 (a, b & c)

Signage at this location indicates the waterbodies are associated with the Coal Authority workings. EGL06a&b comprise four rectangular artificial waterbodies with varying levels of pollution evident. The upslope and downslope waterbodies are connected via pipe but there is no water transfer between the eastern (EGL06a) and western (EGL06b) waterbodies. The downslope waterbodies exhibited less obvious pollution and were densely vegetated with common reed *Phragmites australis* or great reedmace. The upslope waterbodies were highly polluted and more sparsely vegetated with great reedmace. EGL06b had greater floristic diversity, including grasses, mosses, pondweed, and water plantain *Alisma plantago-aquatica*.

EGL06c is a shallow puddle at the base of a slope, downhill from EGL06a&b. This puddle is sparsely vegetated with rushes and common reed, and there are signs of pollution across the surface.

Plate 5. Photograph of EGL06a

Plate 6. Photograph of EGL06b

Plate 7. Photograph of EGL06c

Table 6. HSI results for Pond EGL06a,b&c

Habitat parameter	Field Score	HSI Score (6a)	HSI Score (6b)	HSI Score (6c)
S1 Location	Zone B - Marginal	0.50	0.50	0.50
S2 Pond area	> 2,000 m^2 / 200 m^2	No score	No score	0.40
S3 Pond drying	Never dries / Sometimes dries	0.90	0.90	0.50
S4 Water quality	Bad	0.01	0.01	0.01
S5 Shade	0-60%	1.00	1.00	1.00
S6 Waterfowl	Minor / Absent	0.67	0.67	1.00
S7 Fish	Absent	1.00	1.00	1.00
S8 Pond count	13+	1.00	1.00	1.00
S9 Terrestrial habitat	Poor / Moderate	0.33	0.33	0.67
S10 Macrophyte cover	60% / 40%	0.90	0.90	0.70
Habitat suitability	**Poor** **HSI Score**	**0.46**	**0.46**	**0.46**

A.7 EGL07

Shallow pond at base of railway embankment, fenced off from adjacent livestock pasture. Waterfowl, including mallard *Anas platyrhynchos* and moorhen *Gallinula chloropus* observed using the waterbody. Marginal vegetation surrounding the pond includes abundant soft rush, locally abundant gorse *Ulex europaeus*, frequent creeping buttercup *Ranunculus repens*, and occasional forget-me-not *Myosotis* sp. and broad-leaved dock *Rumex obtusifolius*. A small amount of pondweed and sweet-grass *Glyceria* sp. grows within the pond. Hawthorns *Crataegus monogyna* cast minimal shade on the southern edge.

Plate 8. Photograph of EGL07

Table 7. HSI results for Pond EGL07

Habitat parameter	Field Score	HSI Score
S1 Location	Zone B - Marginal	0.50
S2 Pond area	1,000 m^2	0.95
S3 Pond drying	Rarely dries	0.50
S4 Water quality	Poor	0.33
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	Moderate	0.67
S10 Macrophyte cover	25%	0.55

Habitat suitability	**Average**	**HSI Score**	**0.67**

A.8 EGL08

Pond in topographic depression of cow pasture field. Very muddy and soft substrate with the only emergent vegetation comprising soft rush around the perimeter. All banks are heavily poached by cattle.

Plate 9. Photographs of EGL08

Table 8. HSI results for Pond EGL08

Habitat parameter	Field Score	HSI Score
S1 Location	Zone B - Marginal	0.50
S2 Pond area	1400 m^2	0.89
S3 Pond drying	Rarely dries	1.00
S4 Water quality	Bad	0.01
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	Poor	0.33
S10 Macrophyte cover	20%	0.50
Habitat suitability	**Poor**	**HSI Score**	**0.46**

A.9 EGL09

Swamp in a likely seasonally wet depression within clearing of dense conifer plantation. Dominated by sedges and rushes and dry at time of surveys.

Plate 10. Photographs of EGL09

Table 9. HSI results for Pond EGL09

Habitat parameter	Field Score	HSI Score
S1 Location	Zone B - Marginal	0.50
S2 Pond area	100 m^2	0.20
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	4	0.70
S9 Terrestrial habitat	Moderate	0.67
S10 Macrophyte cover	70%	1.00
Habitat suitability	**Below average**	**HSI Score** **0.56**

A.10 EGL10

Large private pond fenced off from adjacent livestock pasture, surrounded by willow and hawthorn scrub. Waterfowl were present during survey, but no impact was noted. Water appears very deep with waterlilies growing towards the edge. Marginal vegetation includes water mint *Mentha aquatica*, meadowsweet *Filipendula ulmaria*, and water horsetail *Equisetum fluviatile*. "No fishing" sign on broken gate indicates presence of fish.

Plate 11. Photographs of EGL10

Table 10. HSI results for Pond EGL10

Habitat parameter	Field Score	HSI Score
S1 Location	Zone B - Marginal	0.50
S2 Pond area	>2,000 m^2	No score
S3 Pond drying	Never dries	0.90
S4 Water quality	Good	1.00
S5 Shade	80%	0.60
S6 Waterfowl	Minor	0.67
S7 Fish	Major	0.01
S8 Pond count	4	0.70
S9 Terrestrial habitat	Moderate	0.67
S10 Macrophyte cover	35%	0.65
Habitat suitability	**Poor**	**HSI Score** **0.43**

A.11 EGL11

Hollow in livestock pasture housing water buffalo which was dry at the time of surveys. The depression is highly poached and very muddy with a high proportion of grass coverage.

Plate 12. Photographs of EGL11

Table 11. HSI results for Pond EGL11

Habitat parameter	Field Score	HSI Score
S1 Location	Zone B - Marginal	0.50
S2 Pond area	800 m^2	0.98
S3 Pond drying	Dries annually	0.10
S4 Water quality	Poor	0.33
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	Moderate	0.67
S10 Macrophyte cover	50%	0.80
Habitat suitability	**Below average**	**HSI Score** **0.60**

A.12 EGL12

Camilla Loch is a large waterbody that is designated as a SSSI, primarily for the extensive and diverse area of freshwater transition mire at the western end of the loch which is relatively undisturbed and is the best example of its type in west Fife. The eastern end, which is nearer the Site, is bound by dense scrub and improved pasture grazed by water buffalo. Private fishing signs indicate presence of fish.

Plate 13. Photographs of EGL12

Table 12. HSI results for Pond EGL12

Habitat parameter	Field Score	HSI Score
S1 Location	Zone B - Marginal	0.50
S2 Pond area	>2,000 m^2	No score
S3 Pond drying	Never dries	0.90
S4 Water quality	Good	1.00
S5 Shade	0-60%	1.00
S6 Waterfowl	Major	0.01
S7 Fish	Major	0.01
S8 Pond count	3	0.66
S9 Terrestrial habitat	Moderate	0.67
S10 Macrophyte cover	5%	0.35
Habitat suitability	**Poor**	**HSI Score** **0.27**

A.13 EGL13 (a, b & c)

EGL13a&b comprises two adjacent wet depressions within a livestock pasture. Both have muddy substrate and are highly poached. The smaller pond (second photo) is downslope of the larger area and shows signs of erosion on the western bank. Both ponds contained emergent vegetation including soft rush, brooklime *Veronica beccabunga*, and sweet-grass. Great reedmace was also locally abundant north of the upper pond (first photo). HSI assessment was the same for both ponds.

EGL13c is an extremely muddy puddle with no emergent vegetation under a gate between livestock pasture fields. Owing to the lack of suitable egg laying macrophytes and that the puddle is frequently poached by cattle and ATV's, this waterbody is not considered suitable for great crested newts and was scoped out of further survey.

Plate 14. Photographs of EGL13a&b

Table 13. HSI results for Pond EGL13a&b

Habitat parameter	Field Score	HSI Score
S1 Location	Zone B - Marginal	0.50
S2 Pond area	800 m^2	0.98
S3 Pond drying	Sometimes dries	0.50
S4 Water quality	Poor	0.33
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	Moderate	0.67
S10 Macrophyte cover	40%	0.70
Habitat suitability	**Average**	**HSI Score** **0.69**

A.14 EGL14

Three artificial waterbodies associated with apparent water treatment works. This is inhospitable for great crested newts. The waterbodies are situated behind 6 ft security fencing and could not be accessed to carry out HSI assessment.

Table A1. HSI results for Pond EGL01

Habitat parameter	Field Score	HSI Score
SI1 Location	N/A	N/A
SI2 Pond area	N/A	N/A
SI3 Pond drying	N/A	N/A
SI4 Water quality	N/A	N/A
SI5 Shade	N/A	N/A
SI6 Waterfowl	N/A	N/A
SI7 Fish	N/A	N/A
SI8 Pond count	N/A	N/A
SI9 Terrestrial habitat	N/A	N/A
SI10 Macrophyte cover	N/A	N/A
	HSI Score	**N/A**
	Habitat suitability	**N/A**

A.15 EGL15

Large pond within landscaped grounds of Kilrie Granary. Waterfowl including mute swans *Cygnus olor* were observed using the waterbody. Marginal vegetation occurs around 75% of the perimeter and some emergent vegetation was noted within the water. Algal growth was recorded across approximately 17% of the surface.

Plate 15. Photograph of EGL15

Table 2. HSI results for Pond EGL15

Habitat parameter	Field Score	HSI Score
S1 Location	Zone B - Marginal	0.50
S2 Pond area	>2,000 m^2	No score
S3 Pond drying	Rarely dries	1.00
S4 Water quality	Moderate	0.67
S5 Shade	0-60%	1.00
S6 Waterfowl	Major	0.01
S7 Fish	Possible	0.67
S8 Pond count	5	0.72
S9 Terrestrial habitat	Moderate	0.67
S10 Macrophyte cover	15%	0.45
Habitat suitability	**Poor**	**HSI Score** **0.45**

A.16 EGL16

Pond in private landscaped garden with numerous non-native ornamental shrubs and semi-nature to mature trees including birch *Betula* sp. and beech *Fagus sylvatica* around the perimeter.

Plate 16. Photographs of EGL16

Table 3. HSI results for Pond EGL16

Habitat parameter	Field Score	HSI Score
S1 Location	Zone B - Marginal	0.50
S2 Pond area	1,000 m^2	0.95
S3 Pond drying	Never dries	0.90
S4 Water quality	Moderate	0.67
S5 Shade	70%	0.80
S6 Waterfowl	Minor	0.67
S7 Fish	Possible	0.67
S8 Pond count	6	0.80
S9 Terrestrial habitat	Moderate	0.67
S10 Macrophyte cover	15%	0.45
Habitat suitability	**Average**	**HSI Score** **0.69**

A.17 EGL17

Recently drained or seasonally wet pond carpeted by marginal vegetation including soft rush, reed sweet-grass and mint *Mentha* sp.. Occasional crack willows *Salix fragilis* are present at the edge of the pond. This pond was dry at the time of surveys.

Plate 17. Photographs of EGL17

Table 4. HSI results for Pond EGL17

Habitat parameter	Field Score	HSI Score
S1 Location	Zone B - Marginal	0.50
S2 Pond area	1,950 m^2	0.81
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	5	0.72
S9 Terrestrial habitat	Moderate	0.67
S10 Macrophyte cover	70%	1.00
Habitat suitability	**Average**	**HSI Score 0.69**

A.18 EGL18

Deep pond within private estate, surrounded by marginal vegetation including great reedmace, sweet-grass, and reed canary grass, but more suitable egg laying vegetation is lacking. Invertebrates observed indicating good water quality. Major impact by permanent occurrence of waterfowl.

Plate 18. Photographs of EGL18

Table 5. HSI results for Pond EGL18

Habitat parameter	Field Score	HSI Score
S1 Location	Zone B - Marginal	0.50
S2 Pond area	>2,000 m^2	No score
S3 Pond drying	Never dries	0.90
S4 Water quality	Good	1.00
S5 Shade	0-60%	1.00
S6 Waterfowl	Major	0.01
S7 Fish	Possible	0.67
S8 Pond count	6	0.80
S9 Terrestrial habitat	Moderate	0.67
S10 Macrophyte cover	30%	0.60
Habitat suitability	**Poor**	**HSI Score** **0.46**

A.19 EGL19

Pond in garden of private property with a small boathouse on northern bank. Surrounded by neutral grass and scattered scrub, rarely rushes occur along the eastern bank but marginal / emergent vegetation is lacking (except around 5% cover of duckweed). Breeding greylag geese *Anser anser* were observed in May 2025.

Plate 19. Photographs of EGL19

Table 6. HSI results for Pond EGL19

Habitat parameter	Field Score	HSI Score
S1 Location	Zone B - Marginal	0.50
S2 Pond area	>2,000 m^2	No score
S3 Pond drying	Never dries	0.90
S4 Water quality	Moderate	0.67
S5 Shade	0-60%	1.00
S6 Waterfowl	Major	0.01
S7 Fish	Possible	0.67
S8 Pond count	1	0.38
S9 Terrestrial habitat	Moderate	0.67
S10 Macrophyte cover	5%	0.35
Habitat suitability	**Poor**	**HSI Score** **0.38**

A.20 EGL20

Waterbody adjacent to the yard of Grange Farm. Land access constraints mean this waterbody has not been assessed.

Table A1. HSI results for Pond EGL20

Habitat parameter	Field Score	HSI Score
SI1 Location	N/A	N/A
SI2 Pond area	N/A	N/A
SI3 Pond drying	N/A	N/A
SI4 Water quality	N/A	N/A
SI5 Shade	N/A	N/A
SI6 Waterfowl	N/A	N/A
SI7 Fish	N/A	N/A
SI8 Pond count	N/A	N/A
SI9 Terrestrial habitat	N/A	N/A
SI10 Macrophyte cover	N/A	N/A
	HSI Score	**N/A**
	Habitat suitability	**N/A**

Annex B eDNA Sampling Laboratory Analysis

The following Technical Report produced by SureScreen Scientifics provides the results of eDNA analysis. The Site Name matches the pond reference used throughout this document.

Folio No:	1934-2025
Purchase Order:	UK 1704685
Contact:	Aecom Infrastructure & Environment Uk Ltd
Issue Date:	12.06.2025
Received Date:	29.05.2025

GCN Report

Technical Report

SureScreen Scientifics

Folio No: 1934-2025
Purchase Order: UK 1704685
Contact: Aecom Infrastructure & Environment Uk Ltd
Issue Date: 12.06.2025
Received Date: 29.05.2025

SureScreen Scientifics

GCN eDNA Analysis

Summary

When great crested newts (GCN), *Triturus cristatus* , inhabit a pond, they continuously release small amounts of their DNA into the environment. By collecting and analyzing water samples, we can detect these small traces of environmental DNA (eDNA) to confirm GCN habitation or establish GCN absence.

Results

Lab ID	Site Name	OS Reference	Degradation Check	Inhibition Check	Result	Positive Replicates
GCN25 0699	EGL4 - 16		Pass	Pass	Negative	0/12
GCN25 0700	EGL4 - 19		Pass	Pass	Negative	0/12
GCN25 0701	EGL4 - 18		Pass	Pass	Negative	0/12
GCN25 0702	EGL4 - 13		Pass	Pass	Negative	0/12
GCN25 0703	EGL4 - 10		Pass	Pass	Negative	0/12
GCN25 0704	EGL4 - 12		Pass	Pass	Negative	0/12
GCN25 0705	EGL4 - 15		Pass	Pass	Negative	0/12
GCN25 0706	EGL4 - 6c		Pass	Pass	Negative	0/12
GCN25 0707	EGL4 - 6b		Pass	Pass	Negative	0/12
GCN25 0708	EGL4 - 8		Pass	Pass	Negative	0/12

GCN25 0709	EGL4 - 7		Pass	Pass	Negative	0/12
GCN25 0710	EGL4 - 3	NT 20454 96642	Pass	Pass	Negative	0/12
GCN25 0711	EGL4 - 4	NT 19597 95668	Pass	Pass	Negative	0/12
GCN25 0712	EGL4 - 6a		Pass	Pass	Negative	0/12
GCN25 3923	EGL4 - 2	NT 20383 96769	Pass	Pass	Negative	0/12

Matters affecting result: none

Reported by: Amy Bermudez

Approved by: Lauryn Jewkes

SureScreen Scientifics

Methodology

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

If GCN DNA is present, the DNA is amplified up to a detectable level, resulting in positive species detection. If GCN DNA is not present then amplification does not occur, and a negative result is recorded. Analysis of eDNA requires attention to detail to prevent the risk of contamination. True positive controls, negative controls, and spiked synthetic DNA are included in every analysis and these have to be correct before any result is declared and reported. Stages of the DNA analysis are also conducted in different buildings at our premises for added analytical security.

SureScreen Scientifics Ltd is ISO9001 accredited and participates in Natural England's proficiency testing scheme for GCN eDNA testing.

Interpretation of Results

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

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

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

Result: **Presence of GCN eDNA (Positive/Negative/Inconclusive)**
Positive: GCN DNA was identified within the sample, indicative of GCN presence within the sampling location at the time the sample was taken or within the recent past at the sampling location.
Positive Replicates: Number of positive qPCR replicates out of a series of 12. If one or more of these are found to be positive the pond is declared positive for GCN presence. It may be assumed that small fractions of positive analyses suggest low level presence, but this cannot currently be used for population studies. In accordance with the WC1067 Natural England protocol, even a score of 1/12 is declared positive. 0/12 indicates negative GCN presence.
Negative: GCN eDNA was not detected or is below the threshold detection level and the test result should be considered as evidence of GCN absence, however, does not exclude the potential for GCN presence below the limit of detection.
Inconclusive: Controls indicate inhibition or degradation of the sample, resulting in the inability to provide conclusive evidence for GCN presence or absence.