



Alveston Hill Cycle Route

Environmental DNA Survey for Great Crested Newt

South Gloucestershire Council

November 2023





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This document has 19 pages including the cover.

Report Validity

In the event of scope or programme changes or if works do not commence within 12 months of the date of this report, then updates to the surveys may be required to ensure the validity of the data, as per CIEEM guidance¹.

Document history

Document title: Environmental DNA Survey for Great Crested Newt

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		E.Green	C.Llewellyn	S.Cafferty		

Client signoff

Client	South Gloucestershire Council
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¹ <u>https://cieem.net/resource/advice-note-on-the-lifespan-of-ecological-reports-and-surveys/</u> [Accessed February 2023]



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1. Introduction

1.1. Terms of Reference

- 1.1.1. AtkinsRéalis was commissioned by South Gloucestershire Council to carry out a great crested newt (GCN) Habitat Suitability Index (HSI) and Environmental DNA (eDNA) survey, in relation to the Alveston Hill Cycle Route Scheme (hereafter referred to as the Scheme), located near the village of Alveston.
- 1.1.2. This report is to be submitted as a technical appendix to the EcIA and presents the results acquired from the Habitat Suitability Index assessment and Environmental DNA survey and the presence/likely absence of GCN.

1.2. The Site

- 1.2.1. The Site, Alveston Hill, is situated between the village of Alveston and the town of Thornbury in the County of South Gloucestershire. The Site is presented in **Appendix A**.
- 1.2.2. The Site is centred at Ordnance Survey National Grid reference (OSNGR) ST 63442 88851 and comprises two agricultural fields with public footpath adjacent to/east of Alveston Hill road (B4061). The road is lined with trees and shrubs. Thornbury Leisure Centre is located north of the Site.
- 1.2.3. Habitats adjacent to the Site include agricultural fields, broad-leaved trees and hedgerows, a small stream and scrub. Habitats within the wider landscape beyond the Site predominantly comprise fields which are likely in agricultural use, patches of deciduous woodland and amenity grassland associated with Thornbury Golf Centre and residential and commercial buildings associated with Alveston and Thornbury. A large quarry is also located approximately 2.4 km to the east of the Site.

1.3. The Scheme

- 1.3.1. The Alveston Hill scheme proposes the following:
 - 2-way cycle track and footway to run along western side of B4061 between Alveston Hill and the A38;
 - Pedestrian and cycling crossings of Down Road and Alveston Hill; and
 - Off-road segregated walking and cycling path from Alveston Hill to Thornbury Leisure Centre.
- 1.3.2. The proposed works within and alongside the public highway are permitted development under Part 9 Class A(a&b) of the Town and Country Planning General Permitted Development Order (2015) (amended) (GDPO), meaning that formal planning permission is not required. The proposed off-road segregated footpath and cycleway does not fall under permitted development and therefore requires planning permission. Only the off-road segregated footpath and cycleway is included in this assessment and Survey Area.
- 1.3.3. This off-road section proposes a 5 m wide path comprising a 3 m wide 2-way cycleway and 2 m wide footpath. These would be side by side but segregated to facilitate easy movement and prevent obstructions to cyclists and pedestrians. The path will have concrete edging and a stock proof fence is proposed to run alongside the east of the path, serving as a perimeter boundary treatment to the private land beyond. The route includes soft landscaping and opportunities for seating/recreation spaces for visual amenity and functionality purposes.
- 1.3.4. In addition to running through a field, the route passes through existing tree groups and hedgerows. Part of these green infrastructure features will need to be removed to facilitate the development include: a section of hedgerow along the south-west boundary of the Site, a section of the hedgerow which runs roughly east to west across the centre of the Site dividing the two field parcels, and complete removal of a hedgerow and two groups of trees in the northern section.



- 1.3.5. At either end of this section of route, the Scheme will tie into the existing highway.
- 1.3.6. At the time of writing, the preliminary construction design, construction timetable and construction working methods are not finalised.

1.4. Scope of the Assessment

- 1.4.1. The scope for this assessment was to undertake a HSI and eDNA survey of 1 pond, located within 100m of the Scheme, in order to understand the use of the Site by great crested newt and confirm their presence or likely absence, so as to ensure the Scheme complies with relevant legislation.
- 1.4.2. This report presents the findings of the HSI and eDNA survey, as well as the methods used and any further recommendations, including identification of potential avoidance, mitigation and enhancement measures, where appropriate.

1.5. Legislation and Relevant Policy

- 1.5.1. The Scheme has the potential to directly impact on great created newts and the habitat of great crested newts.
- 1.5.2. Great crested newt is a protected species under The Conservation of Habitats and Species (Amendment) (EU Exit) Regulations, 2019. This means it is an offence to:
 - Deliberately² capture, injure or kill any listed wild animal or destroy their eggs;
 - Deliberately disturb³ such an animal;
 - Damage or destroy a breeding site or resting place used by such an animal; or
 - Intentionally or recklessly obstruct access to any structure or place used for shelter or protection or disturb⁴ such an animal in such a place.
- 1.5.3. GCN are a European protected species (EPS) and are listed under section 41 of the '*Natural Environment and Rural Communities (NERC) Act 2006*' as a Species of Principal Importance for Conservation in England. Therefore, GCN and their resting and breeding places have full legal protection. Section 40 of the NERC Act requires that local and regional authorities have regard to the conservation of biodiversity in England, when carrying out their normal functions. GCN are protected under the Wildlife and Countryside Act 1981 (as amended) S.9, meaning it is an offence to intentionally or recklessly obstruct access to any structure or place used for shelter or protection or disturb a great created newt in such a place. GCN are listed as a priority species in the South Gloucestershire Biodiversity Action Plan⁵.
- 1.5.4. Within South Gloucestershire Council's Local Plan Core Strategy⁶, Policy CS9, Managing the Environment and Heritage, sets out the objectives of conserving and enhancing the district's distinctive landscapes, natural environmental resources and biodiversity. This policy states that new development will be expected to conserve and enhance the natural environment, avoiding or minimising impacts on biodiversity and geodiversity and conserve and enhance the character, quality, distinctiveness, and amenity of the landscape. Within South Gloucestershire Council's Policies, Sites

² Deliberate capture or killing is taken to include "accepting the possibility" of such capture or killing.

³ Deliberate disturbance of animals includes in particular any disturbance which is likely a) to impair their ability (i) to survive, to breed or reproduce, or to rear or nurture their young, or (ii) in the case of animals of hibernating or migratory species, to hibernate or migrate; or b) to affect significantly the local distribution or abundance of the species to which they belong.

⁴ Lower levels of disturbance not covered by the Conservation of Habitats and Species Regulations 2017 remain an offence under the Wildlife and Countryside Act 1981 although a defence is available where such actions are the incidental result of a lawful activity that could not reasonably be avoided.

⁵ South Gloucestershire Council, 2016. South Gloucestershire Biodiversity Action Plan 2016-2026 [pdf] Available at: <

https://beta.southglos.gov.uk/static/e25ebdbd66bf0d60a81b9501a5427a7f/Biodiversity-Action-Plan-2016-26.pdf > [Accessed 22 August 2023]

⁶ South Gloucestershire Council, 2013. South Gloucestershire Local Plan: Core Strategy 2006-2027 [pdf] Available at: < https://beta.southglos.gov.uk/static/f149e2bb1bf00a972238eb11eb06d132/South-Gloucestershire-Core-Strategy-2006-2027.pdf> [Accessed 22 August 2023].



and Places (PSP) plan⁷, Policy PSP19 – wider biodiversity, states that biodiversity gain will be sought from development proposals. Land with low to negligible nature conservation value (such as pasture or arable land) can be used for provision of new semi-natural habitat, which would provide gains for local wildlife.

⁷ South Gloucestershire Council, 2017. South Gloucestershire Local Plan: Policies, Sites and Places Plan Adopted November 2017. [pdf] Available at < https://beta.southglos.gov.uk/static/90efa5d673f208a3109ed111ba963a01/PSP-Plan-Nov2017.pdf> [Accessed 22 August 2023]

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2. Methodology

2.1. Great Crested Newt HSI and eDNA Survey

- 2.1.1. The desk study identified one pond within 250m of the Scheme, located 5m west of the Site boundary at OSGR ST 6339388818. The location of this pond is shown on the habitat survey plan in Appendix A. No additional ponds were identified during the walkover survey.
- 2.1.2. This pond was assessed for potential to support GCN using a Habitat Suitability Index (HSI) and eDNA survey, following good practice guidance^{8,9}.
- 2.1.3. A HSI is a numerical index between 0 and 1, where 0 indicates an unsuitable habitat and 1 represents an optimal habitat. A score is calculated based on the results of 10 suitability indices, all of which are factors thought to affect GCN presence. The resulting score categorises the pond based on its suitability to support GCN and can be used to influence the decision as to whether further detailed survey work is required, as shown in Table 2-1 below.

Table 2.1 - Habitat Suitability Index Scoring System

HSI Score	Suitability to support GCN
<0.5	Poor
0.5-0.59	Below Average
0.6-0.69	Average
0.7-0.79	Good
>0.8	Excellent

- 2.1.4. In accordance with HSI methodology, the following features were assessed:
 - Geographical location;
 - Pond area;
 - Pond permanence;
 - Water quality;
 - Pond shading;
 - Number of Waterfowl;
 - Occurrence of fish;
 - Pond density/other ponds within 1km;
 - Terrestrial habitat quality; and,
 - Macrophyte cover.
- 2.1.5. Environmental DNA, or eDNA, refers to DNA that can be extracted from environmental samples, such as water, soil or faeces, without having to isolate the target organism. All living organisms leave traces of their DNA within the environment and this DNA enables the detection of a species regardless of its life stage or gender.
- 2.1.6. eDNA surveys can prove presence or likely absence of GCN without the typical suite of night time presence/ absence surveys being undertaken.

⁸ Amphibian and Reptile Groups of the United Kingdom (2010). ARG UK Advice Note 5: Great Crested Newt Habitat Suitability Index. ARGUK.

⁹ Biggs *et al.*, (2014). Technical Advice Note for Field and Laboratory Sampling of Great Crested Newt eDNA in Analytical and Methodological Development for Improved Surveillance of the Great Crested Newt. Defra Project WC1067. Appendix 5. Freshwater Habitats Trust, Oxford.

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- 2.1.7. The eDNA survey was carried out in June 2023, in accordance with the relevant methods set out in the technical advice note for field and laboratory sampling of great crested newt by Biggs *et al.* (2014)¹⁰.
- 2.1.8. This method involved using a ladle to collect 20 evenly spaced water samples, from around the perimeter of the pond, and then transferring these to a Whirl Pak bag. Before each sample was taken, the water was gently stirred using the ladle. This is because eDNA will often be present in larger quantities at the bottom of the pond as it tends to sink in water. The Whirl Pak bag was then gently shaken to mix eDNA across the whole water sample. A pipette was then used to transfer 15ml of water from the whirl Pak bag into each of the six conical tubes containing a preserving fluid. Each conical tube was then vigorously shaken for ten seconds to mix the water sample and the preservative. The six conical tubes were then labelled and sent to the lab for analysis.
- 2.1.9. The eDNA samples collected were express couriered to SureScreen Scientifics who carried out analysis of the samples.
- 2.1.10. These surveys were conducted by suitably qualified and experienced ecologists including one ecologist who holds a Natural England class licence for surveying GCN.

2.2. Limitations

- 2.2.1. Ecological surveys are limited by factors which affect the presence of species, such as the time of year, migration patterns and behaviour. Therefore, the surveys of this Site have not produced a complete list of species and the absence of evidence of any species should not be taken as conclusive proof that the species is not present or that it will not be present in the future.
- 2.2.2. Access to the pond was restricted, so eDNA samples were only taken from one side of the pond, rather than spread evenly around the perimeter of the waterbody. This is not considered a significant limitation to the survey as the pond was small in size (8mx2m).

¹⁰ Biggs J, Ewald N, Valentini A, Gaboriaud C, Griffiths RA, Foster J, Wilkinson J, Arnett A, Williams P and Dunn F (2014) Analytical and methodological development for improved surveillance of the Great Crested Newt. Appendix 5. Technical advice note for field and laboratory sampling of great crested newt (Triturus cristatus) environmental DNA. Freshwater Habitats Trust, Oxford.

3. Results

- 3.1.1. The waterbody (Pond 1) subject to eDNA sampling tested negative for GCN eDNA, suggesting that GCN are not present within this waterbody.
- 3.1.2. A description of the pond along with the eDNA and HSI results for Pond 1 is provided in Table 3-1 below. The full HSI results can be seen in **Appendix B**.
- 3.1.3. The technical sample analysis report produced by SureScreen Scientifics is provided in **Appendix C.**

Waterbody Reference	Survey Date	Grid Reference	Description	Species Present	Photograph	eDNA Result	HSI Result
Pond 1	08/06/23	ST63393 88818	This a small waterbody (8mx2m) fed by a stream, between an existing woodland and a adjacent road.	watercress (Nasturtiumof ficinale), dogs mercury (Mercurialis perennis), common nettle (Urtica dioica), red campion (Silene dioica), bramble (Rubus fructicosus).		Negative	0.64 (Average)

 Table 3.1 - Waterbody Description and Survey Results

4. Recommendations

4.1. Enhancement

- 4.1.1. The Scheme will provide opportunities for ecological enhancement to align with South Gloucestershire Core Strategy on protecting and enhancing the landscape for biodiversity. This will include planting of hedge and grassland, to provide additional terrestrial habitat and the creation of two new ponds and a ditch network for GCN and other amphibians to increase breeding opportunities. The location of the proposed habitat creation is provided within a landscape general arrangement plan in **Appendix D**.
- 4.1.2. The recommendations below are outlined, further details will be found within a Landscape Ecology Management Plan (LEMP) which will be commissioned once the planning application has been determined.
- 4.1.3. The LEMP plan will include further details on the creation and management of these new habitats described below, including management prescriptions, approach, timings, and recommendations on planting, with specialist input from an aquatic ecologist and landscape architect.

Hedgerow Creation

- 4.1.4. Landscaping should be maximised within the Scheme design and provide additional species rich hedgerows to facilitate the works and reconnect the landscape adjacent, which will provide an important wildlife corridor, and the provision of additional shelter and refuge for amphibians.
- 4.1.5. This should include native flower and fruit bearing tree and shrub species of local provenance and species composition similar to existing hedgerows within the area. Planting a variety of species will help to provide diverse habitat and a species rich community and provide additional food sources for birds and invertebrates. The hedgerows should be managed on a rotational basis to retain the structural integrity of hedgerows and maintain connections with other habitats, such as existing and newly created ponds suitable for amphibians and other wildlife.

Grassland Improvements

4.1.6. Grassland adjacent to woodland and hedgerows will be managed through cutting and monitoring regime to improve the current condition of the grassland, this will provide dense ground level cover for fauna, including refuge for amphibians, and foraging habitat, through an increase in prey availability (. e.g., insects), due to the increase in wildflowers available as food plants as nectar.

Pond Creation

- 4.1.7. Two new ponds will be created within the Scheme, to support amphibians, and aquatic invertebrates. GCN are most suited to ponds with the following characteristics, which would be suitable for other amphibians also:¹¹:
 - Surface area between 100 and 300m²
 - Depth may vary; both deep (up to around 4m) and shallow ponds are suitable also
 - Occasional drying out is not a problem, even if this means a total loss of that year's larvae; the pond should hold water throughout at least one summer in every 3 years
 - Substantial cover of submerged and marginal vegetation
 - Open areas to facilitate courtship behaviour
 - Good populations of invertebrates for prey
 - Ponds in clusters, rather than in isolation
 - Absence of shading on the south side
 - Absence of fish

¹¹English Nature, 2001. Great crested newt mitigation guidelines. English nature, Peterborough



• Absence or low density of waterfowl

Wetland Planting

4.1.8. Marginal and emergent vegetation are important components of an amphibian pond as they provide excellent egg-laying sites. Plants recommended for this purpose include water forget-me-not (*Myosotis scorpioides*), water mint (*Mentha aquatica*) floating sweet grass (*Glyceria fluitans*) and great hairy willowherb (*Epilobium hirsutum*) which should be included within the planting required around each pond, and within the wider vicinity due to the inundated wet conditions of the landscape. It is also recommended that high levels of human or animal disturbance, agricultural or road run-off and significant shading from surrounding trees should be avoided.

Ditch Network

- 4.1.9. The Scheme will include a ditch network either side of the cycleway for drainage purposes. However, this will provide additional connectivity across the landscape for amphibians, including refuge, foraging and dispersal opportunities, including links to creation of new ponds which could provide breeding potential for future amphibian populations in the area.
- 4.1.10. A full recommended plant species list for all created habitats will be required in the LEMP.

5. Conclusion

- 5.1.1. The waterbody was classified with an average HSI score of 0.64 and the eDNA sampling returned a negative result for GCN. Therefore, GCN are considered likely to be absent in this waterbody.
- 5.1.2. It is concluded that the Scheme is unlikely to negatively impact great crested newts. It is recommended that to adhere to local planning policy, that hedgerow, grassland and ditch creation is provided to increase terrestrial habitat for GCN and other amphibians, and that two new waterbodies are created.
- 5.1.3. In the event of scope or programme changes or if works do not commence within 12 months of the date of this report, further surveys may be required to ensure the validity of the data, as per CIEEM guidance¹².

¹² CIEEM, 2019. Advice Note on the Lifespan of Ecological Reports and Surveys.

Appendices

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Appendix A. Current Pond Location

The Site is indicated by the Survey Area shown on the habitat plan below, which provides the current pond location. Pond 1 is located at ST6339388818, shown by TN7 (circled in black) on the habitat survey plan.







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Appendix B. Habitat Suitability Index Results – Pond 1

Location Score	Pond Area Score	Permanence Score	Water Quality Score	Shade Score	Waterfowl Score	Fish Score	Pond Count Score
1	0.05	1	0.67	1	1	1	1





Terrestrial Habitat Score	Macrophyte Score
0.33	1



Appendix C. Environmental DNA Laboratory Reports



Folio No: E18005 Report No: 1 Purchase Order: EHAC08062023 Client: ATKINS LTD Contact: Ellen Harpham

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: Date Reported: Matters Affecting Results:				14/06/2023 21/06/2023 None							
Lab Sample No.	Site Name	O/S Reference	SIC		DC		IC		Result	P Re	ositive plicates
0893	Alveston - Pond 1	ST 63391 88819	Pass	L	Pass	1	Pass	I	Negative	L	0

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

Reported by: Chris Troth

Approved by: Jackson Young



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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.



Appendix D. Proposed Habitat Creation















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