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Tyrer Ecological Consultants Ltd, Formby Business Centre, 42 Duke Street, Formby, L37 4AT

Technical Appendix II

Environmental DNA (eDNA) Survey Results for Great Crested Newt (GCN)

July 2022

**Brook Lodge,
Trough Road,
Scorton,
PR3 1DH**

National Grid Ref: SD526511



Brook Lodge, Trough Road, Scorton, PR3 1DH
eDNA Survey Results for GCN

Document Title	Environmental DNA (eDNA) Survey Results for Great Crested Newt (GCN)
Issue no.	1.0
Prepared for	Graham Anthony Associates
Prepared by	Tyrer Ecological Consultants Ltd

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Survey Date	22/06/2022
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Review date	09/08/2022
Approved by	Mrs. K. Wilding CEnv MIEMA ACIEEM
Date of Issue	09/08/2022

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1.0 Background & Introduction

- 1.1 As part of a proposed planning application for Brook Lodge in Scorton, Tyrer Ecological Consultants Ltd carried out a Preliminary Ecological Appraisal (PEA) in May 2022. – the report was issued in May 2022 (for further information see **Appendix I**).
- 1.2 Proposals are understood to involve the erection of a number of chalets and change of land use.
- 1.3 As part of the Preliminary Ecological Appraisal, twelve ponds were assessed by the surveyor with respect to their potential to offer suitable habitat for GCN, as well as the most appropriate survey methodology. These assessments were carried out by Mr. M. Pritchard ACIEEM, Senior Ecologist at Tyrer Ecological Consultants Ltd, holder of a Great Crested Newt Survey Level 1 / Natural England License: 2018-34062-CLS-CLS, who has worked on a large number of schemes involving both GCN surveying, habitat assessment and mitigation.
- 1.4 Following recommendations provided in the Preliminary Ecological Appraisal, Tyrer Ecological Consultants Ltd were recommissioned by Graham Anthony Associates to undertake an Environmental DNA (eDNA) Survey study of the twelve ponds on site during an optimal time of the year to detect aquatic based GCN (optimal period is between 15th April and 30th June).
- 1.5 This report thus details the methodology used, results and conclusions derived from the eDNA sampling of all of the ponds at Brook Lodge and will present any further recommendations, including any avoidance, minimisation or indicative mitigation required to inform the planning application, and/or inform an application to Natural England for a European Protected Species Mitigation Licence (EPSML), if necessary.

Survey Objectives

- 1.6 The eDNA survey aims to determine presence, or reasonably assert absence, of Great Crested Newts at the 12 ponds on site (see **Figure 1.1**). It does not consider any other water bodies either on site or within a 250 metre radial buffer of the site.
- 1.7 If GCN were detected during the survey and / or may be affected by the development proposals, then a European Protected Species Mitigation Licence (EPSML) or District Level Licence (DLL) may be required to proceed with the development.
- 1.8 This report should be read, understood and presented to the local authority as an addendum document to **Appendix I** (see Contents page).



Figure 1.1 – Location of Ponds 1-12 on site and one additional woodland pond (Pond 13) within 250 metres (Blue infill). There are several former ponds in the woodland now dried up.

2.0 Great Crested Newt (GCN) – Legislation & Policy

2.1 GCN are fully protected in all life stages by Schedule 2 of The Conservation of Habitats and Species (Amendment) (EU Exit) Regulations 2019 (SI 2019/579) and the Wildlife and Countryside Act (1981) (as amended). They are listed a priority species under section 41 (s.41) of the Natural Environment Rural Communities Act 2006 (NERC Act) and are also listed on the UK Biodiversity Action Plan (UKBAP). The legislation makes it a punishable offence to:

- Deliberately, intentionally or recklessly kill, injure or take a GCN,
- Deliberately, intentionally or recklessly take or destroy GCN eggs,
- Possess or control any live or dead specimen, or anything derived from a GCN,
- Deliberately, intentionally or recklessly damage, destroy or obstruct access to any structure or place used for shelter or protection by a GCN,
- Deliberately, intentionally or recklessly disturb a GCN while it is occupying a structure or place which it uses for that purpose

2.2 GCN are also protected by the Protection of Animals Act (1911), which prohibits any acts of cruelty or mistreatment.

- 2.3 In summary, Great Crested Newts are protected from disturbance, killing, injuring or possession at any life stage, whilst confirmed breeding ponds and resting places are afforded the same protection once identified. Habitat for this species is protected when within certain range of a breeding pond. This places an obligation on local authorities to fully assess the impacts of development on this species prior to determining a planning application.
- 2.4 GCN populations have declined within the UK over recent years due to destruction and fragmentation of breeding ponds and terrestrial habitat. They spend a high percentage of their adult life in terrestrial habitat such as woodland, and shelter in environments with piles of rubble, log piles, tree roots, miscellaneous other for safety, refuge and hibernation. In Lancashire, GCN's are widespread and can be found where favourable habitat coincides with connectivity to breeding pools.

NB: *Research presented by Natural England provides evidence that GCN are most likely to use terrestrial habitat within 100 metres of their breeding ponds, whilst the Great Crested Newt Method Statement for EPSML (Natural England) describes terrestrial habitat zones - the 50metres range of any GCN pond is the 'Core' habitat zone and the area most likely to support newts in a lifetime; the area between 51-250m is the 'Intermediate' zone and 250m+ is the 'Distant' zone; impacts to these zones, from individual ponds, are considered by the ecologist; both permanent and temporary impacts are taken into account.*

Policy

- 2.5 Paragraph 180 of the National Policy Planning Framework (as revised in July 2021) states:

180. When determining planning applications, local planning authorities should apply the following principles:

a) if significant harm to biodiversity resulting from a development cannot be avoided (through locating on an alternative site with less harmful impacts), adequately mitigated, or, as a last resort, compensated for, then planning permission should be refused;

b) development on land within or outside a Site of Special Scientific Interest, and which is likely to have an adverse effect on it (either individually or in combination with other developments), should not normally be permitted. The only exception is where the benefits of the development in the location proposed clearly outweigh both its likely impact on the features of the site that make it of special scientific interest, and any broader impacts on the national network of Sites of Special Scientific Interest;

c) development resulting in the loss or deterioration of irreplaceable habitats (such as ancient woodland and ancient or veteran trees) should be refused, unless there are wholly exceptional reasons and a suitable compensation strategy exists; and,

d) development whose primary objective is to conserve or enhance biodiversity should be supported; while opportunities to improve biodiversity in and around developments should be integrated as part of their design, especially where this can secure measurable net gains for biodiversity or enhance public access to nature where this is appropriate.

- 2.6 The Office of the Deputy Prime Minister (ODPM) Circular 06/2005 provides administrative guidance on the application of the law in relation to planning and nature conservation:

"It is essential that the presence or otherwise of protected species, and the extent that they may be affected by the proposed development, is established before planning permission is granted, otherwise all relevant material considerations may not have been addressed in making the decision.

2.7 Policy CDMP4 of the Wyre Local Plan, titled Environmental Assets, echoes this national focus on preservation of biodiversity, stating:

“1. Development proposals should, where possible: a) Provide enhancements in relation to the environmental assets in this policy; and b) Seek to minimise or eliminate net environmental impact.

2. Development will be required to be accompanied by proposals to mitigate the overall environmental impact and maximise further opportunities to improve the environmental outcomes. Where mitigation measures are not considered adequate, appropriate on or off site compensation measures will be sought to off-set the environmental impact of the development.

3. Development will be permitted where, following implementation of any required mitigation, there is no unacceptable impact on environmental assets or interests, including, but not limited to, green infrastructure, habitats, species, soils, water quality and resources and trees and hedgerows.

10. The Borough’s designated and undesignated ecological assets will be protected, enhanced and managed with the aim of establishing and preserving functional networks which facilitate the movement of species and populations and protect the Borough’s biodiversity. Development should contribute to the restoration, enhancement connection of natural habitats through the provision of appropriate Green Infrastructure and to a net gain in biodiversity where possible.

13. Development that would result in the further fragmentation of, or compromises the function of, Wyre’s ecological network will not be permitted unless: a) The harm caused is significantly and demonstrably outweighed by other planning considerations; and b) An appropriate mitigation and compensation strategy can be secured.

21. Development will be expected to incorporate existing trees and hedgerows into the design and layout of the scheme where possible unless their loss is essential to allow the development to go ahead and is supported by evidence in a tree or hedgerow survey.

22. Where tree and hedgerow loss is unavoidable, an equivalent amount of new trees and hedgerows of suitable species should be proposed unless a clear justification is provided for not doing so. Where appropriate, opportunities to increase tree and hedgerow cover should be explored.

23. Development and planting schemes must be designed so as to avoid: a) Damage to existing trees which are to be retained; or b) The potential for future conflict between buildings and trees.

24. Where development is proposed which would result in the loss of ancient woodland, protected tree(s) or veteran tree(s), planning permission will only be granted where: a) The removal of one or more trees would be in the interests of good arboriculture practice; or b) It is demonstrated that the benefits of the proposed development outweighs the amenity and/or nature conservation value of the tree(s).”

2.8 In addition, in 2022 Natural England updated the four policies which are relevant to European Protected Species (EPS), such as GCN, which were launched originally in 2016. The policies seek to achieve better conservation outcomes for EPS and reduce unnecessary costs, delays and uncertainty that can be inherent in the current system.

Policy 1: Reduce mitigation measures for impacts on EPS

Defra considers that compensation for EPS impacts can be delivered without the need to relocate or exclude populations, where all of the following apply:

- exclusion or relocation measures are not necessary to maintain the conservation status of the local population
- the avoid-mitigate-compensate hierarchy is followed
- compensation provides greater benefits to the local population than would exclusion, or relocation, or both

Policy 2: Location of compensation habitats

If the licensing tests are met and the avoid-mitigate-compensate hierarchy is followed, off-site compensation measures may be preferred to on-site compensation measures, where both of these conditions apply:

- there are good reasons for maximising development on the site of EPS impacts
- an off-site solution provides greater benefit to the local population than an on-site solution

Policy 3: Let EPS use temporary habitats

Where development (such as mineral extraction) will temporarily create habitat which is likely to attract EPS, Defra favours proposals which enable works to proceed without the exclusion of EPS, where the conservation status of the local population would not be detrimentally affected.

On completion of development, such sites must contribute to the conservation status of the local population as much as or more than the land use which preceded development.

The measures to achieve this should be set out in a management plan and secured by a legal agreement.

Policy 4: Alternative sources of evidence to reduce standard survey requirements

Natural England will be expected to ensure that licensing decisions are properly supported by survey information, taking into account industry standards and guidelines. It may however accept a lower than standard survey effort where all the following apply:

- costs or delays associated with carrying out standard survey requirements would be disproportionate to the additional certainty that it would bring
- ecological impacts of development can be predicted with sufficient certainty
- mitigation or compensation will ensure that the licensed activity does not detrimentally affect the conservation status of the local population of any EPS

2.9 These policies should only be applied by a suitably qualified ecologist to support suitable development schemes and/or applications for EPSMLs.

3.0 Environmental DNA (eDNA) Survey Results

Sample Collection Protocol

- 3.1 A daytime survey visit for the collection of eDNA samples from the pond was carried out on the 22nd June 2022 in sunny conditions (28°C), 0% Cloud, wind 1/12 Beaufort by Mrs. K. Wilding ACIEEM, Principal Level Ecologist at Tyrer Ecological Consultants Ltd, Mr. M. Pritchard ACIEEM, Senior Ecologist, Mr. J. Pescod Qualifying CIEEM, Consultant Ecologist, and Miss. T. Hesketh, Junior Ecologist.
- 3.2 All of the surveying team are extensively trained in undertaking eDNA surveying visits as well as traditional GCN surveys and are Accredited Agents on the licence of Mr. M. Pritchard (2018-34062-CLS-CLS). Mr. M. Pritchard has extensive training in GCN surveying, licencing and mitigation, having previously carried out x30+ eDNA surveys to date, and having also been involved in a large number and variety of development schemes, many of which have involved Great Crested Newt surveys, implications and mitigation.
- 3.3 All eDNA sampling was carried out in accordance with the stringent survey methodologies defined within Natural England's accepted protocol (*Biggs J., et al., 2014*).

Summary of Technical Sampling Procedure

- 3.4 A total of 20 water samples were taken respectively from each of the 12 ponds on site to form the basis of the DNA samples. The samples were taken using a sterile ladle and emptied into a sterile self-supporting Whirl-Pak bag. All samples were taken from locations around the margins of each pond in areas which could be utilised by GCN for egg laying or displaying. Once all 20 samples were collected, the sterile self-supporting bag was shaken to mix any DNA across the whole pond sample. A sterile plastic pipette was used to transfer approximately 15.0 ml of the mixed pond sample water into a sterile conical tube. This was undertaken for each of the six sterile conical tubes in the kit, per pond. Each sterile conical tube(s) contained 35.0 ml of ethanol to preserve any DNA within the samples. The box of six sterile conical tubes per pond were returned within 48 hours at ambient air temperature to the ADAS eDNA testing service for laboratory analysis.

eDNA Analysis

- 3.5 eDNA analysis by ADAS was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

eDNA Survey Constraints

- 3.6 Any constraints such as access issues at the water body, likely contamination, weather problems or other limitations are given below, where applicable.
- The site had become heavily overgrown since the Preliminary Ecological Appraisal was carried out. This meant that many of the ponds could not be easily accessed, with just 5% of Pond 1 being accessible, 15% of Pond 3, 30% of Pond 5, 35% of Pond 6, 40% of Pond 4, and 70% of Ponds 2 and 7. Additionally, Pond 3 was very scummy.
- 3.7 In considering possible limitations, there were no significant survey constraints that may adversely affect the results, findings or recommendations of this report.

eDNA Survey Results

3.8 Following analysis of samples provided to, and by, ADAS of the ponds on site as described in this report, the eDNA presence/absence results are presented below (see **Figures 3.1-3.12**). The raw data returned by ADAS is also provided as **Appendix II**.



Sample ID: ADAS-5568	Condition on Receipt: Good	Volume: Passed	
Client Identifier: Pond 1, Brook Lodge	Description: pond water samples in preservative		
Date of Receipt: 27/06/2022	Material Tested: eDNA from pond water samples		
Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	29/06/2022
Degradation Control [‡]	Within Limits	Real Time PCR	29/06/2022
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	29/06/2022
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [§]	4 of 4	Real Time PCR	As above for GCN
Report Prepared by:	Dr Helen Rees	Report issued by:	Dr Ben Maddison
Signed:		Signed:	
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	01/07/2022	Date of issue:	01/07/2022

Figure 3.1 – eDNA results from Pond 1 on site


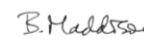
Sample ID: ADAS-5563	Condition on Receipt: Low Sediment	Volume: Passed	
Client Identifier: Pond 2, Brook Lodge	Description: pond water samples in preservative		
Date of Receipt: 27/06/2022	Material Tested: eDNA from pond water samples		
Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	29/06/2022
Degradation Control [‡]	Within Limits	Real Time PCR	29/06/2022
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	29/06/2022
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [§]	4 of 4	Real Time PCR	As above for GCN
Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:		Signed:	
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	01/07/2022	Date of issue:	01/07/2022

Figure 3.2 – eDNA results from Pond 2 on site



Sample ID: ADAS-5566	Condition on Receipt: Medium Sediment	Volume: Passed	
Client Identifier: Pond 3, Brook Lodge	Description: pond water samples in preservative		
Date of Receipt: 27/06/2022	Material Tested: eDNA from pond water samples		
Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	0 of 2	Real Time PCR	29/06/2022
Degradation Control [‡]	Evidence of degradation or residual inhibition	Real Time PCR	29/06/2022
Great Crested Newt*	Indeterminate	Real Time PCR	29/06/2022
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [§]	4 of 4	Real Time PCR	As above for GCN
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Figure 3.3 – eDNA results from Pond 3 on site

Brook Lodge, Trough Road, Scorton, PR3 1DH
eDNA Survey Results for GCN



Sample ID: ADAS-5567	Condition on Receipt: Low Sediment	Volume: Passed	
Client Identifier: Pond 4, Brook Lodge	Description: pond water samples in preservative		
Date of Receipt: 27/06/2022	Material Tested: eDNA from pond water samples		
Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	29/06/2022
Degradation Control [§]	Within Limits	Real Time PCR	29/06/2022
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	29/06/2022
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [¶]	4 of 4	Real Time PCR	As above for GCN
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Figure 3.4 – eDNA results from Pond 4 on site



Sample ID: ADAS-5562	Condition on Receipt: Good	Volume: Passed	
Client Identifier: Pond 5, Brook Lodge	Description: pond water samples in preservative		
Date of Receipt: 27/06/2022	Material Tested: eDNA from pond water samples		
Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	29/06/2022
Degradation Control [§]	Within Limits	Real Time PCR	29/06/2022
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	29/06/2022
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [¶]	4 of 4	Real Time PCR	As above for GCN
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Figure 3.5 – eDNA results from Pond 5 on site


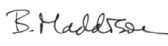
Sample ID: ADAS-5565	Condition on Receipt: Good	Volume: Passed	
Client Identifier: Pond 6, Brook Lodge	Description: pond water samples in preservative		
Date of Receipt: 27/06/2022	Material Tested: eDNA from pond water samples		
Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	29/06/2022
Degradation Control [§]	Within Limits	Real Time PCR	29/06/2022
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	29/06/2022
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [¶]	4 of 4	Real Time PCR	As above for GCN
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Figure 3.6 – eDNA results from Pond 6 on site

Brook Lodge, Trough Road, Scorton, PR3 1DH
eDNA Survey Results for GCN

Sample ID: ADAS-5564	Condition on Receipt: Good	Volume: Passed
Client Identifier: Pond 7, Brook Lodge	Description: pond water samples in preservative	
Date of Receipt: 27/06/2022	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	28/06/2022
Degradation Control [§]	Within Limits	Real Time PCR	28/06/2022
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	28/06/2022
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [¶]	4 of 4	Real Time PCR	As above for GCN


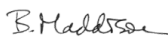
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Figure 3.7 – eDNA results from Pond 7 on site

Sample ID: ADAS-5573	Condition on Receipt: Good	Volume: Passed
Client Identifier: Pond 8, Brook Lodge	Description: pond water samples in preservative	
Date of Receipt: 27/06/2022	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	29/06/2022
Degradation Control [§]	Within Limits	Real Time PCR	29/06/2022
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	29/06/2022
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [¶]	4 of 4	Real Time PCR	As above for GCN



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Figure 3.8 – eDNA results from Pond 8 on site

Sample ID: ADAS-5572	Condition on Receipt: Good	Volume: Passed
Client Identifier: Pond 9, Brook Lodge	Description: pond water samples in preservative	
Date of Receipt: 27/06/2022	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	29/06/2022
Degradation Control [§]	Within Limits	Real Time PCR	29/06/2022
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	29/06/2022
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [¶]	4 of 4	Real Time PCR	As above for GCN


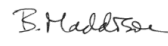
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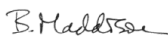
Figure 3.9 – eDNA results from Pond 9 on site

Brook Lodge, Trough Road, Scorton, PR3 1DH
eDNA Survey Results for GCN

Sample ID: ADAS-5571	Condition on Receipt: Good	Volume: Passed
Client Identifier: Pond 10, Brook Lodge	Description: pond water samples in preservative	
Date of Receipt: 27/06/2022	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	29/06/2022
Degradation Control [§]	Within Limits	Real Time PCR	29/06/2022
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	29/06/2022
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [¶]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

Signed:  Signed: 

Position: Director: Biotechnology Position: MD: Biotechnology



Date of preparation: 01/07/2022 Date of issue: 01/07/2022

Figure 3.10 – eDNA results from Pond 10 on site

Sample ID: ADAS-5570	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: Pond 11, Brook Lodge	Description: pond water samples in preservative	
Date of Receipt: 27/06/2022	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	29/06/2022
Degradation Control [§]	Within Limits	Real Time PCR	29/06/2022
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	29/06/2022
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [¶]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

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Position: Director: Biotechnology Position: MD: Biotechnology



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Figure 3.11 – eDNA results from Pond 11 on site

Sample ID: ADAS-5569	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: Pond 12, Brook Lodge	Description: pond water samples in preservative	
Date of Receipt: 27/06/2022	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	29/06/2022
Degradation Control [§]	Within Limits	Real Time PCR	29/06/2022
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	29/06/2022
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [¶]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

Signed:  Signed: 

Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 01/07/2022 Date of issue: 01/07/2022

Figure 3.12 – eDNA results from Pond 12 on site

4.0 Impact Assessment

- 4.1 Following the DNA analysis of samples taken from the twelve ponds on site, as described, the eDNA presence/absence test concluded a result of '**Negative**' for the presence of GCN in 11 of the ponds. The test for Pond 3 returned an '**Indeterminate**' result, with evidence of degradation or residual inhibition. This result was likely caused by the heavy overgrowth and presence of scum on the surface of the pond. However, given that the other 11 ponds on the site produced negative results, it is deemed highly improbable that Pond 3 could support any GCN.
- 4.2 It is thus concluded absence of GCN can be reasonably asserted at all of the ponds at Brook Lodge and that GCN do not form an ecological constraint against the proposals for which the applicant seeks consent.

5.0 Conclusions & Recommendations

- 5.1 No impacts are anticipated in relation to GCN as a result of the proposed works, therefore no further surveys, licensing or mitigation for this species is required.
- 5.2 With absence of GCN DNA recorded within the surveyed ponds, their presence in the landscape is unlikely, given the site-specific factors described in this report; impacts to this species through habitat loss / modification from the proposals can therefore be reasonably discounted.
- 5.3 Notwithstanding the above, RAMS for general amphibian species is recommended in the site associative Inspection & Assessment in Relation to Bats, Breeding Birds & GCN report due to other newt records in the immediate vicinity; any RAMS conditioned should act as a sufficient safeguard for herpetofauna. It is recommended that a detailed and bespoke working Method Statement should be applied outlining key reasonable avoidance measures (RAMS) and working protocols to be in effect during the proposed work schedule once confirmed. The recommended RAMs should be drafted by a suitably experienced and qualified GCN licenced Ecologist/Ecological Clerk of Works (ECoW). Upon gaining understanding of the details contained within the RAMS, the applicant and associated contract team would be expected to comply with the measures recommended within it.
- 5.4 Any RAM's drafted should be subject to the approval of the local planning authority – following approval, adherence to the terms of the RAM's would be a requirement of the named authority and the applicant would be the liable party, with the Ecologist/ECoW acting for and on behalf of the applicant.
- 5.5 The applicant and all contractors would be aware that if at any stage GCN are encountered during works, or at any other stage of the work programme, works would be required to immediately cease and the Ecologist/ECoW be made aware as to provide further guidance if an Ecologist is not already present.

6.0 Bibliography

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Appendix I: *Preliminary Ecological Appraisal*
(Tyrer Ecological Consultants Ltd, May 2022)

Appendix II: *Environmental DNA (eDNA) raw data* (Sample ID: ADAS-5562 – 5573; ADAS, July 2022)