

Great Crested Newt Habitat Suitability Index Assessment & eDNA Survey

MIRA NW08

A Report To: Horiba Mira Ltd
Report Number: RT-MME-160692
Date: July 2023



Quality Assurance

Date	Version	Author	Checked and approved by
31/07/2023	Final	Helen Griffin ACIEEM (Principal Ecological Consultant)	Hannah Train ACIEEM (Principal Consultant)

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

The contents of this report are the responsibility of Middlemarch Environmental Ltd. It should be noted that, whilst every effort is made to meet the client’s brief, no site investigation can ensure complete assessment or prediction of the natural environment. Middlemarch Environmental Ltd accepts no responsibility or liability for any use that is made of this document other than by the client for the purposes for which it was originally commissioned and prepared.

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 June 2023, Horiba Mira Ltd commissioned Middlemarch to undertake a Habitat Suitability Index (HSI) Assessment and eDNA survey for great crested newts *Triturus cristatus* at the site of a proposed development at NW08, located at Watling Street in Nuneaton. This survey was required assess the potential for the development of NW08 to impact on great crested newts.

Scope of Survey

Middlemarch has carried out surveys for great crested newts across the wider MIRA site since 2016, including of ponds in proximity to adjacent developments at TIC-IT and NW07. The focus of the current survey was three ponds (P7, P8 and a SUDS feature) within a 50 m radius of the NW08 plot. Great crested newt monitoring survey results from 2022 were also reviewed to inform the assessment.

Survey Results

The HSI Assessment classed Ponds P7 and P8 as having 'average' suitability to support great crested newts, and the SUDS as having 'good' suitability. Positive eDNA results were returned for Pond P8 and the SUDS, which indicates that newts had utilised these waterbodies in 2023. Given that no great crested newts were identified within Pond P8 or the SUDS during previous surveys (between 2018 and 2022), it is considered most likely that the positive result is indicative of a low number of transient individuals.

A negative result was returned for Pond P7 and great crested newt individuals are therefore deemed to be absent from this pond at the time of survey.

Potential Impacts on Great Crested Newts

No great crested newt breeding habitat will be lost as a result of the proposed development. However, immediate (<50 m from breeding ponds) and intermediate (>50 m and <250 m from breeding ponds) great crested newt terrestrial habitat will be lost to accommodate the proposed development. In the absence of mitigation this could lead to the killing or injury of great crested newts and a breach of wildlife legislation.

Recommendations

In order to ensure compliance with wildlife legislation, the following recommendations are made (further details are provided in Chapter 5):

- **Mitigation Strategy and Licence:** Due to the presence of a suspected small population class of great crested newts in Pond P8 and the SUDS, prior to any works commencing on site a Natural England great crested newt development licence will be required.
- **Scheme Design:** The proposed development should be designed in accordance with the ecological mitigation hierarchy as set out in the National Planning Policy Framework (NPPF), and the National Planning Practice Guidance (NPPG).

Contents

1. Introduction	5
1.1 Project Background	5
1.2 Site Description and Context	5
1.3 Survey Area and Justification	6
1.4 Documentation Provided	7
2. Methods.....	8
2.1 Field Survey	8
2.3 Constraints.....	9
3. Survey Results	10
3.1 Habitat Suitability Index Assessment	10
3.2 Site Suitability and Connectivity Assessment	10
3.3 eDNA Survey	10
4. Impact Assessment	12
4.1 Summary of Proposals	12
4.2 Summary of Key Features for Great Crested Newts.....	12
4.3 Potential Impacts on Great Crested Newts	13
5. Recommendations	14
6. Drawings.....	15
Appendix 1	17
Relevant Legislation	17
Appendix 2	19
eDNA Survey Report.....	19

1. Introduction

1.1 Project Background

In June 2023, Horiba Mira Ltd commissioned Middlemarch to undertake a Habitat Suitability Index Assessment and eDNA survey for great crested newts *Triturus cristatus* at the site of a proposed development at NW08, located at Watling Street in Nuneaton.

Middlemarch has carried out surveys for great crested newts across the wider MIRA site since 2016. Updated surveys were undertaken in 2018 to inform the development and subsequent great crested newt mitigation licences for adjacent developments at TIC-IT and NW07. These developments are now complete, and the first two years of licence monitoring were carried out in 2020 and 2022. The findings of these surveys are detailed within the following reports:

- Great Crested Newt Survey, RT-MME-121221-02-03 (2016);
- Great Crested Newt Survey, RT-MME-127241-01 (2018);
- Great Crested Newt Monitoring, RT-MME-151974 (2020); and,
- Great Crested Newt Monitoring, RT-MME-157275 (2022).

In addition to Great Crested Newt Surveys, an Ecological Walkover of NW08 was also commissioned, the results of which can be found within Report RT-MME-160215 Rev B.

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	Plot NW08, Horiba MIRA, Watling Street, Nuneaton
National Grid Reference	SP 3695 9661
Site Area (ha)	3.4
Topography	Flat
Land Cover (on site)	The site comprised an area of unmanaged neutral grassland with encroaching tall ruderal and willow scrub. A large damp area dominated by soft and compact rushes was noted in the east, while in the south the sward was noticeably finer with frequent moss and a gravelly substrate below. Other habitats on site included a line of young trees along the southern boundary, a species-rich hedgerow which delineated the eastern boundary, and a band of dense scrub which was encroaching from the hedgerow base.
Land Cover (site surrounds)	NW08 is situated in the north of the Mira landholding. The recently developed NW07 and TIC-IT plots are located adjacent to the south and west respectively. The main track is located to the east, and to the north is a mosaic of grassland and scrub.

Table 1.1: Summary of Site and Surroundings

1.3 Survey Area and Justification

The Ecological Walkover, carried out by Middlemarch in April and June 2023, included the area proposed for Phase 1 of the development works. During subsequent discussions with the client and design team, it was found that a second phase of works is proposed within a period of approximately 2 years. In order to minimise the risk of double handling of any great crested newt populations on site, it was proposed that the whole area including both phases should be considered concurrently as part of this assessment.

It should also be noted for context that this area was previously identified within a Great Crested Newt Masterplan, submitted to Natural England as part of the TIC-IT licence in 2019. NW08 and a large area of land to the north were identified as areas of future development, for which a great crested newt licence – then referred to as ‘Licence 3’ – would be required.

Three ponds are located within 50 m of the NW08 proposed development area. These ponds were surveyed in 2020 and 2022, and no great crested newts were identified. However, given the suitability of terrestrial habitat on site and the proximity to known populations within the vicinity, eDNA was undertaken in 2023 to provide supplementary information to determine the potential use of on-site terrestrial habitats by great crested newts.

Reference to Ordnance Survey mapped data indicates the potential presence of up to 19 other ponds within a 500 m radius of the site. Of the ponds identified, 18 have been subject to survey since 2018 either as part of presence/absence or population assessment surveys undertaken to inform development, as part of associated licence monitoring surveys (most recently carried out in 2022), or both. Therefore, contextual information on great crested newt populations within the vicinity is known and updated assessment of these ponds was not deemed necessary. Given the small size of NW08 and availability of data from previous years, the primary focus of this assessment is the ponds situated within a 50 m radius of the proposed development area. The 2023 survey results are detailed within Chapter 3.

The presence, connectivity and great crested newt status of ponds located within a 250 m radius has been considered as part of the wider assessment, and these ponds are discussed further in Chapter 4.

The distances of ponds within 250 m of the development boundary are provided in Table 1.2, along with the justification for inclusion or exclusion from the survey effort. The locations of these ponds in relation to the proposed development site are shown on Drawing C160692-01 in Chapter 6.

Pond Reference	Distance from Development Boundary	Most recent survey type	Date of most recent survey	Justification
SUDS	On site	eDNA	2023	Ponds situated on site, and connected to ponds in the north via grassland, scrub and tree lines.
P8	On site	eDNA	2023	
P7	30 m	eDNA	2023	Pond is connected to the site and to ponds in the west via grassland and tree lines.

Table 1.2: Distance of Ponds from Development Boundary (continues)

Pond Reference	Distance from Development Boundary	Most recent survey type	Date of most recent survey	Justification
P10	50 m	Monitoring	2022	Connectivity is present between these ponds and the site / on-site ponds via grassland, scrub and tree lines.
P10a	60 m	Monitoring	2022	
P9	150 m	Monitoring	2022	Connectivity is limited by the TIC-IT track to the west; however, this is unlikely to completely sever connectivity and these ponds are known to support a population of great crested newts.
P21	210 m	Monitoring	2022	
P11	300 m	Monitoring	2022	Pond 11 is situated over 250 m away and connectivity between this pond and the site is limited; however, this pond is included within this assessment as it has been identified as a potential receptor area location.

Table 1.2 (Cont.): Distance of Ponds from Development Boundary

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 1.3.

Document / Drawing Number	Author
Phase 2 Initial Option / 7045-SK-2023-07-24 Rev P1	BCAL Consulting

Table 1.3: Documentation Provided by Client

2. Methods

2.1 Field Survey

The survey comprised the following elements:

Habitat Suitability Index Assessment

All surveyed ponds were visually assessed for their suitability to support great crested newts by a suitably qualified ecologist. Ponds were assessed utilising the great crested newt Habitat Suitability Index (HSI) developed by Oldham *et al* in 2000¹ and revised by ARG UK in 2010. The HSI is a numerical index between 0 and 1, wherein a score of 1 represents optimal habitat for great crested newts. The HSI score is used to define the suitability of the pond on a categorical scale (Table 2.1). It should be noted, however, that the system is not precise enough to allow the conclusion that a pond with a high score will definitely support great crested newts whilst those with a low score will not.

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

Table 2.1: Great Crested Newt HSI Scoring

The HSI is given by assigning a quantitative figure to each of 10 variables, including pond area, water quality and level of shading, which are all factors thought to affect great crested newts. The tenth root of the product of these variables is then calculated, giving a figure for habitat suitability.

The field assessment was undertaken on 14th June 2023 by James Calow MPhil CEnv MCIEEM MIEMA (Senior Biodiversity Consultant, licence number 2016-20384-CLS-CLS). Table 2.2 details the weather conditions at the time of the assessment.

Parameter	Condition
Temperature (°C)	24
Cloud (%)	0
Wind (Beaufort)	F0-1
Precipitation	Nil

Table 2.2: Weather Conditions During Field Survey

¹ 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 (4), 143-155.

Site Suitability and Connectivity Assessment

As part of the Ecological Walkover (Report RT-MME-160215 Rev B) the habitats on site were assessed for their suitability 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 30ml 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 2.

2.3 Constraints

At the time of survey, the SUDS was almost dry with only a shallow pool of water remaining in the base. Access to P8 was restricted by the presence of dense scrub and trees around the periphery. The surveyor gained access to strategic locations around the banks using secateurs to lightly prune bramble branches where needed to provide access for sample collection.

3. Survey Results

3.1 Habitat Suitability Index Assessment

The HSI score for each of the ponds assessed is detailed in Table 3.1. Pond locations are shown on Middlemarch Drawing C160692-01 in the Chapter 6.

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	
SUDS	1.0	0.9	1.0	0.33	1.0	1.0	1.0	1.0	0.33	0.4	0.72 (Good)
P8	1.0	0.3	0.9	0.67	0.2	1.0	1.0	1.0	0.33	0.9	0.64 (Average)
P7	1.0	0.4	0.9	0.67	0.2	1.0	1.0	1.0	0.33	0.5	0.62 (Average)
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 3.1: Habitat Suitability Index of Ponds Within 250m of Study Area

3.2 Site Suitability and Connectivity Assessment

The site provides suitable opportunities for refuge, connectivity and foraging within the unmanaged neutral and wet grassland, tall ruderal and scrub. The hedgerows which delineate the northern, western and eastern boundaries also provide suitable terrestrial refuge and connectivity opportunities at their bases. The SUDS and P8 are located on site, adjacent to the proposed development area with no barriers to dispersal. P7 is located on the opposite side of a narrow asphalt access road, which is likely to limit but not sever connectivity.

Connectivity to the wider landscape is predominantly present to the north, where the site abuts wider areas of grassland and scrub which is likely to be unmanaged. Areas of grassland, scrub, vegetated boundaries and shrub planting, as well as other ponds, are present to the west, south and east. Connectivity to these areas is present, but is deemed less extensive than to the north, with areas of built environment and tightly mown grassland likely to impact upon the availability of commuting and foraging habitat.

3.3 eDNA Survey

A summary of the eDNA analysis results provided by SureScreen Scientifics is given in Table 3.2.

Pond Ref.	SIC	DC	IC	Result
P1 (SUDS)	Pass	Pass	Pass	Positive
P2 (P8)	Pass	Pass	Pass	Positive
P3 (P7)	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 3.2: eDNA Analysis Results

4. Impact Assessment

4.1 Summary of Proposals

The works proposed at NW08 are considered in two phases. Initially, this assessment is required in the context of a planning application for Phase 1 only. Proposals comprise the construction of a multi-use concrete pad with associated access, landscaping, and a small service building. The second phase is considered in the context of potential risk to great crested newt populations through double handling. Provisional plans have been made available to facilitate this assessment, and the exact details will be clarified in due course.

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 of 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;
- Frequent movement of heavy goods vehicle, cars and other forms of transportation;
- Use of lighting associated with roads and buildings;
- Presence of and movement of site personnel; and,
- Maintenance of landscaping.

4.2 Summary of Key Features for Great Crested Newts

Three ponds were included within updated surveys in 2023 due to their proximity to the proposed development site. The Habitat Suitability Index Assessment found the SUDS was of good suitability, while P7 and P8 were of average suitability for great crested newts.

eDNA analysis of these waterbodies identified great crested newt DNA within the SUDS and P8.

P7 and P8 were subject to presence/absence surveys in 2018, 2020 and 2022, and the SUDS was included within the 2020 and 2022 surveys. During these surveys, no great crested newts were identified within these ponds. During the 2023 eDNA survey, great crested newt DNA was detected within the SUDS and P8, indicating that newts had utilised these waterbodies in 2023. As individuals have never been recorded in these ponds before, it is considered most likely that the positive result is indicative of a low number of transient individuals.

No great crested newt DNA was detected within P7, and individuals are therefore deemed to be absent from this pond at the time of survey.

4.3 Potential Impacts on Great Crested Newts

No waterbodies will be lost or directly impacted by the proposals; however, the proposed development will lead to the permanent loss of neutral and marshy grassland, tall ruderal and scrub vegetation, providing terrestrial refuge, foraging and commuting habitat for great crested newts. In addition, there is potential that retained habitats could be subject to temporary disturbance during the construction phase as a result of pollution, vibration, movement of plant and contractors around the site or insensitive storage of materials.

Populations of great crested newts are known to be present within a 250 m radius of the site. The nearest of these were within Ponds P9 and P10, where peak counts of 1 no. individual was recorded within each pond in 2022, and within P21 and P10a, where peak counts of 4 no. and 3 no. were recorded in 2020 and 2018 respectively. Given the proximity between these ponds, the SUDS and P8, it is likely they form a metapopulation with some movement of individuals between them.

Long term impacts resulting from habitat loss and/or fragmentation are not deemed likely due to the small scale of the project at NW08 and presence of suitable terrestrial habitat to the north. All ponds will be retained, while suitable commuting and foraging habitat will remain at the boundaries. However, in the absence of mitigation, clearance works to the vegetative habitats on site have the potential to cause death or harm to individuals. A recommendation for a great crested newt mitigation licence has been made in Chapter 5.

5. 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 Mitigation Strategy and Licence:** Due to the presence of a suspected small population class of great crested newts in Pond P8 and the SUDS, prior to any works commencing on site a Natural England great crested newt development licence will be required. A great crested newt mitigation strategy will need to be developed to minimise potential impacts on great crested newts and / or their breeding and resting places and to ensure adequate mitigation is provided as part of the development proposals.

Natural England great crested newt development licences can only be applied for once outline planning permission (with reserved matters relating to wildlife or habitat issues resolved) or full planning permission has been granted, and where any conditions related to ecology have been discharged.

- R2 Scheme Design:** The proposed development should be designed in accordance with the ecological mitigation hierarchy as set out in the National Planning Policy Framework (NPPF), and the National Planning Practice Guidance (NPPG). The mitigation hierarchy requires all development schemes to apply the following principles:

- *Avoidance and Mitigation* – the proposed development should seek to avoid/minimise losses of neutral and marshy grassland, vegetated boundaries, and scattered scrub areas, in the first instance and incorporate these features in the landscaping layout of the scheme accordingly.
- *Compensation* – where unavoidable losses occur and mitigation cannot be provided, compensation for significant residual harm will be required as a last resort or planning permission could be refused. Compensation should include the creation of new great crested newt habitat such as pond enhancement, grassland creation, and replacement refugia and hibernacula, with linkages to suitable offsite opportunities to ensure the conservation status of great crested newts on site can be maintained.

6. Drawings

Drawing C160692-01 – Pond Location Plan



C160692-01

Legend

Approximate site boundary

250m from site boundary

500m site boundary

GCN positive results:

GCN eDNA positive 2023

GCN positive 2022

GCN positive 2020

GCN positive 2018

GCN negative results:

GCN eDNA negative 2023

GCN negative 2022 and 2020

GCN negative 2018 (no further surveys undertaken)

No results:

No access

Project

MIRA NW08

Drawing

Pond Map

Client

HORIBA MIRA Ltd.

Drawing Number

C160692-01

Revision

00

Scale @ A3

1:5,000

Date

July 2023

Approved By

HG

Drawn By

VO

MIDDLEMARCH

Triumph House, Birmingham Road, Allesley, Coventry CV5 9AZ

T:01676 525880

E:admin@middlesmarch-environmental.com

This map is reproduced from the Ordnance Survey material with the permission of Ordnance Survey on behalf of The Controller of Her Majesty's Stationary Office. © Crown copyright. Unauthorised reproduction infringes Crown copyright and may lead to prosecution of civil proceedings.

Licence Number: 100040519

Maxar, Microsoft, Contains OS data © Crown Copyright and database right 2022

Contains data from OS Zoomstack

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

eDNA Survey Report

Folio No: E18086
Report No: 1
Purchase Order: POR001554
Client: MIDDLEMARCH
ENVIRONMENTAL LTD
Contact: James Calow

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: 16/06/2023
Date Reported: 19/06/2023
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
5560	Mira NW08 - 160692 - Pond P1	SP 37058 96729	Pass	Pass	Pass	Positive	2
5561	Mira NW08 - 160692 - Pond P2	SP 37019 96652	Pass	Pass	Pass	Positive	10
5562	Mira NW08 - 160692 - Pond P3	SP 36951 96529	Pass	Pass	Pass	Negative	0

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

Reported by: Gabriela Danickova

Approved by: Jackson Young



METHODOLOGY

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

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

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

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

INTERPRETATION OF RESULTS

- SIC:** **Sample Integrity Check** [Pass/Fail]
When samples are received in the laboratory, they are inspected for any tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to inconclusive results.
- DC:** **Degradation Check** [Pass/Fail]
Analysis of the spiked DNA marker to see if there has been degradation of the kit or sample between the date it was made to the date of analysis. Degradation of the spiked DNA marker may lead indicate a risk of false negative results.
- IC:** **Inhibition Check** [Pass/Fail]
The presence of inhibitors within a sample are assessed using a DNA marker. If inhibition is detected, samples are purified and re-analysed. Inhibitors cannot always be removed, if the inhibition check fails, the sample should be re-collected.
- Result:** **Presence of GCN eDNA** [Positive/Negative/Inconclusive]
Positive: GCN DNA was identified within the sample, indicative of GCN presence within the sampling location at the time the sample was taken or within the recent past at the sampling location.
Positive Replicates: Number of positive qPCR replicates out of a series of 12. If one or more of these are found to be positive the pond is declared positive for GCN presence. It may be assumed that small fractions of positive analyses suggest low level presence, but this cannot currently be used for population studies. In accordance with Natural England protocol, even a score of 1/12 is declared positive. 0/12 indicates negative GCN presence.
Negative: GCN eDNA was not detected or is below the threshold detection level and the test result should be considered as evidence of GCN absence, however, does not exclude the potential for GCN presence below the limit of detection.

