Great Crested Newt

IAMP

September 2022

Durham Wildlife Services
Rainton Meadows
Chilton Moor
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Quality Control

Report Status: Draft

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Great Crested Newt Report

International Advanced Manufacturing Plant (IAMP)

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1.0 EXECUTIVE SUMMARY

1.0.1 Durham Wildlife Services (DWS) was commissioned by Ecology Solutions on behalf of IAMP LLP in June 2022 to undertake a great crested newt *Triturus cristatus* eDNA surveys across the International Advanced Manufacturing Park (IAMP) area, north of Nissan Car Manufacturing Plant, in Sunderland. The approximate National Grid Reference for the centre of the site is NZ335593. The area covered by the site is proposed for development, and includes a Ecological and Landscape Mitigation Area (ELMA). IAMP is a joint venture between Sunderland City Council and South Tyneside Council will deliver a nationally significant infrastructure project to create a new hub for advanced manufacturing, automotive and technology business within the area.

1.0.2 A number of surveys have already been completed across the site by several ecological companies including White Young and Green (WYG) in 2014 and 2015, ARUP in 2016-2017, and DWS in 2018/2019 & 2020. This report focuses on great crested newts.

1.0.3 The ponds on site were found to be negative for great crested newts, and the site does not fall within 500 metres of a known GCN pond. Overall, GCN do not pose a constraint to development.

2.0 INTRODUCTION

2.1 Background

2.1.1 Durham Wildlife Services (DWS) was commissioned by Ecology Solutions on behalf of IAMP LLP in June 2022 to undertake a great crested newt *Triturus cristatus* eDNA surveys across the International Advanced Manufacturing Park (IAMP) area, north of Nissan Car Manufacturing Plant, in Sunderland. The approximate National Grid Reference for the centre of the site is NZ335593. The area covered by the site is proposed for development, and includes a Ecological and Landscape Mitigation Area (ELMA). IAMP is a joint venture between Sunderland City Council and South Tyneside Council will deliver a nationally significant infrastructure project to create a new hub for advanced manufacturing, automotive and technology business within the area.

2.1.2 A number of surveys have already been completed across the site by several ecological companies including White Young and Green (WYG) in 2014 and 2015, ARUP in 2016-2017, and DWS in 2018/2019 & 2020. This report focuses on great crested newts.

2.2 Site Description

The site is a mixture of arable and pasture farmland, with small pockets of woodland, located to the north of the Nissan Car Manufacturing Plant, in Sunderland. Two watercourses flow across site, the River Don and into the River Don flows the Usworth Burn. The site also includes a number of farm steadings, and cottages and the IAMP ONE development area. Nissan CMP lies immediately south (Figure 1, Appendix A).

2.3 Survey Objectives

EDNA surveys were carried out for great crested newts on all ponds within the site and within 500 metres of the site to establish presence and likely impacts for this species.

2.4 Legislation

Great Crested Newt (GCN) is fully protected through its inclusion in Schedule 5 of the Wildlife and Countryside Act 1981 (as amended) and in Schedule 2 of the The Conservation of Habitats and Species Regulations 2017 (as amended) as a European protected species. Under the legislation, it is an offence to intentionally

kill, injure or take a great crested newt as well as intentionally or recklessly damage, destroy or obstruct access to any structure or place used for shelter or protection by a great crested newt or disturb an animal while it is occupying a structure or place which it uses for that purpose. The legislation applies to great crested newts in both aquatic and terrestrial habitats and to all life stages. Great Crested Newts are also subject to a national Biodiversity Action Plan (BAP).

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3.0 METHODOLOGY

3.1 Desk Based Study

The Environmental Records Centre Northeast (ERIC NE) were contacted for records of protected species and sites within 2km of the site.

3.2 Sampling Methodology

Fourteen water bodies were identified within the survey area/ within 500 metres. Six were found to be dry at the time of the eDNA survey. The rest were tested using the recommended eDNA collection and analysis methodology. The sample collection was carried out by Sacha Elliott (Licence Number 2017-30847-CLS-CLS) and Laura Thompson (Licence Number 2018-33469-CLS-CLS) on the 23rd June 2022, and Karen Devenney (Licence Number 2015-17181-CLS-CLS) and Ian Craft (Licence Number 2015-18706-CLS-CLS) on the 24th June 2022. The laboratory testing adhered to strict guidelines laid down in *WC1067 Analytical and Methodological Development for Improved Surveillance of the Great Crested Newt Triturus cristatus, Version 1.1.* The location of these waterbodies can be found in Figure 2, Appendix A.

3.3 Constraints and Assumptions

There were no constraints to the surveys carried out.

4.0 SURVEY RESULTS

4.1 Desk Based Study

ERIC NE provided 68 records of GCN within 2km of the site. Most of these records are for Severn Houses LWS, with records as recently as 2019. 2020 records found GCN along the road verge adjacent to Severn Houses. Although Severn Houses LWS lies within 500 metres of the site, the pond itself lies 600 metres away. The road verge where GCN were found lies in excess of 600 metres. There are no records from within the site itself, nor within 500 metres. Previous eDNA surveys have all come back negative (DWS 2018, 2021, WYG 2015)

4.2 Water Bodies

A large number of SuDS ponds have been installed over the last two years around the new developments in IAMP ONE. This has resulted in 13 water bodies now being present within the IAMP area, with an addition pond lying just outside the site boundary by My Pet Stop (Figure 2).

Ponds 1 – 3 (Photographs 1-3)

These are a series of wader scrapes, less than two years old, which were not holding enough water to eDNA sample at the time of the survey. They lack in vegetation both within the ponds and on the banksides.

Pond 4 (Photograph 4)

This is a wet depression within a horse pasture, it is dominated by floating sweet-grass *Glyceria fluitans* and water starwort *Callitriche stagnalis*. This pond was dry at the time of the eDNA survey.

Pond 5 (Photograph 5)

This pond is within the grounds of My Pet Stop. It is dominated by common reed *Phragmites australis*. This pond was dry at the time of the 2022 survey, although previously negative in 2020.

Pond 6 (Photograph 6)

This is a dry SuDS basin within the Faltec business grounds.

Pond 7 (Photograph 7)

This is a newly created SuDS ponds adjacent to the A19, and, at present, lacks vegetation both aquatic and on its banksides.

Pond 8 (Photograph 8)

This a well developed SuDS pond within the SNOP business grounds. Water levels were low, but a sample was still able to be taken. This pond has silted up quite a lot. A sown wildflower meadow is doing well around the pond boundary.

Pond 9 (Photograph 9)

This is good quality established SuDS pond within the grounds of Faltec. Aquatic and bankside vegetation is well established and invertebrates such as dragonflies and damselflies were seen during the survey.

Ponds 10-12 (Photographs 10-12)

These are a series of SuDS ponds running through the centre of the IAMP ONE development. They are all well established with aquatic and bankside vegetation.

Ponds 13 & 14 (Photographs 13 & 14)

These two linked SuDS ponds are present within the grounds of the former Nightingale Hospital. The eastern is of higher quality, with better water quality and more aquatic vegetation. The western lacked in aquatic vegetation and water quality was poor.

4.3 eDNA Results

Water samples were sent away for eDNA analysis collected from ponds 7-14. The results indicated that these ponds are all negative for the presence of GCN eDNA. Appendix C provides the results from the laboratory.

4.4 Habitat Suitability Index (HSI)

Ten key habitat criteria were assessed using objective habitat measurements to produce a HSI for the ponds identified, based on the methodology detailed by Oldham et al., (2000). The following bullet points provide a summary of this information together with a summary of the criteria fully outlined by Oldham et al., (2000).

1. Geographic Location – The site falls within the optimal zone for the known newt distribution, based on existing maps of newt distribution, therefore the ponds scored 1.

2. Pond Area – The pond area is a determinant of the magnitude of biological productivity of the pond ecosystem on which the newt population depends. The ponds vary widely from 100 m^2 through to 5800 m^2. Resulting score, therefore, range from 0.1 through to 1.

3. Pond Permanence – Pond permanence is crucial to permit completion of metamorphosis in any given year. Several of the SuDS ponds within the new businesses hold water all year round, whereas some of the shallower SuDS and the new scrapes, as well as the two ponds to the northwest all dry out most years or every year. Scores again vary, with ponds scoring between 0.1 to 0.9.

4. Water Quality – The adult GCN is capable of using atmospheric oxygen and is relatively tolerant of eutrophic conditions. The gill-breathing larva is more vulnerable and shares the need for reasonably well-aerated water with a number of aquatic invertebrates. The water quality of the ponds on site are largely poor, or assumed poor if dry. Some of the more established SuDS ponds were assessed as moderate water quality.

5. Pond Shading – Shading by trees may increase the organic content through leaf fall and cause eutrophication. All but one of the ponds have no shading and receives the maximum score of 1. The pond at My Pet Stop has some shading from scrub but still scores 1.

6. Number of Waterfowl – Common waterfowl may damage the habitat, partly by mechanical interference, but also by excessive nutrient enrichment. Most of the ponds had no sign of waterfowl and scored 1. The exception was the three scrapes, which have mostly bare ground banksides, but this is partly due to their newness. These scrapes were given the score of 0.67.

7. Occurrence of Fish – The effect of fish varies with species, but some (such as Stickleback) may be predatory and competitive. Most of the ponds that are new or dry frequently were absent of fish so scored 1. The more established SuDS ponds were given a score of 0.67 because fish were possible.

8. Pond Density – Swan and Oldham, (1993) suggested that a minimum pond density threshold of 0.7 ponds / km-2 for great crested newts to occur in the area. Great crested newts generally exhibit metapopulation dynamics and population persistence depends, in part, upon the distance separating breeding sites (Halley et al., 1996). The ponds are all in close proximity to at least 3 other

ponds, but most have 11 within 1km. Most ponds, therefore, scored 0.95, with the lowest scoring 0.65.

9. Proportion of “Newt Friendly” Habitat – The habitat occupied by GCN is highly variable, but newts are frequently found on land of low intensity use (scrub, woodland), rather than on pasture and arable land (Swan and Oldham, 1993). The habitat surrounding the ponds varies. Most are surrounded by roads and new development, with limit suitable terrestrial habitat, thus scoring 0.33. The scrapes and the ponds to the northwest are surrounded by undeveloped land and scored a better 0.67.

10. Macrophyte Content – Although not a direct food source for GCN, macrophytes fulfil a number of roles. They provide a food source (direct and indirect) for prey organisms, cover from predators and a substrate for egg attachment. Beyond a certain density however, they restrict space for courtship. Several of the new SuDS lack vegetation, as do the scrapes. More established SuDS ponds had around 40% macrophytes, with one up to 95%. Scores ranged from 0.3 through to 1.

The resulting score can be seen in Table 1 below, with the full HSI calculations shown in Appendix D. The ponds range from below average to excellent, with the established SuDS ponds within the IAMP ONE development scoring especially well and providing good habitat for this species, despite lacking in surrounding terrestrial habitat.

Table 1 HSI Results

Pond	HSI	Pond suitability
1	**0.66**	Average
2	**0.66**	Average
3	**0.66**	Average
4	**0.56**	Below Average
5	**0.60**	Average
6	**0.53**	Below Average
7	**0.72**	Good
8	**0.75**	Good
9	**0.80**	Excellent
10	**0.79**	Good
11	**0.77**	Good
12	**0.80**	Excellent
13	**0.77**	Good
14	**0.68**	Average

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Great Crested Newts

The ponds on site were found to be negative for great crested newts, and the site does not fall within 500 metres of a known GCN pond. Overall, GCN do not pose a constraint to development.

5.2 Table 2 Summary of Ecological Impacts and Recommendations

Ecological Factor	Potential Impacts	Recommendations	Mitigation and Enhancements
Great Crested Newts	This species was not found on site, and the site does not fall within 500 metres of a pond containing GCN. No breeding ponds will be impacted by the proposals.	None. The proposed development should not impact on this species.	Pond and wetland creation on site will benefit this species.

6.0 REFERENCES

Department for the Environment, Food and Rural Affairs (DEFRA) (2002) Working With the Grain of Nature. A Biodiversity Strategy for England. HMSO: London.

Durham Wildlife Services (2018) *IAMP Ecological Report*

Durham Wildlife Services (2021) *Great Crested Newt Survey Report*

ERIC NE Consultation Data

HMSO. (1981) Wildlife and Countryside Act. http://www.legislation.gov.uk/

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

WYG (2015) Land North of Nissan

APPENDIX A

Figures

Legend

★ Site Location

DWS ECOLOGICAL SERVICES

Rainton Meadows
Chilton Moor
Houghton-le-Spring
Tyne and Wear
DH4 6PU

info@dwsecology.co.uk
www.dwsecology.co.uk

Project	IAMP
Title	Location Plan
Client	Ecology Solutions & IAMP LLP
Date	30/08/2022
Ref	Figure 1

Legend

- Ponds Surveyed
- Ponds Too Dry To Survey

DWS ECOLOGICAL SERVICES

Rainton Meadows
Chilton Moor
Houghton-le-Spring
Tyne and Wear
DH4 6PU

info@dwsecology.co.uk
www.dwsecology.co.uk

Project	IAMP
Title	Pond Locations
Client	Ecology Solutions & IAMP LLP
Date	30/08/2022
Ref	Figure 2

APPENDIX B

Photographs

Photograph 1, Scrape 1.

Photograph 2, Scrape 2.

Photograph 3, Scrape 3.

Photograph 4, Pond 4, dry pond to the northwest of the site.

Photograph 5, Pond 5 at My Pet Stop outside the site boundary.

Photograph 6, Pond 6 – dry SuDS pond within Faltec.

Photograph 7, Pond 7 by the A19.

Photograph 8, Pond 8 by SNOP.

Photograph 9, Pond 9 within Faltec.

Photograph 10, Pond 10 middle SuDS Pond.

Photograph 11, Pond 11 southern SuDS Pond.

Photograph 12, Pond 12 northern SuDS Pond.

Photograph 13, Pond 13 eastern pond within former Nightingale Hospital.

Photograph 14, Pond 14 western pond within former Nightingale Hospital.

APPENDIX C

EDNA Results

SNOP IAMP = Pond 8

IAMP A19 = Pond 7

Faltec IAMP = Pond 9

IAMP SuDS North, South & Middle = Ponds 12, 11 & 10

IAMP Pond 1 Former Nightingale = Pond 13

IAMP Pond 1 Former Nightingale = Pond 14

SureScreen Scientifics

Folio No: E14712
Report No: 1
Purchase Order: IAMP3
Client: TOTAL ECOLOGY
Contact: Karen Devenney

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: 30/06/2022
Date Reported: 11/07/2022
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
6332	SNOP IAMP		Pass	Pass	Pass	Negative	0
6340	IAMP A19	NZ 34478 59177	Pass	Pass	Pass	Negative	0

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

Reported by: Chelsea Warner **Approved by:** Gabriela Danickova

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.

SureScreen Scientifics

Folio No: E14715
Report No: 1
Purchase Order: IAMP1/2
Client: TOTAL ECOLOGY
Contact: Karen Devenney

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: 30/06/2022
Date Reported: 13/07/2022
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
6329	IAMP Pond 2 Former Knightingale	NZ 33712 59342	Pass	Pass	Pass	Negative	0
6330	IAMP Pond 1 Former Knightingale	NZ 33712 59342	Pass	Pass	Pass	Negative	0

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

Reported by: Chris Troth **Approved by:** Chelsea Warner

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.

SureScreen Scientifics

Folio No: E14718
Report No: 1
Purchase Order: IAMP
Client: TOTAL ECOLOGY
Contact: Karen Devenney

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: 30/06/2022
Date Reported: 12/07/2022
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
6336	IAMP Suds North	NZ 33410 59195	Pass	Pass	Pass	Negative	0
6346	IAMP Suds South	NZ 33737 58882	Pass	Pass	Pass	Negative	0
6349	IAMP Suds Middle	NZ 33673 58962	Pass	Pass	Pass	Negative	0

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

Reported by: Chris Troth **Approved by:** Chris Troth

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.

SureScreen Scientifics

Folio No: E14719
Report No: 1
Purchase Order: IAMPFALTEC
Client: TOTAL ECOLOGY
Contact: Karen Devenney

TECHNICAL REPORT

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

SUMMARY

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RESULTS

Date sample received at Laboratory: 30/06/2022
Date Reported: 12/07/2022
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
6328	FALTEC IAMP	NZ 33768 58978	Pass	Pass	Pass	Negative	0

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

Reported by: Chris Troth **Approved by:** Chris Troth

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.

APPENDIX D

HSI Results

				Location	Area	Drying	Vater qualit	Shade	Fowl	Fish	Ponds	Terrestrial	Macrophytes		
Pond	**Site boundary distance (m)**	**Maximum area (m^2)**	**Ponds /1km^2**	**SI1**	**SI2**	**SI3**	**SI4**	**SI5**	**SI6**	**SI7**	**SI8**	**SI9**	**SI10**	**HSI**	**Pond suitability**
1	0	3000.00	11.0	1	0.8	0.5	0.33	1	0.67	1	0.95	0.67	0.3	**0.66**	Average
2	0	5800.00	10.0	1	0.8	0.5	0.33	1	0.67	1	0.95	0.67	0.3	**0.66**	Average
3	0	2100.00	11.0	1	0.8	0.5	0.33	1	0.67	1	0.95	0.67	0.3	**0.66**	Average
4	0	160.00	7.0	1	0.2	0.1	0.33	1	1	1	0.85	0.67	0.8	**0.56**	Below Average
5	15	100.00	3.0	1	0.1	0.5	0.33	1	1	1	0.65	0.67	0.8	**0.60**	Average
6	0	370.00	11.0	1	0.6	0.1	0.33	1	1	1	0.95	0.33	0.3	**0.53**	Below Average
7	0	800.00	7.0	1	0.98	0.9	0.67	1	1	0.67	0.85	0.33	0.33	**0.72**	Good
8	0	950.00	11.0	1	0.95	0.9	0.33	1	1	1	0.95	0.33	0.6	**0.75**	Good
9	0	2000.00	11.0	1	0.8	0.9	0.67	1	1	1	0.95	0.33	0.7	**0.80**	Excellent
10	0	1300.00	11.0	1	0.9	0.9	0.67	1	1	0.67	0.95	0.33	0.8	**0.79**	Good
11	0	3000.00	11.0	1	0.8	0.9	0.67	1	1	0.67	0.95	0.33	0.7	**0.77**	Good
12	0	1400.00	11.0	1	0.9	0.9	0.67	1	1	0.67	0.95	0.33	1	**0.80**	Excellent
13	0	350.00	11.0	1	0.7	0.9	0.67	1	1	0.67	0.95	0.33	0.85	**0.77**	Good
14	0	500.00	11.0	1	1	0.9	0.33	1	1	0.67	0.95	0.33	0.35	**0.68**	Average

APPENDIX E

Report Conditions

DURHAM WILDLIFE SERVICES

REPORT CONDITIONS

IAMP

This report is produced solely for the benefit of Ecology Solutions & IAMP LLP and no liability is accepted for any reliance placed on it by any other party unless specifically agreed in writing otherwise.

This report is prepared for the proposed uses stated in the report and should not be used in a different context without reference to Durham Wildlife Services. In time improved practices, fresh information or amended legislation may necessitate a re-assessment. Opinions and information provided in this report are on the basis of Durham Wildlife Services using due skill and care in the preparation of the report.

This report refers, within the limitations stated, to the environment of the site in the context of the surrounding area at the time of the inspections. Environmental conditions can vary and no warranty is given as to the possibility of changes in the environment of the site and surrounding area at differing times.

This report is limited to those aspects reported on, within the scope and limits agreed with the client under our appointment. It is necessarily restricted and no liability is accepted for any other aspect. It is based on the information sources indicated in the report. Some of the opinions are based on unconfirmed data and information and are presented as the best obtained within the scope for this report.

Reliance has been placed on the documents and information supplied to Durham Wildlife Services by others but no independent verification of these has been made and no warranty is given on them. No liability is accepted or warranty given in relation to the performance, reliability, standing etc of any products, services, organisations or companies referred to in this report.

Whilst skill and care have been used, no investigative method can eliminate the possibility of obtaining partially imprecise, incomplete or not fully representative information. Any monitoring or survey work undertaken as part of the commission will have been subject to limitations, including for example timescale, seasonal and weather related conditions.

Although care is taken to select monitoring and survey periods that are typical of the environmental conditions being measured, within the overall reporting programme constraints, measured conditions may not be fully representative of the actual conditions. Any predictive or modelling work, undertaken as part of the commission will be subject to limitations including the representativeness of data used by the model and the assumptions inherent within the approach used. Actual environmental conditions are typically more complex and variable than the investigative, predictive and modelling approaches indicate in practice, and the output of such approaches cannot be relied upon as a comprehensive or accurate indicator of future conditions.

The potential influence of our assessment and report on other aspects of any development or future planning requires evaluation by other involved parties.

The performance of environmental protection measures and of buildings and other structures in relation to acoustics, vibration, noise mitigation and other environmental issues is influenced to a large extent by the degree to which the relevant environmental considerations are incorporated into the final design and specifications and the quality of workmanship and compliance with the specifications on site during construction. Durham Wildlife Services accept no liability for issues with performance arising from such factors

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