

GREAT CRESTED NEWT SURVEY REPORT

MAY 2025

North Manchester Golf Club

Manchester Old Road,
Middleton,
Manchester,
M24 4PE

U R B A N
G R E E N



QUALITY MANAGEMENT

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1 Executive Summary

- 1.1.1.1 Duchy Homes is proposing to develop land at the North Manchester Golf Club, Middleton (hereafter referred to as “the site”).
- 1.1.1.2 The proposals include the demolition of the existing clubhouse and a residential dwelling; the creation of a residential development comprising 56 dwellings; the erection of a replacement club house (including pro-shop and function room) and greenkeepers store; the relocation of the existing practice area; construction of a replacement car park; deforesting of woodland and the creation of a driving range and associated landscaping and infrastructure.
- 1.1.1.3 Urban Green has been appointed to complete a suite of great crested newt surveys of the ponds on site and within 250m, identified during the desk study phase.
- 1.1.1.4 Three ponds were found to hold water on the day of survey within 250m of the site and were subsequently subject to a great crested newt HSI and eDNA survey.
- 1.1.1.5 All ponds returned a negative eDNA result, indicating that great crested newt are likely absent from all three waterbodies.
- 1.1.1.6 It is recommended that the works proceed in a timely manner without the requirement for further surveys or ecological supervision in the context of ponds within 250m of the site and great crested newt.



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Legend: Red Line Boundary		0.85 Kilometers		U R B A N G R E E N A: Ground Floor, The Tower, Deva City Office Park, Trinity Way, Manchester M3 7BF T: +44 (0) 161 312 3131 weareurbangreen.co.uk
Client:	North Manchester Golf Club	Issue:	01	
Project:	North Manchester Golf Club	Scale @ A4	1:10,000	
Title:	Site Context	Approved by:	SB	Checked by: TM
Drawing Ref:	UG_284_SITE_CONTEXT	Author:	CL	Date: 05/06/2024



U R B A N
G R E E N

Legend

- Red Line Boundary
- 250m Buffer

Ponds

GCN eDNA results

- Negative
- Unsurveyed



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Client: North Manchester Golf Club		
Project: North Manchester Golf Club		
Title: Great Crested Newt Survey Results		
Issue: 01		Figure: 02
Drawn: CL	Checked: BG	Approved: BG
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Dwg No: UG_284_ECO_GCNR_01		Revision: 01

Figure 3. Photographs of the Site



Photograph 1: Pond P1 – HSI Score is average with a negative eDNA Result



Photograph 3: Pond P2 – Unsurveyed for a lack of water



Photograph 2: Pond P3 – HSI Score is excellent with a negative eDNA Result



Photograph 4: Pond P4 – HSI Score is average with a negative eDNA Result

2 Introduction

2.1 Scope

- 2.1.1.1 Urban Green has been instructed by Duchy Homes Ltd. to carry out great crested newt (*Triturus cristatus*) (GCN) surveys at North Manchester Golf Club in Middleton and produce our findings in a technical report.
- 2.1.1.2 The proposals include the demolition of the existing clubhouse and residential dwelling; the creation of a residential development comprising 56 dwellings; the erection of a replacement club house (including pro-shop and function room) and greenkeepers store; the relocation of the existing practice area; construction of a replacement car park; deforesting of woodland and the creation of a driving range and associated landscaping and infrastructure.

2.2 Site Context

- 2.2.1.1 The site is located at National Grid Reference SD 85884 05522 and comprises a total area of approximately 4.8ha (see Figure 1).
- 2.2.1.2 The site is located on the outskirts of a densely urban area of Middleton, approximately 7km north of the centre of Manchester. Alkrington Woods Nature Reserve is located immediately south of the site on the southern aspect of the A576. The M62 is present 1.7km north of the site and the M60 is present 500m south of the site. Residential housing bounds the site to the north, east, and southwest.

2.3 Purpose of this Report

- 2.3.1.1 This report has been produced to document the methods, results and conclusions of the GCN surveys. The objective of the assessment includes a desk study exercise and field survey of all waterbodies on site and within 250m, where accessible. The surveys include a Habitat Suitability Index (HSI) assessment and environmental DNA (eDNA) analysis of each waterbody.
- 2.3.1.2 The assessment will attempt to determine whether GCN are present or likely absent from the surveyed waterbodies and the suitability of each waterbody to support GCN.
- 2.3.1.3 Relevant UK legislation of the protection of GCN are detailed in Appendix 1.

3 Methods

3.1 General Approach

3.1.1 In order to accurately complete the GCN surveys, the survey effort was split into four stages:

1. Desk study exercise
2. Ground truthing
3. Habitat Suitability Index assessment
4. eDNA sampling

3.1.1.2 The 25th April 2025 was selected for the field survey element to be undertaken and was completed by Barnaby Gardner, Ecologist and Siobhan Gandee, Assistant Ecologist.

3.2 Stage 1 – Desk Study Exercise

3.2.1.1 The primary focus of the desk study exercise is to identify ponds that are suitable for GCN within a buffer zone proportionate to the scope of works. Moreover, biological information relevant to GCN is additionally collected to provide further context about this species within the local landscape.

3.2.1.2 Sources of information used in the desk study are presented in Table 1.

Table 1. – Desk Study Sources of Information

Source	Date Consulted	Information Sought
Online aerial imagery	27/05/2025	Review of satellite imagery.
MAGIC website (www.magic.gov.uk)	27/05/2025	Locations of granted EPSL within 5km of the site.
GMLRC – Greater Manchester Local Record Centre	10/05/2024	Records of GCN within 1km of the site boundary.

Defining an Appropriate Buffer Zone

3.2.1.3 Natural England's standing advice for GCN (2021) recommends a 500m standard radius for assessing potential impacts on GCN (particularly for large or high-impact developments). This radius is based on known dispersal distances and represents a conservative approach. However, it is commonly accepted that a 250m buffer zone is suitable for small-scale developments (Oldham *et al.*, 2000).

3.2.1.4 In the context of this development, a 250m buffer zone was selected and was deemed appropriate due to the small-scale, contained operations of the proposed works.

Waterbody Identification

- 3.2.1.5 In order to identify waterbodies within 250m of the site, aerial imagery and OS map data was reviewed to select waterbodies that were deemed suitable for survey.
- 3.2.1.6 Any waterbodies that were assessed as being separated from the site by the presence of significant dispersal barriers or (for example) being fast-moving waterbodies/stocked with fish screened out from further survey effort at this stage.

3.3 Stage 2 – Ground Truthing

- 3.3.1.1 During the field survey, surveyors conducted a ground truthing exercise which aimed to confirm the suitability of waterbodies identified during Stage 1.
- 3.3.1.2 Each waterbody was examined to assess its suitability for GCN and were screened out from further assessment where they met one or more of the following exclusion criteria:
 - Permanently infilled or exhibiting seasonal desiccation rendering them dry for the majority of the year;
 - Functioning as slurry storage lagoons associated with agricultural operations;
 - Artificial structures containing treated or chlorinated water, such as swimming pools;
 - Actively managed angling lakes or ponds known to be stocked with fish species;
 - Linear, flowing watercourses including streams, ditches, and rivers;
 - Standing open water bodies exceeding two hectares in surface area; or
 - Demonstrating saline characteristics incompatible with GCN ecology.
- 3.3.1.3 Where waterbodies were not able to be ruled out, they were scoped in for further assessment.

3.4 Stage 3 - Habitat Suitability Index Assessment

- 3.4.1.1 An HSI assessment was carried out for each waterbody scoped in (at Stage 2) for further survey.
- 3.4.1.2 The HSI assessment was undertaken to evaluate the suitability of aquatic and surrounding terrestrial habitat for GCN, following the standard methodology described in ARG UK Advice Note 5 (2010), based on Oldham et al. (2000).
- 3.4.1.3 The HSI provides a numerical index (ranging from 0 to 1), where values closer to 1 indicate that the habitat is more suitable for GCN. The assessment involved both field-based data collection and desk-based research and considered ten key habitat parameters:
 - SI1: Geographic location
 - SI2: Pond surface area
 - SI3: Pond permanence (likelihood of drying)
 - SI4: Water quality (based on invertebrate diversity and other indicators)
 - SI5: Extent of perimeter shading
 - SI6: Presence and impact of waterfowl
 - SI7: Presence and density of fish
 - SI8: Density of ponds within 1 km (excluding ponds separated by significant barriers)
 - SI9: Quality of surrounding terrestrial habitat

- **SI10:** Extent of aquatic macrophyte cover

3.4.1.4 Data for SI1 and SI8 were gathered through desktop review using 1:25,000 Ordnance Survey maps and aerial imagery. All other indices were determined through on-site observations during a field survey conducted in the active season for GCN (March to September).

3.4.1.5 Each habitat factor was scored individually following the guidance criteria. Scores were converted to a standard scale from 0.01 (highly unsuitable) to 1.0 (optimal) and combined using the geometric mean to calculate the final HSI value:

$$HSI = (SI1 \times SI2 \times SI3 \times SI4 \times SI5 \times SI6 \times SI7 \times SI8 \times SI9 \times SI10)^{1/10}$$

3.4.1.6 See Table 2 for a breakdown of different possible HSI scores.

3.4.1.7 Similarly, waterbodies that were dry at the time of survey, the water quality index was omitted; dry waterbodies or waterbodies supporting less than 5cm of water were also scoped out from Stage 4 (eDNA sampling) as a water sample could not feasibly be collected. All other waterbodies were scoped in for a Stage 4 assessment.

Table 2. HSI Scores

HSI Score	Pond Suitability
<0.5	Poor
0.5 – 0.59	Below average
0.6 – 0.69	Average
0.7 – 0.79	Good
>0.8	Excellent

3.4.1.8 Whilst a high HSI score suggests greater habitat suitability, it does not confirm GCN presence; therefore, the HSI assessment is used to inform the likelihood of presence and the need for further survey effort.

3.5 Stage 4 - eDNA Assessment

3.5.1.1 Waterbodies scoped in for further assessment at Stage 3 were subject to an eDNA assessment. To detect the presence of great crested newt, eDNA sampling was conducted following the standard protocol provided by SureScreen Scientifics. The sampling was performed during the strict survey window, between 15th April and 30th June, as instructed by Natural England.

3.5.1.2 To collect the samples a strict sampling procedure was followed at each waterbody to comply with Natural England's sampling methodology. The sampling procedure followed is detailed below:

1. **Site Selection:** Twenty evenly spaced sampling points were identified around the pond perimeter, including areas with vegetation and likely GCN occupancy.
2. **Sample Collection:**
 - Sterile gloves were worn throughout the process to prevent contamination.

- At each of the 20 points, a water sample was collected using a sterile ladle, reaching a minimum depth of 5–10 cm while avoiding sediment disturbance.
- Each sample was transferred into a sterile collection bag.

3. Sample Mixing:

- After collecting all 20 subsamples, the collection bag was sealed and shaken for 10 seconds to homogenise the sample.

4. Sample Preservation:

- Using a sterile pipette, 15 mL of the homogenized water was transferred into each of six 50mL tubes, pre-filled with GCN RNA and a preservative solution.
- Tubes were sealed tightly and shaken for 3 seconds to mix the contents.

5. Sample Submission:

- Tubes were placed in the provided rack and packaging.
- A completed sample submission form was included.
- Samples were stored in a refrigerator (if not immediately shipped) and sent to the laboratory for analysis within the recommended timeframe.

3.5.1.3 The laboratory analysis was conducted by SureScreen Scientifics, a provider with a consistent 100% pass rate in Natural England's proficiency testing from 2021 to 2024.

3.6 Constraints to the Survey

- 3.6.1.1 Where a lack of records is found during the desk search for a defined geographical area, it does not necessarily mean that there is a lack of ecological interest; the area may be simply under-recorded.
- 3.6.1.2 The conclusions and recommendations detailed in this report are based upon the site redline boundary and the development proposals as outlined by the client at the time of writing. Should there be any changes to the site redline boundary or development proposals at a later stage, this assessment should be reviewed to determine whether any amendments or additional survey work is required.

3.7 Lifespan for Report

- 3.7.1.1 In accordance with CIEEM's Advice Note on the Lifespan of Ecological Reports and Surveys (CIEEM, 2019), the details of this report will remain valid for a period of **18 months** from the date of the survey (i.e. until October 2026). After this date, this assessment should be reviewed to determine whether any update surveys are required.

4 Results

4.1 Desk Study Exercise

Stage 1

- 4.1.1.1 A total of nine waterbodies were identified within 250m of the site boundary during Stage 1 of the assessment. These were refined down to four ponds, based on suitability to support GCN as well as true proximity and connectivity to the site. Pond P5 was discounted as it is beyond 250m of the main construction site; it is still situated within 250m of minor vegetation clearance works in the north of the site, not assessed to be impactful concerning GCN. Ponds P6-9 were discounted as they are situated beyond a major road and are identifiably fish-stocked ponds.
- 4.1.1.2 To that end, ponds P5-9 were discounted and, therefore, not subject to any further survey effort.

Stage 2

- 4.1.1.3 During the field survey, pond P2 supported a low level of water. Water depth must be between 5cm to 10cm for a valid water sample to be collected. Pond P2 did not meet this minimum threshold and therefore was ruled out from further survey.
- 4.1.1.4 Therefore, a total of three ponds (P1, P3, P4) were subject to full HSI and eDNA assessment within 250m of the site.

Biological Records

- 4.1.1.5 No records of GCN were returned in a data search undertaken as part of the Preliminary Ecological Appraisal (Urban Green, 2024).
- 4.1.1.6 A MAGIC Map search returned no granted EPSL relating to GCN present within 1km of the site.

4.2 Habitat Suitability Index

- 4.2.1.1 A total of three ponds were subject to an HSI assessment, the results of which are detailed in Table 3.
- 4.2.1.2 All three ponds shared similar characteristics: moderate-large ponds with shallow, gradual slopes, vegetated embankments (partially bordered by woodland) and a good level of water in the basin of the ditch. Water quality was assessed as poor; all three were highly turbid and visibly polluted, including litter debris. Fish were assessed as possible and waterfowl presence as minor. The ponds received moderate-to-high shading from surrounding woodlands. Though macrophyte cover was relatively sparse throughout the length of both ditches, with only minor amounts of submerged vegetation.

Table 3. HSI Results

Pond Reference	HSI Score	Pond Suitability	Grid Reference
P1	0.62	Average	SD 85664 05701
P3	0.67	Average	SD 85817 05674
P4	0.66	Average	SD 85924 05713

4.3 eDNA Assessment

- 4.3.1.1 The same three ponds were also subject to eDNA analysis, all of which returned negative results for GCN DNA, which is equivalent to 'likely absence' of GCN from these waterbodies.

Table 4. eDNA Results

Pond Reference	eDNA Result
P1	Negative
P3	Negative
P4	Negative

5 Ecological Assessment & Recommendations

5.1 Overview

- 5.1.1.1 All three ponds returned a negative eDNA result, indicating that great crested newt are likely absent from these waterbodies. Moreover, all waterbodies identified within 250m of the site boundary are either unsuitable for great crested newt or are, by association with the eDNA survey results, are unlikely to support breeding great crested newt.
- 5.1.1.2 It is recommended that the works proceed in a timely manner without the requirement for further surveys or ecological supervision in the context of ponds within 250m of the site and great crested newt.

6 References

Amphibian and Reptile Groups of the United Kingdom (2010). ARG UK ADVICE NOTE 5. Great Crested Newt Habitat Suitability Index.

CIEEM (2019). Advice Note on the Lifespan of Ecological Reports and Surveys. CIEEM.

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

Urban Green (2024). Preliminary Ecological Appraisal. UG_284_ECO_PEA_04

Appendix 1 – Relevant Legislation

Great Crested Newt

Great crested newt are designated as a European Protected Species and their resting places are protected under the Wildlife and Countryside Act 1981 (as amended), the Countryside and Rights of Way (CROW) Act 2000, and the Conservation of Habitats and Species Regulations 2017.

The Conservation of Habitats and Species Regulations 2017 transpose the European Union's 'Habitats Directive' (Council Directive 92/43/EEC) on the Conservation of Natural Habitats and of Wild Fauna and Flora (EC Habitats Directive) into UK law. The Regulations provide for the designation and protection of 'European Sites', the protection of 'European Protected Species' (EPS), and the adaptation of planning and other controls for the protection of European Sites. EPS are listed on Schedule 2 of the Conservation of Habitats and Species Regulations 2017.

Under the Wildlife and Countryside Act 1981 (as amended) it is an offence to:

- Intentionally kill, injure or take certain animals listed in Schedule 5;
- Intentionally or recklessly damage or destroy any structure or place which any wild animal specified in Schedule 5 uses for shelter or protection;
- Intentionally or recklessly disturb any such animal while it is occupying a structure or place which it uses for shelter or protection; or
- Intentionally or recklessly obstruct access to any structure or place which any such animal uses for shelter or protection.

In addition, under this legislation there are offences relating to sale, possession and control of wild animals listed in Schedule 5.

Under the Conservation of Habitats and Species Regulations 2017 it is an offence to:

- Deliberately capture, injure or kill any wild animal listed as a European Protected Species;
- Deliberately disturb wild animals of any such species in such a way as to be 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.
- Deliberately take or destroy the eggs of such an animal, or;
- Damage or destroy a breeding site or resting place of such an animal.

In addition, under this legislation there are offences relating to possession, control sale and exchange of an EPS.

Great crested newt are listed as a SoPI under Section 41 of the Natural Environment and Rural Communities (NERC) Act 2006.

Appendix 2 - eDNA Laboratory Results

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Issue Date: 28.05.2025
Received Date: 14.05.2025

GCN Report

Technical Report



SureScreen Scientifics

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Purchase Order: UG1516
Contact: Urban Green
Issue Date: 28.05.2025
Received Date: 14.05.2025

GCN eDNA Analysis

Summary

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

Results

Lab ID	Site Name	OS Reference	Degradation Check	Inhibition Check	Result	Positive Replicates
GCN25 0993	North Manchester Golf - P4	SD 85924 05713	Pass	Pass	Negative	0/12

Matters affecting result: none

Reported by: Amy Bermudez

Approved by: Lauryn Jewkes

Methodology

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

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

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

Interpretation of Results

Sample Integrity Check:

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

Degradation Check:

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

Inhibition Check:

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

Result:

Presence of GCN eDNA (Positive/Negative/Inconclusive)

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

Positive Replicates: Number of positive qPCR replicates out of a series of 12. If one or more of these are found to be positive the pond is declared positive for GCN presence. It may be assumed that small fractions of positive analyses suggest low level presence, but this cannot currently be used for population studies. In accordance with the WC1067 Natural England protocol, even a score of 1/12 is declared positive. 0/12 indicates negative GCN presence.

Negative: GCN eDNA was not detected or is below the threshold detection level and the test result should be considered as evidence of GCN absence, however, does not exclude the potential for GCN presence below the limit of detection.

Inconclusive: Controls indicate inhibition or degradation of the sample, resulting in the inability to provide conclusive evidence for GCN presence or absence.



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Technical Report



SureScreen Scientifics

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GCN eDNA Analysis

Summary

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

Results

Lab ID	Site Name	OS Reference	Degradation Check	Inhibition Check	Result	Positive Replicates
R25 0345	North Manchester Golf - P1	SD 85664 05701	Pass	Pass	Negative	0/12

Matters affecting result: none

Reported by: Amy Bermudez

Approved by: Consuela Sopronyi

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.



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GCN Report

Technical Report



SureScreen Scientifics

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GCN eDNA Analysis

Summary

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

Results

Lab ID	Site Name	OS Reference	Degradation Check	Inhibition Check	Result	Positive Replicates
R25 0348	North Manchester Golf - P3	SD 85817 05674	Pass	Pass	Negative	0/12

Matters affecting result: none

Reported by: Amy Bermudez

Approved by: Lauryn Jewkes

Methodology

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

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

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

Interpretation of Results

Sample Integrity Check:

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

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.

