

Great Crested Newt eDNA Survey

Twycross Zoo, Burton Road, Atherstone

A Report To: Baker Mallett LLP
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Quality Assurance

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Declaration of Compliance

This study has been undertaken in accordance with British Standard 42020:2013 “Biodiversity, Code of Practice for Planning and Development”. The information which we have prepared is true, and has been prepared and provided in accordance with the Chartered Institute of Ecology and Environmental Management’s Code of Professional Conduct. We confirm that the opinions expressed are our true and professional bona fide opinions.

Disclaimer

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Validity of Data

The findings of this study are valid for a period of two to four years, depending upon the nature of works proposed and the potential for these works to impact upon great crested newts. If works have not commenced within two years of this study, it may be necessary to undertake an updated survey to allow any changes in the status of great crested newts on site to be assessed, and to inform a review of the conclusions and recommendations made.

Non-Technical Summary

Project Background

In April 2024, Baker Mallett LLP commissioned Middlemarch to undertake an eDNA survey of a single pond (Pond P1) situated within the site of a proposed development at Twycross Zoo, Atherstone in Leicestershire. This assessment is required to inform a planning application associated with the demolition of the Lemur House, removal of several animal enclosures and clearance of trees and vegetation to enable the construction of a National Science Conservation Centre.

Middlemarch has previously carried out a range of ecological and arboricultural surveys and assessments for Baker Mallett LLP at this site in 2019, 2022 and 2023. This included a Great Crested Newt Habitat Suitability Index Assessment in 2023 (Report RT-MME-160474-05 Rev A) which recommended further survey effort to determine likely presence/absence of great crested newts within the vicinity of the proposed development area.

Scope of Survey

To fulfil the above brief, a Great Crested Newt eDNA survey of Pond P1 was carried out on 22nd April 2024 by Charline Hue MSc (Senior Ecological Consultant) and Thomas Weston BSc (Hons) (Ecological Project Officer).

Survey Results

The eDNA analysis of Pond P1 returned a negative result for great crested newts.

Potential Impacts on Great Crested Newts

Pond P1 is situated within the proposed development area and therefore has the potential to be directly and indirectly impacted by the proposed works. However, as the eDNA analysis of Pond P1 returned a negative result, it is considered that the potential impacts on great crested newt at this site is negligible.

Recommendations

Survey Data: If works have not commenced within two years, then an updated assessment should be carried out.

Unexpected Occurrence of Great Crested Newts: In the unlikely event that a great crested newt is discovered during any works at the site, activities should cease immediately, and a licensed great crested newt ecologist be contacted immediately for advice to determine an appropriate way forward.

Common amphibians: Vegetation clearance/removal should be undertaken in a sensitive manner to avoid harming common amphibians. If any are found during the course of site-based works, they should be removed carefully to a suitably undisturbed location within the near vicinity. This should be completed when these species are active (between March and October inclusive).

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1. Introduction

1.1 Project Background

In April 2024, Baker Mallett LLP commissioned Middlemarch to undertake an eDNA survey for great crested newts *Triturus cristatus* at the site of a proposed development at Twycross Zoo, Atherstone in Leicestershire. This assessment is required to inform a planning application associated with the demolition of the Lemur House, removal of several animal enclosures and clearance of trees and vegetation to make way for the construction of a National Science Conservation Centre.

Middlemarch has previously carried out a suite of surveys and assessments at this site, the details of which are detailed in the following reports:

- Preliminary Arboricultural Assessment, 2022, report RT-MME-159277-01;
- Preliminary Ecological Appraisal, 2022, report RT-MME-159277-04;
- Preliminary Bat Roost Assessment, 2022, report RT-MME-159277-05;
- Habitats Regulation Assessment Stage 1 Screening, 2023, report RT-MME-160474-01;
- Preliminary Ecological Appraisal, 2023, report RT-MME-160474-02 Rev A;
- Preliminary Bat Roost Assessment, 2023, report RT-MME-160474-03 Rev A;
- Biodiversity Metric Assessment, 2024, report RT-MME-160474-04; and,
- Habitat Suitability Index Assessment, 2023, report RT-MME-160474-05.

Middlemarch have also been commissioned to undertake additional surveys/assessments for Baker Mallett LLP for this project including Bat Surveys on buildings and trees, Biodiversity Metric Assessment and further Ecological and Arboricultural support.

Great crested newts are a legally protected species and are capable of being a material consideration in the planning process. A summary of the legislation protecting great crested newts is included within Appendix 1.

1.2 Site Description and Context

Table 1.1 provides a brief summary of the site and its surroundings.

Attribute	Description
Location	Twycross Zoo, Burton Road, Atherstone
National Grid Reference	SK 31559 06068
Site Area (ha)	2.19
Topography	Generally flat, slightly sloping to the south.
Land Cover (on site)	The survey area forms the south-west corner of the larger site of Twycross Zoo and a stretch of land immediately south of the current zoo boundary. Approximately one third of the site was composed of semi-improved grassland with scattered scrub. The remaining land comprised a mosaic of dense scrub, amenity grass, conifer plantation, broad-leaved woodland, scattered and groups of planted trees, hedgerow, ditch, pond, hardstanding paths and animal enclosures, mainly containing bark chippings and grasses.

Table 1.1: Summary of Site and Surroundings (continues)

Attribute	Description
Land Cover (site surrounds)	Twycross Zoo dominates the landscape to the north/north-east of the proposed development area with the wider area comprising intensively managed arable farmland dotted with small farmsteads and residential properties. The M42 lies around 3 km to the west.

Table 1.1 (continued): Summary of Site and Surroundings

1.3 Survey Area and Justification

Reference to Ordnance Survey mapped data indicates the potential presence of up to twenty-eight ponds within a 500 m radius of the site.

Only one pond (P1) was situated within the development site and has the potential to be directly impacted by proposals.

A large number of ponds occur within a 250 m radius of the proposed development area. However, these ponds are largely associated with Zoo enclosures, are manmade structures and subject to regular draining, filling and maintenance works. They are also often subject to use by aquatic birds, both native and exotic captive species, and are generally devoid of vegetation. Taking these factors into consideration, and previously 'Low' Habitat Suitability scores recorded during assessment in 2023, it was deemed appropriate to only sample Pond P1 for presence/absence of great crested newt DNA.

As there were limited few historical records of great crested newt within a 1 km radius of the site, the habitats between the on-site pond and the positive great crested newt pond comprise the Zoo Grounds consisting of highly managed animal enclosures, closely mown amenity grassland, formal landscaping and a series of buildings/structures served by a network of formal pathways, all of which are considered sub-optimal for use by amphibians. Between the Zoo Grounds and the positive great crested newt pond, land use is dominated by intensively managed arable farmland with the A444 (Burton-Nuneaton Road) between these two areas. It is therefore considered that the intervening habitats are likely to be less suitable as commuting habitats for amphibians.

The locations of all ponds in relation to the proposed development site are shown on Middlemarch Drawing C160474-05-01 in Chapter 7.

1.4 Documentation Provided

The conclusions and recommendations made in this report are based on information provided by the client regarding the scope of the project. Documentation made available by the client is listed in Table 1.2.

Document / Drawing Number	Author
Soft Landscape Strategy / TWZ-DZU-00-00-DR-L-00400-P05	Dearadh Zú
Tree Removal Plan / TWZ-DZU-00-00-DR-L-00103-P01	Dearadh Zú

Table 1.2: Documentation Provided by Client

2. Methods

2.1 Desk study

As part of the Preliminary Ecological Appraisal (Report RT-MME-159277-04) an ecological desk study was undertaken. The consultees for the desk study were:

- Natural England - MAGIC website for statutory conservation sites;
- Warwickshire Biological Records Centre; and,
- Leicestershire and Rutland Environmental Records Centre.

Middlemarch then assimilated and reviewed the desk study data provided by these organisations. Relevant newt data are discussed in Chapter 3. In compliance with the terms and conditions relating to its commercial use, the full desk study data is not provided within this report.

2.2. Field Survey

The survey comprised the following elements:

Site Suitability and Connectivity Assessment

As part of the Preliminary Ecological Appraisal (Report RT-MME-160474-02 Rev A) a Phase 1 Habitat Survey was undertaken of the proposed development site/works area. Middlemarch used this habitat survey data to undertake an assessment of the suitability of the proposed development site to support great crested newts. An assessment was also made of the habitat connectivity between ponds and the site, based on a review of habitat survey data (if available), aerial imagery and mapped sources.

eDNA Survey

When great crested newts inhabit a pond, they deposit traces of their DNA in the water as evidence of their presence. By sampling the water present within the ponds using a specific protocol, it is possible to analyse these small environmental traces in order to detect great crested newt presence. The protocol involves collecting 20 x 30 mL samples of water from a surveyed pond using strict protocols to prevent cross-contamination. These 20 samples are then combined and separated into 6 tubes, containing a preservative liquid that prevents the sample degrading in transit. Laboratory analysis is then carried out and involves pooling of the six samples to extract as much eDNA as possible and testing the pooled sample. A full description of the methodology can be found in the eDNA report from SureScreen Scientifics in Appendix 3.

The field assessment was undertaken on 22nd April 2024 by Charline Hue MSc (Senior Ecological Consultant and Natural England Great Crested Newt License Holder) and Thomas Weston BSc (Hons) (Ecological Project Officer) Table 2.2 details the weather conditions at the time of the assessment.

Parameter	Condition
Temperature (°C)	11
Cloud (%)	100%
Wind (Beaufort)	F1
Precipitation	Very light rain

Table 2.2: Weather Conditions During Field Survey

2.3 Constraints

No constraints were experienced during the field survey.

3. Desk Study

3.1 Species Records

The data search was carried out on 10th November by Leicestershire and Rutland Environmental Records Centre. Records of great crested newt within a 1 km radius of the survey area provided by the consultee are summarised in Table 3.1.

Date	Grid Reference	Proximity of Record	Additional Information
2021	N/A	640 m north-east	Two records.

Table 3.1: Great Crested Newt Records Within 1 km of the Development Boundary

3.2 Previous Great Crested Newt Surveys

Great Crested Newt surveys were conducted in 2021 on the land to the north of Twycross Zoo by Middlemarch. A small population of great crested newt was recorded to be breeding in the pond located to the north-east of Twycross Zoo, on the other side of Burton Road. This pond did not fall within a 500 m radius of the current survey area and is located 600 m north-east of it.

In September 2023, Middlemarch carried out great crested newt habitat suitability index assessments of the waterbodies within 250 m of the site and shown on Drawing C160474-05-01 in Chapter 7. The HSI scores for the surveyed ponds are presented in Table 3.2. Details of ponds are provided in Appendix 2.

Pond Ref.	HSI Category										HSI Score
	SI 1	SI 2	SI 3	SI 4	SI 5	SI 6	SI 7	SI 8	SI 9	SI 10	
P1	1	0.2	0.5	0.67	1	1	1	1	0.67	0.8	0.72 (Good)
P2	1	0.05	0.9	0.33	1	1	1	1	0.67	0.3	0.56 (Below average)
P3	1	0.05	0.9	0.33	1	1	1	1	0.33	0.3	0.56 (Below average)
P4	1	0.4	0.9	0.67	1	1	0.67	1	0.67	0.3	0.71 (Good)
P5	1	0.1	0.1	0.33	1	1	1	1	0.33	0.3	0.45 (Poor)
P6	1	0.2	0.9	0.01	1	0.01	1	1	0.33	0.3	0.27 (Poor)
P7	1	0.05	0.1	0.33	0.6	1	1	1	0.33	0.3	0.40 (Poor)

Table 3.2: Habitat Suitability Index of Ponds Within 250 m of Study Area (continues)

Pond Ref.	HSI Category										HSI Score
	SI 1	SI 2	SI 3	SI 4	SI 5	SI 6	SI 7	SI 8	SI 9	SI 10	
P8	1	0.05	1	0.33	0.7	0.67	1	1	0.33	0.3	0.49 (Poor)
P14	1	0.05	0.5	0.33	1	1	1	1	0.67	0.3	0.53 (Below average)
P16	1	0.9	0.9	0.33	1	0.67	1	1	0.67	0.3	0.72 (Good)
P17	1	0.8	0.9	0.33	1	0.67	1	1	0.67	0.3	0.71 (Good)
P18	1	0.8	0.9	0.33	1	0.67	1	1	0.67	0.3	0.71 (Good)
P19	1	0.9	0.9	0.33	1	0.67	1	1	0.67	0.3	0.72 (Good)
P22	1	0.4	0.1	0.33	1	1	1	1	0.33	0.3	0.51 (Below average)
P27	1	0.05	0.9	0.33	0.2	1	1	1	0.33	0.3	0.44 (Poor)
Key: SI 1 – Location SI 2 – Pond Area SI 3 – Pond Drying SI 4 – Water Quality SI 5 – Shade						SI 6 – Waterfowl SI 7 – Fish SI 8 – Ponds Within 1km SI 9 – Terrestrial Habitat SI 10 – Macrophytes					

Table 3.2 (continued): Habitat Suitability Index of Ponds Within 250 m of Study Area

4. Survey Results

4.1 Site Suitability and Connectivity Assessment

Although some suitable terrestrial habitat was present in the form of woodland, hedge, log piles, deadwood and rough grassland, most of the site is intensively managed as an attraction for the public which reduces its suitability and limits the connectivity between ponds within a 250 m radius.

4.2 eDNA Survey

A summary of the eDNA analysis results provided by SureScreen Scientifics is given in Table 4.2.

Pond Ref.	SIC	DC	IC	Result
P1	Pass	Pass	Pass	Negative
Key: SIC- Sample Integrity Check. Refers to quality of packaging, absence of tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to results errors. Inspection upon receipt of sample at the laboratory. To check if the sample is of adequate integrity when received. Pass or Fail. DC- Degradation Check. Analysis of the spiked DNA marker to see if there has been degradation of the kit since made in the laboratory to sampling to analysis. Pass or Fail. IC- Inhibition Check- PCR inhibitors can cause false results. Inhibitors are analysed to check the quality of the result. Every effort is made to clean the sample pre-analysis however some inhibitors cannot be extracted. An unacceptable inhibition check will cause an indeterminate sample and must be sampled again. Result- NEGATIVE means that GCN eDNA was not detected or is below the threshold detection level and the test result should be considered as no evidence of GCN presence. POSITIVE means that GCN eDNA was found at or above the threshold level and the presence of GCN at this location at the time of sampling or in the recent past is confirmed. Positive or Negative.				

Table 4.2: eDNA Analysis Results

5. Impact Assessment

5.1 Summary of Proposals

The proposals require the demolition of the Lemur House, removal of several animal enclosures and clearance of trees and vegetation to make way for the construction of a National Science Conservation Centre. Therefore, the permanent loss of the Lemur House, areas of amenity grassland, trees and hedgerow is anticipated, as well as permanent and temporary loss of grasslands and scrub. Pond P1 is not going to be directly impacted.

Construction Phase

The following activities are likely to be associated with the proposed development during the construction phase.

- Site clearance and ground preparation;
- Use and movement of heavy goods vehicles and machinery;
- Storage of plant, materials and waste; and,
- Presence and movement of site personnel.

Operational Phase

The following activities are likely to be associated with the operational phase of the proposed development.

- Permanent siting of buildings, structures and roads/paths;
- Frequent movement of vehicles;
- Use of lighting associated with roads and buildings;
- Presence and movement of site personnel; and,
- Maintenance of landscaping.

5.2 Summary of Key Features for Great Crested Newts

The desk study provided two records of great crested newt within a 1 km radius of the site. Both records were located in excess of 500 m from the proposed development area. These records relate to a pond which is separated from the proposed development area by intensively managed arable farmland and the highly managed zoo grounds.

A total of fifteen ponds were located within a 250 m radius of the development boundary. The Habitat Suitability Index assessment completed in 2023 (Report RT-MME-160474-05 Rev A) concluded that six of the ponds (including Pond P1) had 'Good' suitability to support great crested newts, four had 'Below Average' suitability and five had 'Poor' suitability.

Pond P1 had good suitability for great crested newts and there are records of great crested newts within 1 km radius of the development boundary. The connectivity between the positive great crested newt Pond P1 and the records of great crested newts is considered sub-optimal. The habitats within the zoo are intensively managed rendering them less suitable for use by great crested newts. Pond P1 is a further 285 m beyond the Zoo boundary and separated from it by the busy A444 (Burton/Nuneaton Road) and an extensive area of highly managed arable farmland. Pond P1 returned a negative result for the presence of great crested newt DNA.

The majority of the ponds assessed were contained within animal enclosures. Therefore, they are likely to be subject to frequent draining and re-filling as part of routine maintenance and maintaining animal health. They are known to be used by a variety of waterbirds, both native and exotic, including many predatory species. This would render them as being largely unsuitable for use by amphibians, potentially including great crested newts.

5.3 Potential Impacts on Great Crested Newts

None of the Ponds (P2 - P8, P14, P16, P17, P18, P19, P22 and P27) within 250 m of the site will be directly impacted. Due to their artificial nature, their lack of vegetation and/or low suitability score, these ponds were deemed to provide negligible habitat for great crested newts.

Taking all of the field evidence and information obtained for the proposed development area into account it is considered that there will be no potential impacts on great crested newts and the probability of injury or death of a great crested newt is negligible.

6. Recommendations

All recommendations provided in this section are based on Middlemarch's current understanding of the site proposals, correct at the time the report was compiled. Should the proposals alter, the conclusions and recommendations made in the report should be reviewed to ensure that they remain appropriate.

- R1 Survey Data:** The eDNA analysis revealed that great crested newts are not currently a material consideration in relation to the proposed development. If works have not commenced within two years from the date of this survey, then an updated assessment should be carried out.
- R2 Unexpected Occurrence of Great Crested Newts:** In the unlikely event that a great crested newt is discovered during any works at the site, activities should cease immediately, and a licensed great crested newt ecologist be contacted immediately for advice to determine an appropriate way forward.
- R3 Common amphibians:** Vegetation clearance/removal should be undertaken in a sensitive manner to avoid harming common amphibians. If any are found during the course of site-based works they should be removed carefully to a suitably undisturbed location within the near vicinity. This should be completed when these species are active. This is weather dependent but generally extends between March and October inclusive.

7. Drawings

Drawing C160474-05-01 – Pond Location Plan



- Legend**
- Site boundary
 - Pond proposed to survey
 - 250m from site boundary
 - 500m from site boundary

Project	Twycross Zoo	
Drawing	Pond Location Map	
Client	Baker Mallett c/o Twycross Zoo	
Drawing Number	C160474-05-01	Revision 00
Scale @ A3	1:5,000	Date September 2023
Approved By	CH	Drawn By VO/BD
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C160474-05-01

Appendix 1

Relevant Legislation

Great crested newts (GCN) and the places they use for shelter or protection receive legal protection under the Conservation of Habitats and Species Regulations 2017, (Habitats Regulations 2017) and the Conservation of Habitats and Species Regulations (Amendment) (EU Exit) Regulations 2019 (Habitats Regulations 2019). They receive further legal protection under the Wildlife and Countryside Act (WCA) 1981, as amended. This protection means that GCN, and the places they use for shelter or protection, are capable of being a material consideration in the planning process.

Regulation 41 of the Habitats Regulations 2017, states that a person commits an offence if they:

- deliberately capture, injure or kill a GCN;
- deliberately disturb GCN;
- deliberately take or destroy eggs of a GCN; or
- damage or destroy a GCN breeding site or resting place.

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

It is an offence under the Habitats Regulations 2017 for any person to have in his possession or control, to transport, to sell or exchange or to offer for sale, any live or dead GCN, part of a GCN or anything derived from GCN, which has been unlawfully taken from the wild. This legislation applies to all life stages of GCN.

Changes have been made to parts of the Habitats Regulations 2017 so that they operate effectively from 1st January 2021. The changes are made by the Habitats Regulations 2019, which transfer functions from the European Commission to the appropriate authorities in England and Wales.

All other processes or terms in the 2017 Regulations remain unchanged and existing guidance is still relevant.

The obligations of a competent authority in the 2017 Regulations for the protection of species do not change. A competent authority is a public body, statutory undertaker, minister or department of government, or anyone holding public office.

Whilst broadly similar to the above legislation, the WCA 1981 (as amended) differs in the following ways:

- Section 9(1) of the WCA makes it an offence to *intentionally* kill, injure or take any protected species.
- Section 9(4)(a) of the WCA makes it an offence to *intentionally or recklessly** damage or destroy, or *obstruct access to*, any structure or place which a protected species uses for shelter or protection.
- Section 9(4)(b) of the WCA makes it an offence to *intentionally or recklessly** disturb any protected species *while it is occupying a structure or place which it uses for shelter or protection*.

*Reckless offences were added by the Countryside and Rights of Way (CROW) Act 2000.

The reader should refer to the original legislation for the definitive interpretation.

GCN are listed a Species of Principal Importance for Nature Conservation in England which means they are a material consideration in the planning process. The list of species is derived from Section 41 list of the Natural Environmental and Rural Communities (NERC) Act 2006.

Appendix 2

Pond Descriptions and Assessment of Habitat Features - 2023 Data

Attribute	Description	
Pond P1		
Description	Small manmade pond near woodland.	
Grid Reference	SK 31523 06078	
Pond Area	100 m ²	
Permanence	Sometimes dries. Pond was dry at the time of the 2023 survey but contained water during first survey visit in 2022 and during the eDNA survey in 2024.	
Water Quality	Moderate, water was clear with some invertebrates present.	
Shade	50% shaded by woodland and scrub.	
Waterfowl	Absent.	
Pond Count	62	
Fish	Absent.	
Surrounding Terrestrial Habitat	Moderate suitability – immediate suitable terrestrial habitat included woodland, scrub, and rough grassland.	
Macrophytes	50% cover included bullrush, yellow flag iris, teasel, goat willow, great willowherb and redshank.	
Open Courtship Area	Open courtship areas are present as only 50% of the surface is covered by macrophytes.	
Egg Laying Habitat	Aquatic vegetation is present and submerged leaf litter may be suitable for egg laying.	

Attribute	Description
Pond P2	
Description	Small manmade pond in the west of the tiger enclosure, concrete base and no vegetation present in the water. 
Grid Reference	SK 31630 06106
Pond Area	35 m ²
Permanence	Never dries – normal water level.
Water Quality	Likely poor, moderate water clarity with no vegetation and no signs of invertebrates.
Shade	50% shaded by bamboo and concrete wall.
Waterfowl	Absent.
Pond Count	59
Fish	Absent.
Surrounding Terrestrial Habitat	Moderate suitability. Immediate suitable terrestrial habitat included rough grassland, scrub, logs and rocks.
Macrophytes	0%
Open Courtship Area	Open courtship areas were present (0% macrophyte cover).
Egg Laying Habitat	No aquatic vegetation present for egg laying.

Attribute	Description
Pond P3	
Description	Small manmade concrete pond with no vegetation. Concrete rocks forming edges of feature. 
Grid Reference	SK 31656 06220
Pond Area	8 m ²
Permanence	Never dries – low water level.
Water Quality	Poor – moderate clarity, leaf litter and a bit of scum present.
Shade	40% shaded by concrete and rock wall.
Waterfowl	Absent.
Pond Count	61
Fish	Absent.
Surrounding Terrestrial Habitat	Poor suitability. Short mown grassland, hardstanding and buildings, only a small amount of scrub in the immediate surroundings.
Macrophytes	0%
Open Courtship Area	Open courtship areas were present (0% macrophyte cover).
Egg Laying Habitat	No aquatic vegetation is present for egg laying.

Attribute	Description
Pond P4	
Description	Manmade pond with concrete bank on one side, concrete wall on opposite side and grassy banks on the other two sides. Shallow incline on one grassy bank, steeper (up to 45°) on the other. 
Grid Reference	SK 31599 06319
Pond Area	200 m ²
Permanence	Likely never dries. Medium water depth. Pump present to reduce water level when needed.
Water Quality	Moderate. Water appeared muddy but there was no scum on the surface and lots of dragonflies were present.
Shade	5%
Waterfowl	Absent
Pond Count	58
Fish	Possible
Surrounding Terrestrial Habitat	Moderate suitability. Amenity grassland, rough grassland, dead wood/logs, sand, trees and rocks.
Macrophytes	5% cover, comprising yellow flag iris <i>Iris pseudacorus</i> , rush <i>Juncus</i> sp. and water plantain <i>Alisma plantago-aquatica</i> .
Open Courtship Area	Open courtship areas were present.
Egg Laying Habitat	Water plantain and leaf litter were present.

Attribute	Description
Pond P5	
Description	Manmade concrete pond, nearly dry at time of survey. Margins comprised short, grazed grassland. 
Grid Reference	SK 31741 06256
Pond Area	60 m ²
Permanence	Dries annually – low water level.
Water Quality	Poor – clear water but no vegetation or sign of invertebrates.
Shade	20% shaded by a non-native ornamental tree.
Waterfowl	Absent
Pond Count	61
Fish	Absent
Surrounding Terrestrial Habitat	Poor suitability. Short mown grassland, hardstanding and buildings, only a small amount of scrub in the immediate surroundings.
Macrophytes	0%
Open Courtship Area	Open courtship areas were present (0% macrophyte cover).
Egg Laying Habitat	No aquatic vegetation is present for egg laying. Submerged leaf litter may provide suboptimal egg laying opportunities.

Attribute	Description
Pond P6	
Description	Small concrete manmade lined pond. Chlorinated and filtered (including sand filter). Surrounded by stones and rocks with a concrete wall all around the penguin enclosure. 
Grid Reference	SK 31778 06311
Pond Area	100 m ²
Permanence	Never dries – normal water level.
Water Quality	Excellent clarity but chlorinated and filtered.
Shade	50% shaded by trees including ash and Norway maple.
Waterfowl	Major. Pool houses the zoo collection of penguins and is also regularly visited by grey heron <i>Ardea cinerea</i> .
Pond Count	61
Fish	Absent.
Surrounding Terrestrial Habitat	Poor suitability. Immediate surrounding terrestrial habitat included amenity grassland and hardstanding, suitable habitat was limited and included rocks, scrub and trees. High walls surrounding the enclosure would limit access to the area by amphibians.
Macrophytes	0%
Open Courtship Area	Open courtship area was present (0% macrophyte cover).
Egg Laying Habitat	No suitable egg laying features present. Chlorinated water makes it unsuitable for GCN/amphibians to live in.

Attribute	Description
Pond P7	
Description	Small concrete manmade lined pond in former bush dog enclosure, dry at the time of survey, surrounded by hardstanding and building. 
Grid Reference	SK 31725 06395
Pond Area	8 m ²
Permanence	Dries annually, dry at the time of 2023 survey.
Water Quality	Likely poor.
Shade	80% shaded by trees and building.
Waterfowl	Absent.
Pond Count	58
Fish	Absent.
Surrounding Terrestrial Habitat	Poor suitability. Arable land further afield and amenity grassland within the zoo, immediate habitat included building and hardstanding which are unsuitable.
Macrophytes	0%
Open Courtship Area	Open courtship area was present (0% macrophyte cover).
Egg Laying Habitat	No aquatic vegetation present for egg laying but some leaf litter.

Attribute	Description
Pond P8	
Description	Small concrete manmade pond in bird enclosure, surrounded by small rocks then gravel beyond. 
Grid Reference	SK 31750 06383
Pond Area	8 m ²
Permanence	Likely rarely dries, water level was low.
Water Quality	Likely poor.
Shade	75% shaded by trees.
Waterfowl	Minor.
Pond Count	58
Fish	Absent.
Surrounding Terrestrial Habitat	Poor suitability. Arable land further afield and amenity grassland within the zoo, suitable immediate terrestrial habitat included scrub and ornamental trees.
Macrophytes	0%
Open Courtship Area	Open courtship areas were present (0% macrophyte cover).
Egg Laying Habitat	No aquatic vegetation present for egg laying but some leaf litter.

Attribute	Description
Pond P14	
Description	Small manmade pond in the east of the tiger enclosure, concrete base and no vegetation present in the water. 
Grid Reference	SK 31719 06137
Pond Area	15 m ²
Permanence	Likely sometimes dries – low water level.
Water Quality	Likely poor, moderate water clarity with no vegetation and no signs of invertebrates.
Shade	10% shaded by cherry <i>Prunus</i> sp.
Waterfowl	Absent.
Pond Count	60
Fish	Absent.
Surrounding Terrestrial Habitat	Moderate suitability. Immediate suitable terrestrial habitat included rough grassland, scrub, logs and rocks.
Macrophytes	0%
Open Courtship Area	Open courtship areas present (0% macrophyte cover).
Egg Laying Habitat	No aquatic vegetation present for egg laying.

Attribute	Description
Pond P16	
Description	Manmade concrete and lined pond, forming a moat within the gibbon enclosure. No vegetation present in the pond, gentle sloped short banks with no vegetation. Artificially fed. 
Grid Reference	SK 31828 06115
Pond Area	495 m ²
Permanence	Never dries – normal water level.
Water Quality	Likely poor, poor water clarity with green algae on the surface in areas, no vegetation and no signs of invertebrates.
Shade	20% shaded by building.
Waterfowl	Minor – mallard ducks and moorhen.
Pond Count	61
Fish	Absent.
Surrounding Terrestrial Habitat	Moderate suitability. Suitable immediate terrestrial habitat included rough grassland, scrub, logs and rocks.
Macrophytes	0%
Open Courtship Area	Open courtship areas present (0% macrophyte cover).
Egg Laying Habitat	No aquatic vegetation present for egg laying.

Attribute	Description
Pond P17	
Description	Manmade concrete and lined pond, forming moat within the gibbon enclosure. No vegetation present in the pond, gentle sloped short banks with no vegetation. Artificially fed, P12 feeds into P13 via manmade stream with rocky bottom and bullrush, willowherb and broadleaved dock. 
Grid Reference	SK 31873 06105
Pond Area	450 m ²
Permanence	Never dries – normal water level.
Water Quality	Likely poor, clear water but no vegetation and no signs of invertebrates.
Shade	20% shaded by building and scrub.
Waterfowl	Minor – mallard ducks and moorhen.
Pond Count	61
Fish	Absent.
Surrounding Terrestrial Habitat	Moderate suitability. Suitable immediate terrestrial habitat included rough grassland, scrub, logs and rocks.
Macrophytes	0%
Open Courtship Area	Open courtship areas present (0% macrophyte cover).
Egg Laying Habitat	No aquatic vegetation present for egg laying.

Attribute	Description
Pond P18	
Description	Manmade concrete and lined pond, forming a moat within the gibbon enclosure. No vegetation present in the pond, gentle sloped short banks with no vegetation. Artificially fed. 
Grid Reference	SK 31812 06077
Pond Area	450 m ²
Permanence	Never dries – normal water level.
Water Quality	Likely poor, clear water but no vegetation and no signs of invertebrates.
Shade	20% shaded by building.
Waterfowl	Minor – mallard ducks and moorhen.
Pond Count	61
Fish	Absent.
Surrounding Terrestrial Habitat	Moderate suitability. Suitable immediate terrestrial habitat included rough grassland, scrub, logs and rocks.
Macrophytes	0%
Open Courtship Area	Open courtship areas present (0% macrophyte cover).
Egg Laying Habitat	No aquatic vegetation present for egg laying.

Attribute	Description
Pond P19	
Description	Manmade concrete and lined pond, forming a moat within the gibbon enclosure. No vegetation present in the pond, gentle sloped short banks with no vegetation. Artificially fed. 
Grid Reference	SK 31873 06063
Pond Area	480 m ²
Permanence	Never dries – normal water level.
Water Quality	Likely poor, poor water clarity with green algae on the surface in areas, no vegetation and no signs of invertebrates.
Shade	10% shaded by building and scrub.
Waterfowl	Minor – mallard ducks and moorhen.
Pond Count	61
Fish	Absent.
Surrounding Terrestrial Habitat	Moderate suitability. Immediate suitable terrestrial habitat included rough grassland, scrub, logs and rocks.
Macrophytes	0%
Open Courtship Area	Open courtship areas present (0% macrophyte cover).
Egg Laying Habitat	No aquatic vegetation present for egg laying.

Attribute	Description
Pond P22	
Description	Pond being restored through the Countryside Stewardship Scheme at the time of 2023 survey. Pond was being cleared of debris, desilted, cleared of some scrub and dead wood. A 10 m buffer zone on either side will be implemented in the future, it will be fenced and naturally regenerated. Dry at time of survey, hedge laying is planned to be carried out on either side. 
Grid Reference	SK 31479 05862
Pond Area	200 m ²
Permanence	Dries annually – only holds water 5-6 months of the year according to the landowner.
Water Quality	Likely poor.
Shade	50% shaded by oak, ash, cherry, blackthorn and hawthorn.
Waterfowl	Absent.
Pond Count	64
Fish	Absent.
Surrounding Terrestrial Habitat	Poor suitability, surrounding habitat is mostly arable, suitable habitat included hedge.
Macrophytes	0%
Open Courtship Area	N/A – no water.
Egg Laying Habitat	No egg laying opportunities.

Attribute	Description
Pond P27	
Description	Small woodland pond, fed by rain water, very silty and shaded. 
Grid Reference	SK 31373 05880
Pond Area	25 m ²
Permanence	Likely never dries although the water level was low.
Water Quality	Poor – poor clarity and scum on the surface.
Shade	100% shaded by trees as pond was within a small woodland area, species included mature horse chestnut trees.
Waterfowl	Absent.
Pond Count	52
Fish	Absent.
Surrounding Terrestrial Habitat	Poor suitability. Large areas of arable within 250 m of the pond; immediate suitable terrestrial habitat included woodland, standing deadwood, rough grassland, scrub and logs.
Macrophytes	0%
Open Courtship Area	Open courtship areas were present (0% macrophyte cover).
Egg Laying Habitat	No aquatic vegetation is present for egg laying. Submerged leaf litter likely present as a suboptimal egg laying opportunity.

Table A2.1: Pond Descriptions and Assessment of Habitat Features for Great Crested Newts

Appendix 3

eDNA Survey Report

Folio No: 459-2024
Purchase Order: POR002542
Contact: Middlemarch
Environmental Ltd
Issue Date: 24.04.2024

GCN Report

Technical Report



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
2582	Twycross Zoo, P1	SK3151806078	Pass	Pass	Negative	0/12

Matters affecting result: none

Reported by: Daisy Chambers

Approved by: Christopher Troth



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.

