Loddon Garden Village

Technical Appendix 11.9 – Great Crested Newt

Prepared on behalf of

University of Reading

Final Report

10 September 2025

23/42-13C

Loddon Garden Village

Technical Appendix 11.9 – Great Crested Newt

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Loddon Garden Village

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1. INTRODUCTION

Scope

1.1 This Technical Appendix supports **Chapter 11 (Biodiversity)** of the Environmental Statement (ES). It sets out the detailed methodologies and results of the survey work undertaken to inform:

- The baseline evaluation of the Great Crested Newt (GCN) Triturus cristatus population supported by the Zone of Influence of the Proposed Development;
- The assessment of likely impacts on the Great Crested Newt population;
- The design of impact avoidance and mitigation measures; and
- The design of biodiversity enhancements for Great Crested Newt.

Site and Development Description

1.2 The Site is a large area of land to the west of Wokingham, between the villages of Shinfield, Arborfield and Sindlesham. It is located outside of the Green Belt and is largely made up of agricultural land and grasslands, with pockets of woodland and the River Loddon running through the centre of the Site.

1.3 The description of development for the application is as follows:

"Application for the phased development of a new community at Loddon Garden Village, comprising, in outline:

- *up to 2,800 residential units to include up to 100 custom and self-build plots;*
- *2 primary schools (up to 3 forms of entry) to include early years provision and 1 secondary school (up to 12 forms of entry);*
- *one District Centre, to incorporate up to 11,000m2 of Class E (Commercial, business and Service, to include a food store of around 2,500m2), and Class F (Local Community and Learning);*
- *one Local Centre; to incorporate up to 2,400m2 of Class E;*
- *a Sports Hub to include sports pitches and pavilion space;*
- *up to 4,250m2 of further Class E, Class F, and sui generis development to include commercial, health care and public house;*
- *comprehensive green infrastructure including a Country Park, landscaping and public open space, and ecological enhancement measures;*
- *20 gypsy and traveller pitches;*

- *comprehensive drainage and flood alleviation measures to include Sustainable Urban Drainage Systems (SUDS) and engineering measures within Loddon Valley for the River Loddon;*
- *internal road network including spine road with pedestrian and cycle connections and associated supporting infrastructure;*
- *new and modified public rights of way;*
- *associated utilities, infrastructure, and engineering works, including the undergrounding of overhead lines;*
- *Ground reprofiling to accommodate infrastructure, flood alleviation and development parcels;*
- *Up to 0.5ha of land adjoining St Bartholomew's church for use as cemetery;*
- *Electricity substation (up to 1.5ha).*

All matters reserved other than access, incorporating:

- *a new pedestrian, cycle and vehicular access to Lower Earley Way via a new 4th arm to the Meldreth Way roundabout;*
- *a new pedestrian, cycle and vehicular bridge over the M4;*
- *a new pedestrian, cycle and vehicular bridge over the River Loddon;*
- *a new vehicular access to the A327 Reading Road, via a new arm to the Observer Way roundabout;*
- *a new pedestrian, cycle and vehicular access to Thames Valley Science Park;*
- *an initial phase of internal roads with associated drainage, landscape and engineering works and ground reprofiling, between the A327 and the south eastern boundary of the site.*

Application includes full permission for the change of use of 40.4 hectares of agricultural land to Suitable Alternative Natural Greenspace (SANG), 18.35 hectares of SANG link, and provision of Biodiversity Net Gain measures, the demolition and clearance of 20,809 m2 of buildings and structures at the Centre for Dairy Research (CEDAR) and at Hall Farm, the demolition of 3 existing dwellings on Carter's Hill Lane, and the retention of specified buildings at Hall Farm."

Policy and Legislative Context

Legislation

1.4 Full details of the legislation of relevance to ecology and nature conservation are included in **Appendix 11.1**, however those of particular relevance to GCN are summarised below.

1.5 GCN are legally protected under Schedule 5 of the Wildlife and Countryside Act 1981 (as amended), which protect them from intentional killing, injuring or taking, as well as possession

and trade. They are also a European Protected Species under the Conservation of Habitats and Species Regulations 2017 (as amended), which means that places used for shelter and protection are also safeguarded against intentional or reckless damage, destruction and obstruction of access and disturbance to animals occupying those places. Collectively, this legislation makes it an offence to:

- Intentionally or deliberately kill, injure or capture Great Crested Newts;
- Intentionally, deliberately or recklessly disturb Great Crested Newts in such a way as to be likely to significantly affect the ability of any significant group of Newts to survive, breed, or rear or nurture their young or the local distribution of or abundance the species;
- Intentionally or recklessly damage, destroy or obstruct any place used by Great Crested Newts for shelter or protection, or intentionally or recklessly disturb a Great Crested Newt whilst it is occupying such a place;
- Damage or destroy a breeding site or resting place of a Great Crested Newt; and
- Possess, sell or transport a Great Crested Newt, or anything derived from it.

1.6 GCN are also listed as a Species of Principal Importance in England under S41 of the Natural Environment and Rural Communities (NERC) Act 2006 (as amended).

Planning Policies and Biodiversity Strategies

1.7 Full details of the planning policy of relevance to ecology and nature conservation are included in **Appendix 11.1,** however those of particular relevance to freshwater fish are summarised below.

National Planning Policy Framework

1.8 The National Planning Policy Framework (NPPF) (2024) sets out the Government's planning policies for England and how they should be applied. With regard to protecting the natural environment, Section 15 of the NPPF requires that planning decisions should enhance the natural environment and provide net gains for biodiversity.

Local Planning Policy

1.9 The Wokingham Borough Council Adopted Core Strategy: Development Plan Document (January 2010) sets out the framework for the development of the borough, through a series of policies and strategies. Of particular relevance to Badgers is Policy CP7 – Biodiversity.

1.10 The Wokingham Borough Local Plan Update 2023-2040 was submitted to the Secretary of State for examination by an independent Planning Inspector in February 2025. Whilst not currently enforced, consideration has been given to these emerging policies during the course of the impact assessment, and design of mitigation, compensation and enhancement strategies.

Berkshire Local Nature Recovery Strategy

1.11 The draft Berkshire Local Nature Recovery Strategy was published in February 2025, with finalisation of the strategy anticipated in the summer of 2025. Formed as a requirement of The Environment Act 2021, Local Nature Recovery Strategies aim to identify priority actions for local biodiversity, including habitat and species, to create a collaborative landscape level approach

to nature restoration. GCN are included within the draft species list (Royal Borough of Windsor and Maidenhead, 2025).

2. SURVEY AND ASSESSMENT METHODOLOGY

2.1 The approach to ecological impact assessment taken in this report is in line with guidance from the Chartered Institute of Ecology and Environmental Management Guidelines for Ecological Impact Assessment (CIEEM, 2018), as set out in **Appendix 11.2**.

Defining the Zone of Influence

2.2 The area over which the activities as associated with the Proposed Development are considered to potentially affect the GCN population, the Zone of Influence (ZoI), has been predicted by considering the activities and resultant biophysical changes arising during the construction and operational phases, as summarised below.

Likely Biophysical Changes

2.3 The predicted biophysical changes of relevance to the GCN population are as follows:

Activities and Resultant Biophysical Changes During the Construction Phase

- Dust generation and environmental incidents (e.g. spillages and pollution incidents) reducing suitability of habitats, impacting upon the health and survivability of GCN; and
- Vegetation/habitat clearance which may injure and/or kill individuals, disturb and/or destroy resting places, cause the loss of foraging/supporting habitats and result in habitat fragmentation.

Activities and Resultant Biophysical Changes During the Operational Phase

- Increased levels of public access which may lead to increased levels of disturbance from people and dogs;
- Increased presence of pets, increasing predation risk for GCN;
- Changes in water quality arising from run-off impacting upon the health and survivability of breeding GCN and their eggs;
- Creation of new waterbodies increasing the permeability of the local landscape for GCN; and
- Implementation of habitat management plans resulting in the enhancement of existing habitats for GCN.

2.4 GCN will use terrestrial habitats within a 250m radius of a waterbody, and potentially up to 500m where the habitats are optimal (English Nature, (now Natural England), 2001). Therefore, GCN in any waterbody within 500m of the Site, and not isolated by barriers to movement could be impacted by development in the absence of mitigation. With this in mind, the potential ZoI that has been considered within this Appendix includes all suitable GCN terrestrial habitat and ponds within 250m of the boundary of the Site (and are not constrained by major barriers).

Desktop Study Methodology

2.5 A biological records search was commissioned from Thames Valley Environmental Records Centre (TVERC) in July 2024, in order to obtain existing records of GCN within a 2km radius of the Site, thereby incorporating the potential ZoI and providing context with other GCN populations in the local area.

2.6 A review was undertaken of class licence returns, GCN surveys for district licencing and granted European Protected Species applications available on the Mutli-Agency Geographic Information for the Countryside (MAGIC).

2.7 The Impact Risk Zone maps used by NatureSpace Partnership as the operator of Wokingham Borough Councils District Licence, were reviewed to consider the likely presence of GCN based on habitat suitability. Habitat suitability is modelled to designate land within one of the following risk zones:

- Red – highly suitable habitat – the most important areas for great crested newts;
- Amber – suitable habitat – great crested newts are likely to be present;
- Green – moderate habitat suitability – great crested newts may be present; and
- White – low habitat suitability – low probability of great crested newt presence.

2.8 Ordnance survey maps and aerial imagery were used identify waterbodies within a 250m radius of the Site boundary.

Field Survey Methodology

2.9 Habitat Suitability Index (HSI) assessment is a means by which to assess the quantity and quality of freshwater habitat to support GCN. For each waterbody, scores are allocated to describe the quality of 10 habitat features. These figures are then entered into a calculation, producing an overall index between 0 and 1. HSI values close to 0 indicate unsuitable and those nearer to 1 optimal habitat conditions for GCN. Categories ranging from poor (HSI <0.5) to excellent (HSI >0.8) have been defined to assign approximate waterbody suitability from an overall HSI score.

2.10 To compliment the HSI assessment, water samples were collected to test for environmental DNA (eDNA) from all accessible waterbodies which contained water at the time of sampling.

2.11 HSI surveys were undertaken in early April 2022, with eDNA sampling subsequently undertaken on 20th April 2022 both by Katie Cammack BSc (Hons) MSc MCIEEM (2018-33028-CLS-CLS). The surveys were updated on 18th June 2024 by Siobhan Pryke BSc (Hons) (2022-10577-CL08-GCN). The survey dates therefore fall within Natural England's approved eDNA sampling window of 15th April – 30th June.

2.12 eDNA sampled were analysed by SureScreen Scientifics Ltd.

Survey Limitations and Constraints

2.13 A number of waterbodies (Waterbodies 1-2, 14-24) are located within the potential ZoI but outside of the Site boundary. At the time of the surveys 2022, access had not been sought to survey these additional waterbodies and they were therefore excluded from the survey scope.

2.14 Using OS maps and the Land Registry, land ownership of waterbodies outside of the Site boundary was established. For the update surveys undertaken in 2024, letters to request access to for surveying were sent on 2nd May 2024. Letters were sent to the following addresses:

- Arborfield Grange
- Betty Farm
- Shinfield Grange
- Willow Farm
- Arborfield and Barkham Churches Office

2.15 No responses were received from Betty Farm or Willow Farm and access was denied to Shinfield Grange. Although initial contact was received regarding information sharing for at least one of the ponds surrounding Arborfield Grange/ Arborfield and Barkham Churches Office no further information could be obtained.

2.16 Not all waterbodies will be mapped on OS maps, or visible on aerial imagery. For example, garden ponds, or ephemeral waterbodies are unlikely to be identified outside of the Site boundary. Whilst all efforts have been made to identify waterbodies within the ZoI, it is acknowledged there will be additional waterbodies that have not been considered within this Appendix.

Evaluation Methodology

2.17 The suitability of GCN habitat was assessed following Oldham et al. (2000) and populations can be evaluated following the Great Crested Newt Mitigation Guidelines (English Nature, 2001).

2.18 The evaluation of the GCN population has been undertaken in accordance with the Guidelines for Ecological Impact Assessment in the UK and Ireland: Terrestrial, Freshwater and Marine (CIEEM, 2018).

3. ECOLOGICAL BASELINE

Desktop Study

Records

3.1 Records from TVERC showed substantial numbers of records of GCN to the north-east of the Site around Winnersh and Dinton Pastures Country Park, approximately 1.5km to the nearest record in this cluster.

3.2 The closest EPS licence return was located approximately 2km to the north-east of the Site, with the latest licence granted in 2016.

3.3 The closest record of GCN to the Site was at Nirvana Spa, located approximately 650m to the north-east.

3.4 No pond surveyed to inform district licencing within close proximity to the Site was found to support GCN, although a number of these ponds were considered to provide 'optimal' habitats. The closest pond within which GCN were confirmed as present during the course of these surveys was located near Bill Hill, approximately 4.2km to the north-east.

3.5 Wokingham Borough Council have advised that they are aware of a breeding population of GCN in the ponds surrounding Arborfield Grange/ Arborfield and Barkham Churches Office. During initial contact to discuss access it was confirmed that long-term monitoring of the ponds was taking place.

3.6 Furthermore, the Impact Risk Zone map used by NatureSpace Partnership as the operator of Wokingham Borough Councils District Licence designates the area surrounding the Arborfield Grange/Arborfield and Barkham Churches Office waterbodies as being within a 'red' impact zone, indicating the areas hosts highly suitable habitat for GCN. The remainder of the Site largely lies within a 'green' impact zone whilst limited areas of the Site are included within either 'amber' or 'white' zones.

Waterbodies

3.7 A total of 25 waterbodies were identified within a 250m radius of the Site (**Map 11.9.1**).

3.8 In the local landscape the M4 motorway and the River Loddon can both be considered to be significant barriers to movement.

Field Survey

2022 Survey

3.9 Twenty-five waterbodies were identified within the ZoI, as shown on **Map 11.9.1**. Of these, 12 (Waterbodies 3-13 & 28) fall within the Site boundary and could be accessed for surveys. Waterbody 28, though accessible, was dry at the time of assessment.

3.10 Eleven waterbodies (Waterbodies 3-13) were therefore subject to HSI assessment. HSI scores are shown on **Map 11.9.2** and detailed in **Table 3.1** below.

Table 3.1: 2022 *HSI scores*

Waterbody ID	HSI score	Pond Suitability
3	0.57	Below Average
4	0.32	Poor
5	0.48	Poor
6	0.66	Average
7	0.37	Poor
8	0.34	Poor
9	0.58	Below Average
10	0.34	Poor
11	0.67	Average
12	0.34	Poor
13	0.65	Average

3.11 Of the 11 waterbodies previously surveyed, four waterbodies (Waterbodies 4, 7, 10 and 12) had dried out by the time eDNA sampling commenced. As a result, 7 waterbodies (Waterbodies 3, 5, 6, 8, 9, 11 and 13) were subject to eDNA analysis. All tested negative for the presence of GCN (**Map 11.9.2**). Full results can be found in **Annex 1.**

2024 Survey

3.12 All waterbodies previously subject to a HSI assessment we reassessed during the update surveys. In addition Pond 1 was surveyed from a public road, and Pond 2 was surveyed from at a distance from a public footpath. The results of the updated HSI assessments can be found at **Table 3.2** below.

Table 3.2. 2024 HSI scores and change

Waterbody ID	HSI score	Pond Suitability	Change in suitability since 2022
1	0.44	Poor	n/a
2	0.68	Average	n/a
3	0.70	Good	+
4	0.32	Poor	=
5	0.48	Poor	=
6	0.77	Good	+
7	0.47	Poor	=
8	0.42	Poor	=
9	0.69	Average	+
10	0.34	Poor	=
11	0.67	Average	=
12	0.34	Poor	=
13	0.57	Below Average	-

3.13 Of the 13 waterbodies surveyed, seven were subject to eDNA analysis. All tested negative for the presence of GCN. The full results can be found in **Annex 2.**

4. EVALUATION

4.1 No evidence of GCN was found within waterbodies on the Site, and as a result GCN are considered likely absent from waterbodies.

4.2 However, in the absence of current survey data, on a precautionary basis, for the purposes of this impact assessment GCN are considered as likely present within Waterbodies 14, 15 and 16. There is suitable on-Site terrestrial habitats within 250m of these waterbodies which is considered likely to support during the terrestrial phases of their lifecycle. GCN may therefore be present within terrestrial habitats on the Site.

4.3 In the absence of a population assessment, it is difficult to accurately determine the level of importance which can be attributed to the off-site GCN population. However, given the lack of optimal GCN habitats on-Site, and the lack of connecting waterbodies to the wider landscape, GCN are considered to be of at least **Local Importance**.

5. REFERENCES

CIEEM (2018) Guidelines for Ecological Impact Assessment in the UK and Ireland: Terrestrial, Freshwater, Coastal and Marine version 1.3 updated September 2024. Chartered Institute of Ecology and Environmental Management, Ampfield.

English Nature (2001). Great Crested Newt Mitigation Guidelines. English Nature: Peterborough.

Oldham, R. S., Keeble, J., Swan, M. J. S. and Jeffcote, M. (2000). Evaluating the suitability of habitat for the great crested newt (Triturus cristatus). Herpetological Journal. 10, pp143-155.

Royal Borough of Windsor and Maidenhead (2025). Berkshire Nature Recovery: Berkshire Local Nature Recover Strategy 'Species Priorities List', Draft version 5th February 2025.

MAP 11.9.1 Ponds Identified within Zone of Influence

KEY

- Site boundary
- 250m linear distance from site boundary
- Ponds

SCALE: 1:12,500 at A3

0 100 200 300 400 500 Metres

N

EPR

CLIENT: University of Reading

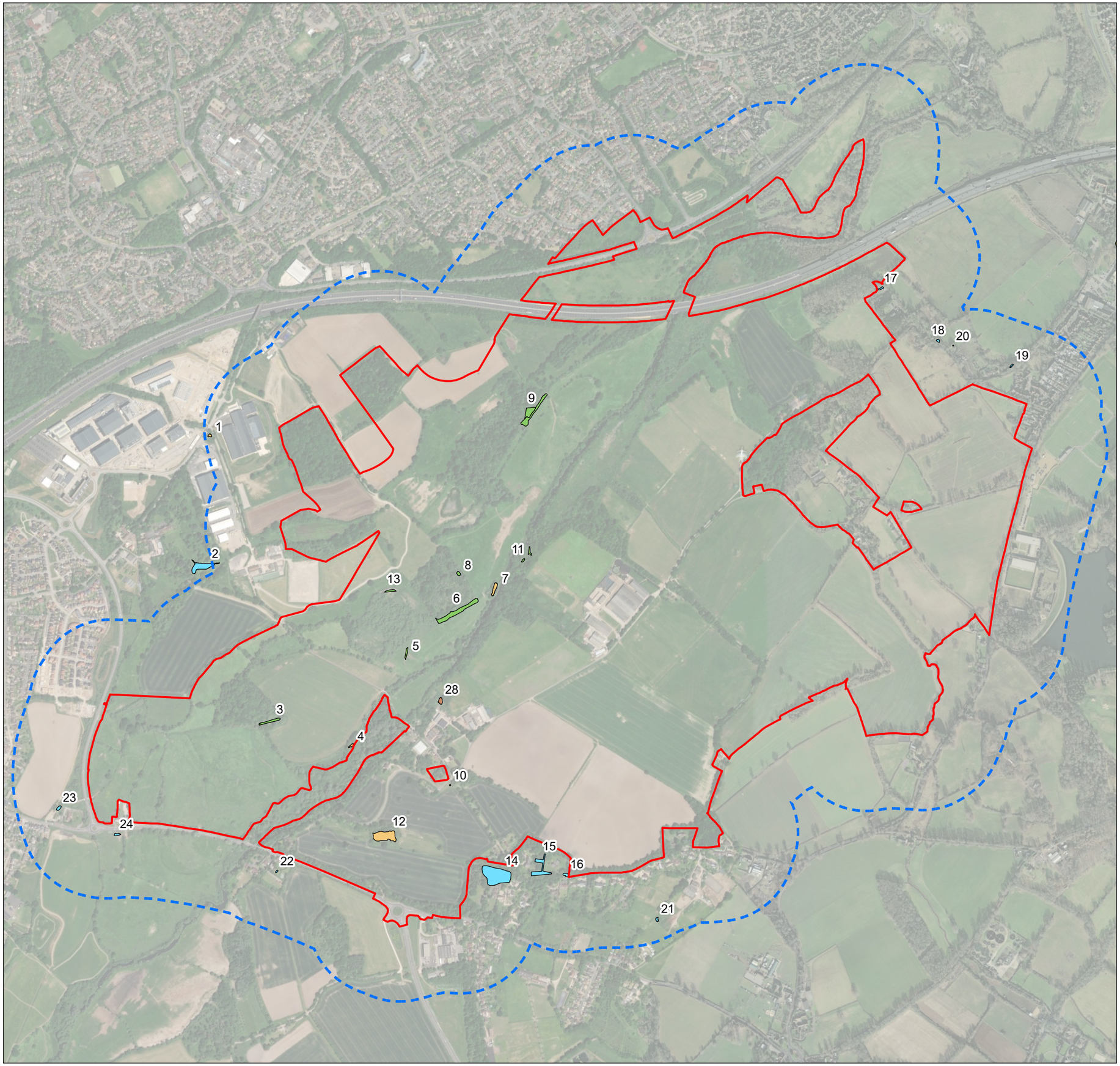
PROJECT: Loddon Garden Village

DATE: 01 August 2025

Y:\Loddon Garden Village, Shinfield 2201\GIS\Planning Application\Technical Appendices\GCN\Map11.9.1_ZOI_Ponds_P2342_3965_010825.aprx

P23/42

Aerial Image Source: Esri, Maxar, Earthstar Geographics, and the GIS User Community



MAP 11.9.2 Great Crested Newt Survey Results

KEY

- Site boundary
- 250m linear distance from site boundary
- Great Crested Newt confirmed
- Pond subject to eDNA survey (negative result)
- Pond subject to HSI Assessment but dry during eDNA survey
- Pond dry at time of assessment
- Pond not surveyed (no access)

SCALE: 1:12,500 at A3

0 100 200 300 400 500 Metres

N

EPR

CLIENT: University of Reading

PROJECT: Loddon Garden Village

DATE: 01 August 2025

Y:\Loddon Garden Village, Shinfield 2201\GIS\Planning Application\Technical Appendices\GCN\Map11.9.2_GCNResults_P2342_3965_010825.aprx

Annex 1

2022 eDNA Survey Results

SureScreen Scientifics

Folio No: E12837
Report No: 1
Purchase Order: 2201
Client: ECOLOGICAL PLANNING AND RESEARCH LTD
Contact: Katie Cammack

TECHNICAL REPORT

ANALYSIS OF ENVIRONMENTAL DNA IN POND WATER FOR THE DETECTION OF GREAT CRESTED NEWTS (TRITURUS CRISTATUS)

SUMMARY

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

RESULTS

Date sample received at Laboratory: 22/04/2022
Date Reported: 28/04/2022
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
0217	8 Loddon Garden Village	SU 75298 68776	Pass	Pass	Pass	Negative	0
0218	3 Loddon Garden Village	SU 74819 68630	Pass	Pass	Pass	Negative	0
0220	5 Loddon Garden Village	SU 75264 69204	Pass	Pass	Pass	Negative	0
0221	7 Loddon Garden Village	SU 75056 68567	Pass	Pass	Pass	Negative	0
0222	2 Loddon Garden Village	SU 74422 68172	Pass	Pass	Pass	Negative	0

0223	4 Loddon Garden Village	SU 74881 68424	Pass	Pass	Pass	Negative	0
0227	6 Loddon Garden Village	SU 75068 68678	Pass	Pass	Pass	Negative	0

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

Reported by: Chris Troth **Approved by:** Esther Strafford

METHODOLOGY

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

If GCN DNA is present, the DNA is amplified up to a detectable level, resulting in positive species detection. If GCN DNA is not present then amplification does not occur, and a negative result is recorded.

Analysis of eDNA requires scrupulous attention to detail to prevent risk of contamination. True positive controls, negative controls and spiked synthetic DNA are included in every analysis and these have to be correct before any result is declared and reported. Stages of the DNA analysis are also conducted in different buildings at our premises for added security.

SureScreen Scientifics Ltd is ISO9001 accredited and participate in Natural England's proficiency testing scheme for GCN eDNA testing. We also carry out regular inter-laboratory checks on accuracy of results as part of our quality control procedures.

INTERPRETATION OF RESULTS

SIC: **Sample Integrity Check** [Pass/Fail]
When samples are received in the laboratory, they are inspected for any tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to inconclusive results.

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

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

Annex 2

2024 eDNA Survey Results

Folio No:	2196-2024
Purchase Order:	LGV8
Contact:	Ecological Planning & Research Ltd
Issue Date:	02.07.2024
Received Date:	20.06.2024

GCN Report

Technical Report

SureScreen Scientifics

Folio No: 2196-2024
Purchase Order: LGV8
Contact: Ecological Planning & Research Ltd
Issue Date: 02.07.2024
Received Date: 20.06.2024

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
6027	Loddon Garden Village, Pond 8	SU7506568672	Pass	Pass	Negative	0/12

Matters affecting result: none

Reported by: Daisy Chambers

Approved by: Lauryn Jewkes

Folio No:	2196-2024
Purchase Order:	LGV8
Contact:	Ecological Planning & Research Ltd
Issue Date:	02.07.2024
Received Date:	20.06.2024

SureScreen Scientifics

Methodology

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

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

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

Interpretation of Results

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

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

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

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

Folio No:	2197-2024
Purchase Order:	LGV6
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GCN Report

Technical Report

SureScreen Scientifics

Folio No: 2197-2024
Purchase Order: LGV6
Contact: Ecological Planning & Research Ltd
Issue Date: 02.07.2024
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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
6029	Loddon Garden Village, Pond 6	SU7506068564	Pass	Pass	Negative	0/12

Matters affecting result: none

Reported by: Daisy Chambers

Approved by: Lauryn Jewkes

SureScreen Scientifics

Methodology

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

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

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

Interpretation of Results

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

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

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

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

Folio No: 2198-2024
Purchase Order: LGV7
Contact: Ecological Planning & Research Ltd
Issue Date: 02.07.2024
Received Date: 20.06.2024

GCN Report

Technical Report

SureScreen Scientifics

Folio No: 2198-2024
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Contact: Ecological Planning & Research Ltd
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Received Date: 20.06.2024

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
6031	Loddon Garden Village, Pond 7	SU7516468655	Pass	Pass	Negative	0/12

Matters affecting result: none

Reported by: Daisy Chambers

Approved by: Lauryn Jewkes

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Contact:	Ecological Planning & Research Ltd
Issue Date:	02.07.2024
Received Date:	20.06.2024

SureScreen Scientifics

Methodology

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

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

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

Interpretation of Results

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

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

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

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

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GCN Report

Technical Report

SureScreen Scientifics

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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
6026	Loddon Garden Village, Pond 5	SU7446168185	Pass	Pass	Negative	0/12

Matters affecting result: none

Reported by: Daisy Chambers

Approved by: Lauryn Jewkes

SureScreen Scientifics

Methodology

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

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

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

Interpretation of Results

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

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

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

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

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GCN Report

Technical Report

SureScreen Scientifics

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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
6030	Loddon Garden Village, Pond 13	SU7479968592	Pass	Pass	Negative	0/12

Matters affecting result: none

Reported by: Daisy Chambers

Approved by: Christopher Troth

Folio No:	2200-2024
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Contact:	Ecological Planning & Research Ltd
Issue Date:	02.07.2024
Received Date:	20.06.2024

SureScreen Scientifics

Methodology

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

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

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

Interpretation of Results

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

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

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

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

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GCN Report

Technical Report

SureScreen Scientifics

Folio No: 2202-2024
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Issue Date: 02.07.2024
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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
GCN6032	Loddon Garden Village, Pond 9	SU752269158	Pass	Pass	Negative	0/12

Matters affecting result: none

Reported by: Daisy Chambers

Approved by: Christopher Troth

Folio No:	2202-2024
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Received Date:	20.06.2024

SureScreen Scientifics

Methodology

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

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

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

Interpretation of Results

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

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

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

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

Folio No:	2203-2024
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Contact:	Ecological Planning & Research Ltd
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GCN Report

Technical Report

SureScreen Scientifics

Folio No: 2203-2024
Purchase Order: LGVA
Contact: Ecological Planning & Research Ltd
Issue Date: 01.07.2024
Received Date: 20.06.2024

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
6028	Loddon Garden Village, Pond A	SU7453768453	Pass	Pass	Negative	0/12

Matters affecting result: none

Reported by: Daisy Chambers

Approved by: Lauryn Jewkes

Folio No:	2203-2024
Purchase Order:	LGVA
Contact:	Ecological Planning & Research Ltd
Issue Date:	01.07.2024
Received Date:	20.06.2024

SureScreen Scientifics

Methodology

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

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

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

Interpretation of Results

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

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

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

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