Results of Great Crested Newt Surveys 2022 - 2024

St Asaph Solar Farm

Anesco

The Green, Easter Park, Benyon Road,
Reading, Berkshire, RG7 2PQ

Prepared by:

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SLR Project No.: 406.065274.00001

29 April 2025

Revision 01

Revision Record

Revision	Date	Prepared By	Checked By	Authorised By
01	29 April 2025	Amy Gill	Emma Clarke	Gary Oliver

Basis of Report

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Appendices

1.0 Introduction

SLR Consulting Ltd were appointed by Anesco Ltd in 2022 to undertake great crested newt (GCN) (*Triturus cristatus*) surveys for the proposed solar farm at St Asaph, Denbighshire. The currently application site boundary is broadly centred on National Grid Reference (NGR) SJ 02443 72771 (hereafter referred to as the 'Site'). However, this report contains information pertaining to earlier site boundaries, and clarification has therefore been provided throughout this report as to which application site is being referred to.

This report presents the findings of the traditional surveys and environmental DNA (eDNA) surveys carried out in 2022, and the update eDNA surveys undertaken in 2024.

The assessment of impacts resulting from the proposed development and the identification of mitigation measures are beyond the scope of this report and will be covered in a separate Ecological Impact Assessment (EcIA) report.

1.1 Background and Context

SLR Consulting Limited were first instructed to provide ecological support in relation to the proposed solar farm at St Asaph in December 2021. Since that date, extensive ecological surveys have been undertaken which have fed into the Site design and final layout.

In 2022, the survey area encompassed parts of the current Site boundary and other fields which are excluded from the current application site; however, all of the information gathered has been presented here, for context, and to show the distribution of GCN ponds in the wider landscape. For the avoidance of doubt Figure 3 – GCN Survey Results (Appendix C) shows the current Site boundary, and all ponds which were surveyed during 2022/2024.

In 2022, surveys were carried out over an extensive area, which encompassed the application site which applied at the time, and land up to 500 metres from this boundary.

The survey approach and extent were scoped and agreed with Senior Species Advisor for Natural Resources Wales (NRW), prior to commencement of survey work, with continued collaboration occurring throughout the process.

1.2 Site Description

The Site is situated in Denbighshire, approximately 1km to the southwest of the settlement of St Asaph, and approximately 8km south of the seaside town of Rhyl. The location of the Site is shown in Drawing C0002452_01 (Appendix A) and the Site boundary is shown in Drawing C0002452_02 (Appendix B). The Site consists of the main Solar Site and the Cable Route, connecting to the St Asaph Substation.

The Solar Site is approximately 35.42ha in extent and is divided into two areas approximately 250m apart. The Western Parcel is a set of four fields approximately centred on NGR SJ 02123 72727 and the Eastern Parcel is a set of three fields approximately centred on NGR SJ 02644 72614. The fields consist of modified grassland, of a short to medium sward length used for cattle and sheep grazing. The boundaries of the fields consist of flailed hedgerows, some containing mature trees, and of semi-natural, lowland mixed deciduous woodland.

The Cable Route is limited to highways that connect the Solar Site to St Asaph Substation. This includes the track to Tyn Y Coed, Glascoed Road, and Cwttir Lane.

The wider landscape is generally farmland used for grazing or arable crops, there are also several woodlands, some of which are ancient.

1.3 Legislation

The great crested newt is fully protected through inclusion on Schedule 5 of the Wildlife and Countryside Act 1981 (as amended) and Schedule 2 of the Conservation of Habitats and Species Regulations 2019 (The Habitats Regulations). In brief, this legislation makes it offence to:

- deliberately kill, injure, or take a great crested newt;
- deliberately disturb a great crested newt in such a way as to be likely to impair their ability to survive, breed or reproduce, or rear or nurture their young; to hibernate or migrate; or to affect significantly the
- local distribution or abundance of that species;
- deliberately take or destroy the eggs of great crested newt;
- damage or destroy the breeding or resting place of a great crested newt;
- intentionally or recklessly obstruct access to a place that great crested newt use for shelter or protection; and
- intentionally or recklessly disturb a great crested newt whilst it is occupying a place which it uses for shelter or protection.

They are also listed on Section 7 of the Environment Act (Wales) as species of principle importance for the purpose of maintaining and enhancing biodiversity in relation to Wales.

2.0 Methods

2.1 Desk study

In 2021, Cofnod the local environmental record centre for North Wales was contacted for pre-existing biological records of protected or otherwise notable species, including great crested newt, for land within a 2km radius of the 2022 Site boundary; this was updated in 2025 to cover all land within 2km of the updated Solar Site boundary; this additional data was obtained on the 12th February 2025.

2.2 Field Survey

2.2.1 Context for 2022 Survey

In agreement with NRW, ponds which lay within the 2022 site boundary, were subjected to 'traditional' survey over the course of three survey visits (refer to Section 2.4 for a description of the methods used), then an eDNA survey was undertaken (if a positive result had not already been determined through traditional methods). If great crested newt were recorded or a positive eDNA result was derived during these surveys, the pond concerned was subject to six survey visits in total, in order to establish its 'population size class'. However, if after three visits no GCN had been recorded and the eDNA test yielded a negative result (i.e. no traces of GCN DNA present), the pond concerned was not subject to further survey.

Ponds which lie outside of but within 250m of the 2022 site boundary were subject to a Habitat Suitability Index (HSI) assessment, one traditional survey and one eDNA survey initially. If the pond scored higher than 'Average' on the HSI survey or returned a positive result in either of the other two surveys initially carried out, two additional surveys using traditional survey methods were undertaken. If the pond scored 'Average' or lower on the HSI survey, one additional survey was carried out. However, if both initial surveys concluded a negative result, the pond was not subject to further survey.

Evident and accessible ponds within 500 metres of the 2022 site boundary, but further than 250m from its boundary were subject to a single eDNA survey.

2.2.2 Habitat Suitability Index (HSI) Assessment

All ponds within the 2022 site boundary and within a 250m buffer, where accessible, were subject to a HSI assessment on the 23rd March 2022. All additional ponds within 500m of the 2022 site boundary, where accessible, were subject to a HSI assessment on the 29th June 2022.

Great crested newt HSI scores are calculated using ten parameters: location; pond area; frequency of pond drying; water quality; shade; waterfowl; fish; presence of other ponds in the area; terrestrial habitat; and macrophyte communities. Each parameter scores a value of between 0.01 and 1. These scores are then multiplied and 'rooted' to produce a geometric mean score, of between 0 and 1.

The categorical scale shown in Table 2-1 was then used to estimate the overall suitability of the water body concerned, for breeding GCN.

Table 2-1 HSI Categories

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

2.2.3 Great Crested Newt eDNA Survey

All eDNA sampling was carried out in accordance with the Natural Resources Wales approved technical advice note[1]. As such twenty samples were collected from each pond using sterile equipment provided by ADAS, at points evenly spread out along the pond perimeters, such that a minimum of 80% of the margins were sampled, where this was safe to do so. The water at each sampling area was gently stirred using a sterile ladle before samples were taken, to mix up DNA which tends to sink, whilst ensuring that sediment on the pond bottom was not disturbed, where historical DNA can persist.

The samples were then fixed in an ethanol preserving solution, and sent to ADAS laboratory for analysis, using the Natural Resources Wales approved method[1]. According to Biggs *et al.* (2014)[1] GCN DNA can be detected within the pond water for up to 21 days after a GCN (including efts) has left the water; a 99.3 % detection rate is achieved when 80 – 90 % of the waterbody margin is sampled.

In order to avoid contamination, the surveyors avoided entering the water. Latex gloves were worn when sampling and only sterile equipment came into contact with the water.

2.2.3.1 2022

All ponds within the 2022 site boundary and those within 250m of it which had not already concluded a positive result using traditional methods on the first two visits (i.e. Ponds 1, 2, 4, 5, 6, 7, 8, 16, 22, 25, 28, 29, 30, 31, and 34) were sampled on 19th and 20th April 2022 (refer to Figure 3 – GCN Survey Results (Appendix C) for pond locations).

The four evident and accessible ponds located within 500m of the 2022 site boundary but beyond 250m of it (Ponds 23, 36, 50 and 51) were subject to eDNA survey on the 29th of June 2022.

Pond 29 returned an 'indeterminate' eDNA result, therefore this pond was subject to an additional survey using traditional methods.

2.2.3.2 2024

In 2024 a further eDNA survey was conducted of ponds within an interim application site boundary (2024 site boundary), and up to 500m of it. This included ponds within the new

[1] Biggs J, Ewald N, Valentini A, Gaboriaud C, Griffiths RA, Foster J, Wilkinson J, Arnett A, Williams P and Dunn F 2014. Analytical and methodological development for improved surveillance of the Great Crested Newt. Appendix 5. Technical advice note for field and laboratory sampling of great crested newt (*Triturus cristatus*) environmental DNA. Freshwater Habitats Trust, Oxford.

buffer zones, ponds where access was not possible in 2022, dry ponds from 2022 if they held water in 2024, and of ponds which has previously tested negative for GCN DNA in 2022, in case of change.

This work updated previous surveys, sampling was carried out on 24th June 2024.

2.2.4 Traditional Survey Methods

Traditional survey methods were carried out between the 23rd of March and the 30th of May 2022, during appropriate weather conditions. A summary of survey timings and weather conditions is provided in Table 2-2 below.

Table 2-2 Summary of Survey Timings and Weather Conditions

Date	Temperature (°C)	Wind (Beaufort scale)	Rain	Wind (0-8)
23/03/22	9	0	None	0
05/04/22	10	3	Light showers	8
19/04/22	9	0	None	5
18/05/22	14	3	Drizzle	6
23/05/22	11	3	Drizzle	7
30/05/22	10	1	None	3

2.2.4.1 Torchlight Counts

The surveyors walked slowly around the perimeter of each pond after dark and scanned the water's edge with a powerful torch, recording the number of great crested newts, additionally making a note of the presence of other amphibian species, if present.

2.2.4.2 Bottle Trapping

In cases where the water had sufficient depth and bottle trapping was considered to be an ineffective or sub-optimal survey technique, newt traps, comprising of open 2 litre plastic drink bottles attached to a bamboo cane, were deployed after sunset around the perimeter of each pond, at 2 metre intervals. Each trap was submerged in the water at an angle, leaving a small air pocket in the bottle (to avoid potentially suffocating any captured newts, or other amphibians). The traps were then checked for newts early the following morning, before being released. and the traps removed from the pond.

2.2.4.3 Egg Search

The surveyors checked the leaves of water plants within each pond for great crested newt eggs (which can be distinguished from the eggs of other newt species by their size and colour). This can be an effective survey technique, as the female folds the leaf over each egg, which can result in giving the leaf a characteristic 'concertina' appearance when multiple eggs are laid.

2.3 Limitations

Several of the ponds were dry or too shallow to survey on all, or a proportion of the visits, as detailed within Section 3.2.

A relatively small number of ponds had restricted access due to either dense marginal vegetation and/or deep, soft sediment at the base of the pond, making them unsafe to enter. Ponds with restricted access from the margin were Ponds 1, 4, 8, 16, 18, 21, 24, 25, 28, 29, and 30. All ponds received either eDNA testing and/or traditional methods of survey where accessible, with GCN presence found in five of the ponds.

A number of ponds were too shallow and/ or had livestock within the field at the time of survey, making it potentially unsafe to bottle trap at risk of the livestock knocking over the traps during the night; specifically this relates to Ponds 2, 5, 7, 16, 17, 19, 22, 28, 31, and 33. Five of these tested negative for GCN eDNA (2, 16, 22, 28, 31) and in Ponds 17, 19 and 33, GCN were found via torching.

A small number of ponds were found to have high levels of aquatic vegetation, obscuring the view into them, these include Ponds 6 and 34. Presence of GCN was found in both. For Pond 6, on three of the survey occasions vegetation cover (duckweed in this case) covered at least 50% of the pond's surface, making torching less effective, however, egg searching and bottle trapping were carried out each time. For Pond 34, on the first survey in April, the pond was chocked with vegetation and by the second survey in May the pond was dry; eDNA testing carried out on 20th April was positive, showing that GCN were present at that time, however any eggs would not have survived the season, due to it drying out shortly afterwards. The pond in its current state is extremely shallow, and not capable of supporting a viable GCN breeding population (see photograph in Table 3-1).

SLR did not have permission to access twelve of the ponds (37 ,38, 41, 42, 54, 55, 56, 57, 58, 59, 60, 61) within the 2022 survey season. Due to changes in the application site boundary, ponds 41, 42, 55, 56, 57 and 58 now lie further than 500m from the current Site boundary. Ponds 54, 59, 60 and 61 were accessed for eDNA survey in 2024. These ponds lie to the northwest of the Western Parcel and are associated with the Gwynt y Mor Offshore Windfarm Substation and have recent records of GCN presence as provided by Cofnod, details provided in Table 3-1.

The locations of all above-mentioned ponds are provided in Figure 3 – GCN Survey Results (Appendix C).

2.4 Quality Assurance and SLR Personnel

All survey work was undertaken by Accredited Agents under the NRW GCN survey licence S090015/1.

All ecologists employed by SLR Consulting Ltd follow the Chartered Institute of Ecology and Environmental Management's (CIEEM) code of professional conduct when undertaking ecological work. All work is subject to internal review as part of SLR's Quality Assurance procedure. This report has been written by Senior Field Ecologist, Amy Gill, qualifying member of CIEEM, and reviewed by Senior Ecologist, Emma Clarke qualifying member of CIEEM, and approved by Principal Ecologist, Mr Gary Oliver, MCIEEM CEnv.

3.0 Results

3.1 Desk Study

3.1.1 2022

The Cofnod data search identified 146 records of GCN within the 2km search area, although no records were supplied for the 2022 application site. Most of the records relate to ponds within the St Asaph Business Park, located approximately 950m to the north-west of the current Site boundary (central OS grid ref: SJ 01567 73930), with the closest of these ponds lying 600m metres from the Site boundary.

The Cofnod data included historic records of GCN from ponds that were included in the 2022 surveys undertaken by SLR, specifically for Ponds 19 and 24, the details of which are included within Table 3-1.

GCN records were also provided for ten further ponds, which were not surveyed by SLR Consulting during 2022, which have been included in Table 3-1 as Ponds 42, and 54 to 61 and illustrated in Figure 3 – GCN Survey Results (Appendix C).

3.1.2 2025

The data search was updated in 2025, Cofnod returned 157 records of great crested newt (GCN) (*Triturus cristatus*) with three records being within 100m of the current Solar Site, these included two records associated with Pond 24, located just outside the Eastern Parcel, and one record 65m outside of the Western Parcel (Pond 19). All the records date from between 2003-2020. There were another five GCN records provided for ponds located within 500m of the Solar Site.

Additionally, Cofnod returned the following records of other amphibians within 2km of the Solar Site boundary:

- 95 records of smooth newt (*Lissotriton vulgaris*), between 1993-2024, with the nearest being 313m northwest from the current Site;
- 61 records of common toad (*Bufo bufo*), between 1993-2020, with the nearest being 434m northwest from the current Site;
- 44 records of palmate newt (*Lissotriton helveticus*), between 2002-2024, with the nearest being 422m north from the current Site; and
- 44 records of common frog (*Rana temporaria)*, between 1968-2024, with the nearest being 11m northeast from the current Site.

3.2 Field Survey

Table 3-1 provides summary details for each pond, including a photograph if it held water, its HSI assessment score and value, along with the peak count of GCN recorded in any of the survey visits.

Full HSI data is provided in Appendix D, whilst Appendix E contains the results for each pond during each of the separate surveys. Appendix F contains the results of the eDNA analysis carried out in 2022 and Appendix G the results of the eDNA analysis carried out in 2024.

Table 3-1 Summary of 2022 Survey Results

Pond Nº	Easting	Northing	Plate	HSI	GCN eDNA	Peak count
1	302383	373469		0.74 - Good	Negative	0
2	302259	373336		0.68 - Average	Negative	0
3	302228	373273	Dry pond			
4	302166	373164		0.61 - Average	Negative	0
5	302085	373142		0.66 – Average	Positive	0

Pond Nº	Easting	Northing	Plate	HSI	GCN eDNA	Peak count
6	302131	372994		0.82 - Excellent	Positive	1
7	302088	372891		0.75 - Good	Positive	0
8	301954	372888		0.72 - Good	Positive	0
9	301828	372819	Dry pond			
10	301841	372729	Dry pond			
11	301681	372501	Dry pond			
12	301750	372349	Dry pond			
13	301745	372318	Dry pond			
14	301904	372327	Dry pond			
15	302177	372095	Dry pond			
16	302207	372669		0.42 - Poor	Negative	0

Pond Nº	Easting	Northing	Plate	HSI	GCN eDNA	Peak count
17	302231	372620		0.66 - Average	N/A	1
18	302259	372855		0.59 - Below Average	N/A	1
19	302313	372955		0.70 - Good	N/A	2 3 positive records from desk study results between 1993 and 2004. No specific numbers provided.
20	302373	373058	Dry pond			
21	302440	373139		0.72 - Good	N/A	2
22	302362	372632		0.64 - Average	Negative	0

Pond Nº	Easting	Northing	Plate	HSI	GCN eDNA	Peak count
23	302645	373296		0.74 - Good	Positive	N/a
24	302730	373036		0.81 - Excellent	N/A	3 3 positive records from desk study results between 1993 and 2004. No specific numbers provided.
25	302740	372972		0.82 - Excellent	Negative	0
26	302591	372806	Dry pond			
27	302727	372701	Dry pond			

Pond Nº	Easting	Northing	Plate	HSI	GCN eDNA	Peak count
28	302953	372526		0.67 - Average	Negative	0
29	303016	372412		0.61 - Average	Indeterminate	0
30	302572	372058		0.66 - Average	Positive	0
31	302433	372163		0.7 - Good	Negative	0
32	302485	372580	Dry Pond			

Pond Nº	Easting	Northing	Plate	HSI	GCN eDNA	Peak count
33	302896	372673		0.67 - Average	N/A	4
34	302068	372795		0.63 - Average	Positive	0
36	303154	372856		0.56 – Below average	Positive	N/A
37	302438	371895	No Access			
38	302362	371908	No Access			
39	302719	372205	Dry Pond			
40	301741	373354	Dry Pond			
41	301728	373488	No Access			
42	301786	373492	No Access Desk study search revealed 8 records within this pond between 2015 and 2019. This a peak count of 2 males and 1 female GCN.			

Pond Nº	Easting	Northing	Plate	HSI	GCN eDNA	Peak count
43	302855	373332	Dry Pond			
44	301774	372185	Dry Pond			
45	301856	371972	Dry Pond			
46	301638	372318	Dry Pond			
47	301457	372563	Two Dry Ponds			
48	301691	372946	Dry Pond			
49	301763	372998	Dry Pond			
50	301782	373101		0.68 - Average	Positive	N/A
51	302839	373489		0.61 - Average	Negative	N/A
52	302931	371766	Dry Pond			
53	302708	373448	Dry Pond			

Pond Nº	Easting	Northing	Plate	HSI	GCN eDNA	Peak count
54	301900	373408	Cofnod data refers to pond as Substation P2 Records between 23/05/18 and 14/05/20 had a peak count of 3x female and 1 x male GCN.			
55	301909	373475	Cofnod data refer to pond as Substation P3 Records between 07/05/15 and 15/05/19 had a peak count of 1x male and 1 x female GCN.			
56	302176	373512	Cofnod data refers to pond as Substation Pond BB9 Records between 14/05/20 and 16/05/2020 had a peak count of 4x male and 3 x female GCN.			
57	302143	373533	Cofnod data refers to pond as Substation Pond BB8 Records between 19/06/18 and 14/05/21 had a peak count of 1x male and 2 x female GCN.			
58	301913	373639	Cofnod data refers to ponds a Substation P4 Records between 07/05/15 and 13/05/16 had a peak count of 5x male and 2x female GCN.			
59	301938	373383	Records between 16/04/20 and 14/05/21 had a peak count of 1 x male and 1 x female GCN.			
60	301945	373407	Record of 1 x male GCN on 16/04/2020.			
61	301948	373426	Peak count of 1 x male and 3 x female GCN between 25/04/2019 and 14/05/2020.			

4.0 Discussions

Of the six ponds found within the final Site boundary, three have been found to support GCN. Of these, GCN were confirmed within two ponds (P34 and P7) via eDNA analysis, with no GCN being detected using traditional survey methods. The remaining pond (P18) had a peak count of one GCN. Ponds 16, 26 and 27 were found to be dry. Within a 250m radius of the main Solar Site boundary an additional eleven ponds have been found to support GCN, of these five (P5, P8, P23, P25 and P30) were confirmed via eDNA analysis. Ponds 5, 8 and 30, were surveyed in 2022 and found to support no GCN via traditional survey. Six ponds (P6, P17, P19, P21, P24, P33) were found to support GCN during traditional survey, with peak counts of between 1 and 4 individuals.

At individual ponds the population of GCN in considered to be 'small' as per the mitigation guidelines[2] i.e. less than 10 individual GCN recorded on one survey. The highest peak count for the whole survey area (all ponds within Site and 250m buffer) was 11 recorded on the 19th- 20th April 2022, indicating a 'medium' population, i.e. between 11-100, albeit at the lower end of this scale.

The surveys undertaken by SLR also confirmed the presence of smooth newt, common frog and common toad within the survey area. Based on the data search results, palmate newt may also be present but was not recorded.

[2] Great crested newt mitigation guidelines

Appendix A Drawing C0002452_01 Site Location

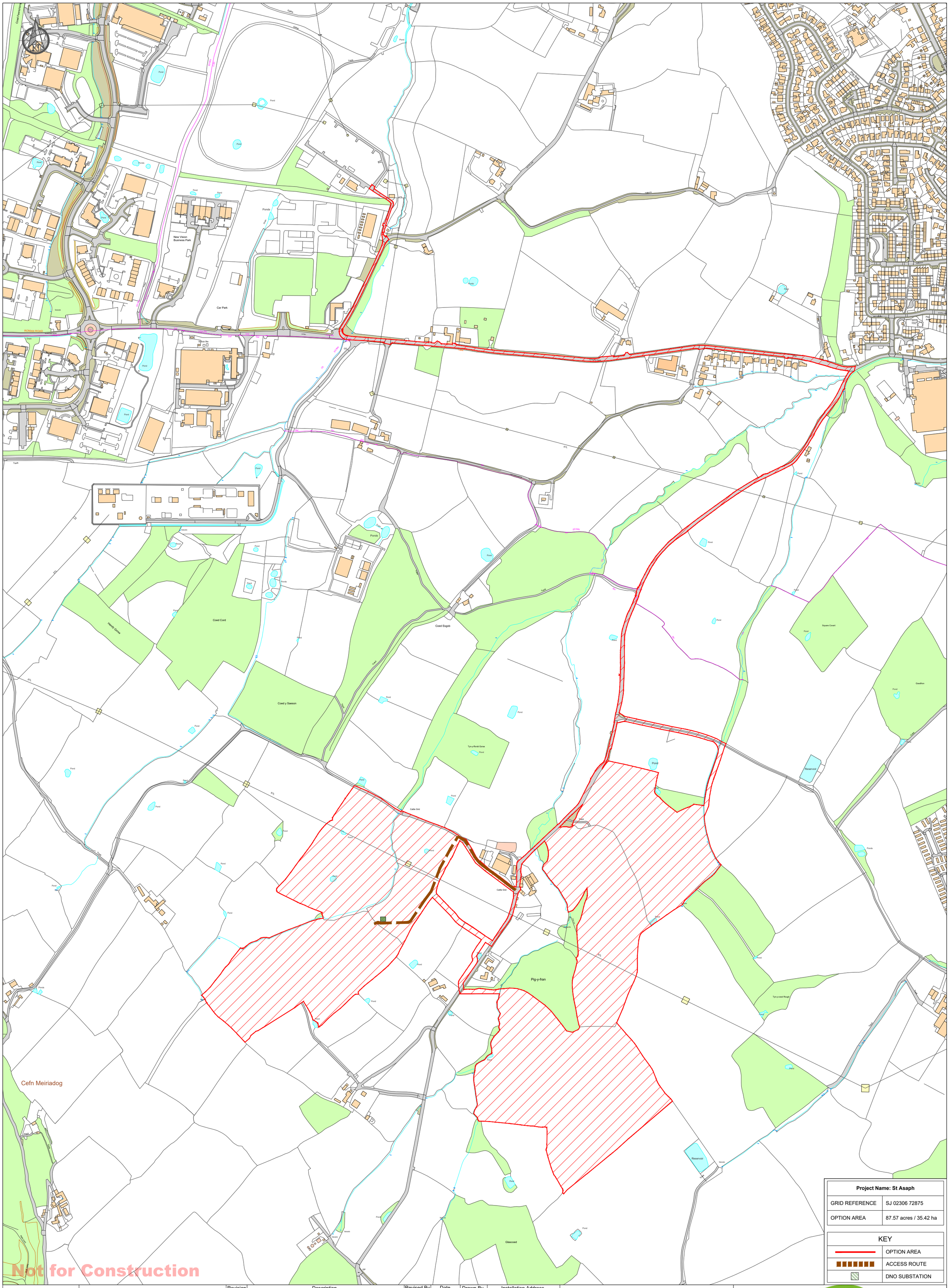
Results of Great Crested Newt Surveys 2022 - 2024

St Asaph Solar Farm

Anesco

SLR Project No.: 406.065274.00001

29 April 2025



Project Name: St Asaph	
GRID REFERENCE	SJ 02306 72875
OPTION AREA	87.57 acres / 35.42 ha

KEY	
	OPTION AREA
	ACCESS ROUTE
	DNO SUBSTATION

Installer Details

Anesco Ltd.
The Green,
Easter Park,
Benyon Road,
Reading,
RG7 2PQ
Tel: 0845 894 4444

Comments

0 50 100 150 200

Revision	Description	Revised By	Date
A	Issued for comment	MS	20/10/2020
B	Land reduced from the North and extended to the South	MS	05/03/2021
C	Area of Land has been altered and the DNO substation moved	MS	04/05/2021
D	Area of Land has been altered and the DNO substation moved	MS	12/05/2021
E	Site area amended	JH	22/11/2023
F	Red Line Boundary amended & access track added in the North	JH	12/12/2023
G	Red Line Boundary amended	MS	10/06/2024
H	Red Line Boundary amended	MS	13/11/2024
J	Red Line Boundary amended	RD	26/03/2025

Drawn By: RD

Scale: 1:5000@ A2

Sheet Size A2

Installation Address

Cefnmeiriadog,
St Asaph,
Denbighshire,
Wales,

LL17 0HF

Project: St Asaph

Title: Location Plan

Drawing No.: C0002452_01

Rev.: J

Anesco™ AMAZING ENERGY

Appendix B Drawing C0002452_02 Site Layout

Results of Great Crested Newt Surveys 2022 - 2024

St Asaph Solar Farm

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SLR Project No.: 406.065274.00001

29 April 2025

Not for Construction

New Vision Business Park

Car Park

Coed Cord

Hendy Gorse

Coed y Saeson

Coed Esgob

Tyn-y-ffordd Gorse

Pig-y-fran

Square Covert

Tyn-y-coed Rough

Reservoir

Glascoed

Ponds

Pond

Track

Drain

FB

Issues

Slurry Pit

Silo

Culverts

Natural

Stream

Overhead Lines

Underground Water Trunk Main

Public Right of Way

Glascoed Road

34.1m

36.0m

41.4m

47.2m

65.9m

LEGEND

	SITE BOUNDARY
	SECURITY FENCE
KV — KV — KV	OVERHEAD ELECTRICAL CABLES (24m/14m OVERALL EASEMENT)
	DNO ACCESS ROUTE
	HV CABLE ROUTE - TRENCH
	LV CABLE ROUTE - TRAY
	PRIVATE HV CABLE ROUTE - TRENCH
BT — BT — BT	BT OVERHEAD CABLE
WM — WM — WM	UNDERGROUND WATER TRUNK MAIN
	UTILITY EASEMENT
	HARDSTANDING AREA
	PROPOSED CULVERT CROSSING
	PROPOSED HEDGE REMOVAL FOR TRACK WIDENING
	FENCE PLACEMENT & HEDGE REGROWTH AREA
	PROPOSED TREE REMOVAL
	PV ARRAY
	INVERTER (SUN2000-330KTL)
	SMART TRANSFORMER STATION (JUPITER-6000K/JUPITER3000K)
DNO	DNO SUBSTATION
CUSTOMER SUBSTATION (PV)	

PROJECT NAME: ST ASAPH	
GRID REFERENCE :	SJ 02300 72873
SITE AREA:	87.57Acres / 35.42 ha
TOTAL INSTALLED CAPACITY:	18.44MWp
MAX INVERTER AC POWER:	18.304MW

0 10 20 40 60 80 100

Installer Details

Anesco Ltd.
The Green,
Easter Park,
Benyon Road,
Reading,
RG7 2PQ
Tel: 0845 894 4444

Comments

Revision	Description	Revised By	Date
A	Issued for comment	MS	19/11/2020
B	Land reduced from the North and extended to the South	MS	05/03/2021
C	Boundary Line Extended	MS	05/05/2021
D	Boundary Line Extended and site maximised	MS	12/05/2021
E	Planning Site Layout	JH	10/11/2021
F	Module Wattage Changed	MS	07/12/2021
G	Site resign to 215KTL & Bifacial Modules	JH	02/03/2022
H	Site redesigned due to tree shading implications	MS	16/06/2022
J	Red Line Boundary amended to include Private HV	JH	03/08/2022
K	Site redesign	JH	15/05/2023
L	Site redesign using new modules and new private HV cable	JH	06/07/2023
M	Site area amended and module redesign	JH	22/11/2023
N	DNO Track Added	LD	28/11/2023
P	Red Line Boundary amended & access track added in the North	JH	12/12/2023
Q	Redesign due to array relocation	JS	19/03/2024
R	Redesign	MS	01/08/2024
S	Access Altered	MS	20/09/2024
T	Hedgerow added and panel locations amended	MS	11/11/2024
U	Inverters added	RD	20/01/2024
V4	Topo and service searches added	RD	31/03/2025

Drawn By: JH

Scale: 1:3000@ A1

Sheet Size A1

Installation Address

Cefnmeiriadog,
St Asaph,
Denbighshire,
Wales,

LL17 0HF

Project: St Asaph

Title: Site layout planning

Drawing No.: C0002452_02

Rev.: V4

Anesco™ AMAZING ENERGY

Appendix C Figure 3 GCN Survey Results

Results of Great Crested Newt Surveys 2022 - 2024

St Asaph Solar Farm

Anesco

SLR Project No.: 406.065274.00001

29 April 2025

301200 301600 302000 302400 302800 303200 303600

373600 373200 372800 372400

N

P58 P57 P56 P55 P41 P42 P61 P54 P60 P59 P40 P1 P53 P51 P64 P43 P67 P23 P2 P3 P4 P5 P21 (2) P20 P50 P68 P49 P48 P69 P6 (1) P19 (2) P24 (3) P25 P63 P66 P65 P8 P7 P9 P18 (1) P34 P10 P26 P36 P16 P17 (1) P22 P32 P27 P33 (4) P28 P29 P47 P11 P12 P46 P13 P14 P44 P31 P39 P15 P30 P45 P38 P37 P52

0 200 400

Metres

406.065274.00001.0005.0 GCN Survey Results

LEGEND

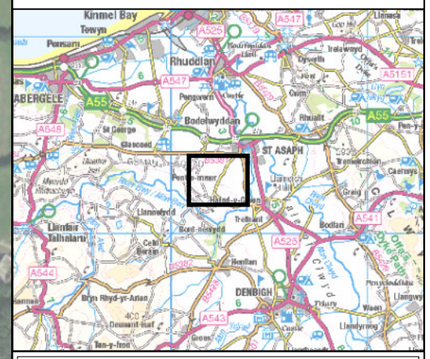
- Site Boundary
- Site Boundary 250m and 500m Buffers

Surveyed Pond 2024

- Dry
- eDNA Negative
- eDNA Positive

Surveyed Pond 2022

- GCN recorded by traditional methods (peak count in brackets)
- eDNA positive but GCN not recorded during traditional survey methods
- eDNA indeterminate, but GCN not recorded using traditional survey methods
- eDNA negative i.e. GCN absent
- Pond dry
- eDNA positive but pond located more than 250m from site boundary, and therefore not subject to population size class counts
- GCN present in Cofnod desk study data, but inaccessible at time of 2022 survey
- No access to survey



Anesco™

SLR

ST ASAPH

406.065274.00001

GCN SURVEY RESULTS

FIGURE 3

Scale 1:9,000 @ A3

Date APRIL 2025

Appendix D Results of Habitat Suitability Index Assessments

Results of Great Crested Newt Surveys 2022 - 2024

St Asaph Solar Farm

Anesco

SLR Project No.: 406.065274.00001

29 April 2025

Pond #	Easting	Northing	Date of HSI	Location	Area	Permanence	Water quality	Shade	Waterfowl effect	Fish	Pond density	Terrestrial habitat	Macrophyte cover	HSI score	HSI rating
1	302383	373469	24/01/22	1	1	0.9	0.67	1	0.67	0.67	1	0.33	0.5	0.73	Good
2	302259	373336	24/01/22	1	0.1	0.5	0.67	0.6	1	1	1	1	1	0.68	Average
4	302166	373164	24/01/22	1	0.1	0.5	0.67	0.6	1	1	1	0.33	1	0.61	Average
5	302085	373142	24/01/22	1	0.1	0.9	0.67	0.8	0.67	0.67	1	1	0.7	0.66	Average
6	302131	372994	24/01/22	1	0.1	0.9	0.67	0.6	1	1	1	1	1	0.82	Excellent
7	302088	372891	24/01/22	1	1	0.5	0.33	1	1	1	1	0.33	1	0.75	Good
8	301954	372888	24/01/22	1	0.2	0.9	0.67	1	1	1	1	0.33	1	0.72	Good
16	302207	372669	24/01/22	1	0.1	0.1	0.33	0.4	1	1	1	0.33	0.4	0.42	Poor
17	302231	372620	24/01/22	1	0.1	0.5	0.67	1	1	1	1	0.67	0.7	0.66	Average
18	302259	372855	24/01/22	1	0.1	0.5	0.33	1	1	1	1	0.33	1	0.59	Below average
19	302313	372955	24/01/22	1	0.4	0.9	0.67	0.8	0.67	0.67	1	0.33	1	0.7	Good
21	302440	373139	24/01/22	1	0.7	0.9	0.33	1	0.67	1	1	0.33	0.8	0.72	Good
22	302362	372632	24/01/22	1	0.3	0.9	0.67	1	0.67	1	1	0.33	0.3	0.64	Average
23	302645	373296	29/06/22	1	0.2	0.9	0.67	1	1	1	1	0.67	0.6	0.74	Good
24	302730	373036	24/01/22	1	0.8	0.9	0.67	1	1	1	1	0.67	0.4	0.81	Excellent
25	302740	372972	24/01/22	1	0.2	1	0.67	1	1	1	1	1	1	0.82	Excellent
28	302953	372526	24/01/22	1	0.07	1	0.67	0.4	1	1	1	1	1	0.67	Average
29	303016	372412	24/01/22	1	0.1	0.1	0.67	1	1	1	1	1	1	0.61	Average
30	302572	372058	24/01/22	1	0.1	0.9	0.67	1	1	1	1	0.67	0.4	0.66	Average
31	302433	372163	24/01/22	1	0.6	0.9	0.67	0.6	0.67	1	0.7	0.67	0.4	0.7	Good
33	302896	372673	24/01/22	1	0.2	1	0.67	0.4	1	1	1	1	0.35	0.67	Average
34	302068	372795	24/01/22	1	0.2	0.5	0.33	1	1	1	1	0.33	0.9	0.63	Average
36	303151	372857	29/06/22	1	0.3	0.5	0.33	0.6	1	1	1	0.33	0.3	0.56	Below average
50	301779	373101	29/06/22	1	0.2	1	0.33	1	1	1	1	0.67	0.5	0.68	Average
51	302833	373490	29/06/22	1	0.2	0.5	0.33	0.6	1	1	1	0.67	0.5	0.61	Average

Appendix E Full Survey Results 2022

Results of Great Crested Newt Surveys 2022 - 2024

St Asaph Solar Farm

Anesco

SLR Project No.: 406.065274.00001

29 April 2025

Pond #	Easting	Northing	Visit #	Date	Vegetation cover	Turbidity (0-5)	GCN torching			GCN bottle trap			GCN egg (+/-)	Other amphibians	Comments
							Male	Female	Juvenile	Male	Female	Juvenile			
1	302383	373469	1	19/04/22	1	3	0	0	0	N/A	N/A	N/A	-	None.	Steep sided pond
2	302259	373336	1	19/04/22	2	2	0	0	0	N/A	N/A	N/A	-	3x smooth newt, 2x undetermined small newt (female palmate or smooth newt).	Too shallow to trap
4	302166	373164	1	23/03/22	3	1	0	0	0	N/A	N/A	N/A	-	None.	Unable to bottle trap due to boggy area around pond edge.
	302166	373164	2	05/04/22	2	0	0	0	0	N/A	N/A	N/A	-	1x common toad.	Light showers throughout survey. Edges drying, grass encroaching.
	302166	373164	3	19/04/22	3	1	0	0	0	N/A	N/A	N/A	-	None.	Difficult to access pond perimeter due to boggy edge.
5	302085	373142	1	19/04/22	2	2	0	0	0	N/A	N/A	N/A	-	None.	Too shallow to bottle trap.
	302085	373142	2	23/05/22	2	1	0	0	0	N/A	N/A	N/A	-	None.	Drizzle rain throughout.
6	302131	372994	1	23/03/22	5	N/A	N/A	N/A	N/A	0	0	0	-	1x smooth newt, 1x common toad.	Duckweed covering >90% of the pond surface so unable to torch and determine turbidity
	302131	372994	2	05/04/22	1	3	0	0	0	0	0	0	-	11x common toad.	Water boatmen, larger beetles present.
	302131	372994	3	19/04/22	5	N/A	N/A	N/A	N/A	0	0	0	-	None.	Duckweed covering >95% of the pond surface so unable to torch and determine turbidity
	302131	372994	4	18/05/22	3	2	1	0	0	0	0	0	-	6x smooth newt.	Very light rain. Duckweed covering 50% of pond. Veg cover score includes this
	302131	372994	5	23/05/22	4	3	0	0	0	0	0	0	-	2x smooth newt, 1x common frog.	-
	302131	372994	6	30/05/22	3	3	0	0	0	1	0	0	-	3x smooth newt, 1x undetermined small newt (female palmate or smooth newt).	-
7	302088	372891	1	19/04/22	2	4	0	0	0	N/A	N/A	N/A	-	None.	Pond quite turbid therefore difficult to survey, unable to bottle trap due to boggy water edge.
	302088	372891	2	23/05/22	3	4	0	0	0	N/A	N/A	N/A	-	None.	Unable to bottle trap due to shallow pond and cattle.
	302088	372891	3	30/05/22	4	4	0	0	0	N/A	N/A	N/A	-	None.	Unable to bottle trap due to shallow pond and cattle.
8	301954	372888	1	19/04/22	2	1	0	0	0	N/A	N/A	N/A	-	1x smooth newt, 1x undetermined small newt (female palmate or smooth newt).	Unable to bottle trap due to boggy area around waters edge
	301954	372888	2	23/05/22	3	1	0	0	0	N/A	N/A	N/A	-	1x undetermined small newt female	Unable to bottle trap due to boggy area around waters edge

Pond #	Easting	Northing	Visit #	Date	Vegetation cover	Turbidity (0-5)	GCN torching			GCN bottle trap			GCN egg (+/-)	Other amphibians	Comments
							Male	Female	Juvenile	Male	Female	Juvenile			
														palmate or smooth newt).	
	301954	372888	3	30/05/22	4	0	0	0	0	N/A	N/A	N/A	-	1x undetermined small newt (female palmate or smooth newt).	-
16	302207	372669	1	23/03/22	1	1	0	0	0	N/A	N/A	N/A	-	None.	Unable to bottle trap due to boggy area around water's edge.
	302207	372669	2	05/04/22	2	2	0	0	0	N/A	N/A	N/A	-	None.	Small pond, very shallow – couldn't bottle trap.
	302207	372669	3	19/04/22	2	2	0	0	0	N/A	N/A	N/A	-	None.	Pond small now, not holding much water.
17	302231	372620	1	19/04/22	2	2	1	0	0	N/A	N/A	N/A	-	None.	-
	302231	372620	2	23/05/22	2	3	0	0	0	N/A	N/A	N/A	-	1x undetermined small newt (female palmate or smooth newt).	Too shallow and boggy at edges to bottle trap.
18	302259	372855	1	23/03/22	2	2	1	0	0	N/A	N/A	N/A	-	2x smooth newt.	Unable to bottle trap due to boggy area around waters edge
	302259	372855	2	05/04/22	2	2	0	0	0	N/A	N/A	N/A	-	None.	Turbid in middle, boggy / grassy at edges.
	302259	372855	3	19/04/22	2	3	0	0	0	N/A	N/A	N/A	-	None.	Boggy around pond but could still get a good view for torching.
	302259	372855	4	18/05/22	3	2	0	0	0	N/A	N/A	N/A	-	1x smooth newt.	Boggy around pond but could still get a good view for torching.
	302259	372855	5	23/05/22	2	3	0	0	0	N/A	N/A	N/A	-	None.	Too shallow and boggy at edges to bottle trap.
	302259	372855	6	30/05/22	3	3	0	0	0	N/A	N/A	N/A	-	1x undetermined small newt (female palmate or smooth newt).	Too shallow and boggy at edges to bottle trap.
19	302313	372955	1	19/04/22	1	3	1	0	0	N/A	N/A	N/A	-	1x smooth newt, 1x undetermined small newt (female palmate or smooth newt).	Too shallow and boggy at edges to bottle trap.
	302313	372955	2	23/05/22	3	1	0	0	0	N/A	N/A	N/A	-	3x smooth newt, 1x undetermined small newt (female palmate or smooth newt), 1x common frog.	Too shallow and boggy at edges to bottle trap.
	302313	372955	3	30/05/22	3	1	0	2	0	N/A	N/A	N/A	-	None.	-
21	302440	373139	1	19/04/22	4	2	2	0	0	N/A	N/A	N/A	-	1x common frog.	-
	302440	373139	2	23/05/22	4	1	0	0	0	N/A	N/A	N/A	-	1x smooth newt, 4x undetermined small newt	Margins clear. Steep sided pond.

Pond #	Easting	Northing	Visit #	Date	Vegetation cover	Turbidity (0-5)	GCN torching			GCN bottle trap			GCN egg (+/-)	Other amphibians	Comments
							Male	Female	Juvenile	Male	Female	Juvenile			
														(female palmate or smooth newt), 2x common frog.	
	302440	373139	3	30/05/22	4	1	0	0	0	N/A	N/A	N/A	-	1x smooth newt, 9x undetermined small newt (female palmate or smooth newt), 3x common frog (+ tadpoles).	-
22	302362	372632	1	19/04/22	0	2	0	0	0	N/A	N/A	N/A	-	5x smooth newt, 19x undetermined small newt (female palmate or smooth newt).	Unable to bottle trap due to livestock.
24	302730	373036	1	20/04/22	3	0	2	1	0	N/A	N/A	N/A	-	1x common frog.	Too boggy at edges to bottle trap
	302730	373036	2	23/05/22	3	3	0	0	0	N/A	N/A	N/A	-	Common frog tadpoles.	-
	302730	373036	3	30/05/22	4	1	0	0	0	N/A	N/A	N/A		None.	-
25	302740	372972	1	20/04/22	4	0	0	0	0	N/A	N/A	N/A	-	None.	Too boggy at edges to bottle trap
28	302953	372526	1	20/04/22	0	3	0	0	0	N/A	N/A	N/A	-	2x smooth newt, 6x undetermined small newt (female palmate or smooth newt), 1x common frog.	Pond perimeter densely vegetated, only 75% accessible in shallower parts of pond
29	303016	372412	1	20/04/22	5	1	0	0	0	N/A	N/A	N/A	-	None.	Pond very inaccessible (95%) due to very densely vegetation.
	303016	372412	2	23/05/22	5	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Water level too low and vegetation cover too high – unable to survey.
30	302572	372058	1	20/04/22	1	3	0	0	0	N/A	N/A	N/A	-	1x smooth newt, 4x undetermined small newt (female palmate or smooth newt).	-
	302572	372058	2	23/05/22	2	3	0	0	0	N/A	N/A	N/A	-	None.	Too boggy at edges to bottle trap
31	302433	372163	1	20/04/22	0	2	0	0	0	N/A	N/A	N/A	-	1x smooth newt, 5x palmate newt, 6x undetermined small newt (female palmate or smooth newt).	Too shallow to trap
33	302896	372673	1	20/04/22	4	2	1	3	0	N/A	N/A	N/A	-	4x smooth newt, 9x smooth newt.	Too shallow to trap
	302896	372673	2	23/05/22	1	1	0	0	0	N/A	N/A	N/A	-	7x undetermined small newt	-

Pond #	Easting	Northing	Visit #	Date	Vegetation cover	Turbidity (0-5)	GCN torching			GCN bottle trap			GCN egg (+/-)	Other amphibians	Comments
							Male	Female	Juvenile	Male	Female	Juvenile			
														(female palmate or smooth newt).	
34	302068	372795	1	20/04/22	5	N/A	N/A	N/A	N/A	0	0	0	-	None.	Unable to torch due to vegetation. Water level has reduced, perimeter very boggy in places
	302068	372795	2	23/05/22	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Pond dry.

Appendix F Great Crested Newt eDNA Survey Results 2022

Results of Great Crested Newt Surveys 2022 - 2024

St Asaph Solar Farm

Anesco

SLR Project No.: 406.065274.00001

29 April 2025

Client: Emma Clark,
SLR Consulting

ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

Tel: 01159 229249
Email: Helen.Rees@adas.co.uk

www.adas.uk

Sample ID: ADAS-3057	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: Pond 31 St Asaph, 424.05075.00154 Phase 0003	Description: pond water samples in preservative	
Date of Receipt: 25/04/2022	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	27/04/2022
Degradation Control[§]	Within Limits	Real Time PCR	27/04/2022
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	27/04/2022
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)[#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:		Signed:	
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	29/04/2022	Date of issue:	29/04/2022

eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.*

[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#]Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

Client: Emma Clark,
SLR Consulting

ADAS
Spring Lodge
172 Chester Road
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WA6 0AR

Tel: 01159 229249
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Sample ID: ADAS-3061	Condition on Receipt: Medium Sediment	Volume: Passed
Client Identifier: Pond 6 St Asaph, 424.05075.00154 Phase 0003	Description: pond water samples in preservative	
Date of Receipt: 25/04/2022	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	27/04/2022
Degradation Control[§]	Within Limits	Real Time PCR	27/04/2022
Great Crested Newt*	8 of 12 (GCN positive)	Real Time PCR	27/04/2022
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)[#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:		Signed:	
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	29/04/2022	Date of issue:	29/04/2022

eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.*

[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#]Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

Client: Emma Clark,
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Tel: 01159 229249
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Sample ID: ADAS-3062	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: Pond 30 St Asaph, 424.05075.00154 Phase 0003	Description: pond water samples in preservative	
Date of Receipt: 25/04/2022	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	27/04/2022
Degradation Control[§]	Within Limits	Real Time PCR	27/04/2022
Great Crested Newt*	11 of 12 (GCN positive)	Real Time PCR	27/04/2022
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)[#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:		Signed:	
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	29/04/2022	Date of issue:	29/04/2022

eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.*

[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#]Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

Client: Emma Clark,
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ADAS
Spring Lodge
172 Chester Road
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Tel: 01159 229249
Email: Helen.Rees@adas.co.uk

www.adas.uk

Sample ID: ADAS-3063	Condition on Receipt: Medium Sediment	Volume: Passed
Client Identifier: Pond 28 St Asaph, 424.05075.00154 Phase 0003	Description: pond water samples in preservative	
Date of Receipt: 25/04/2022	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	0 of 2	Real Time PCR	27/04/2022
Degradation Control[§]	Within Limits	Real Time PCR	27/04/2022
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	27/04/2022
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)[#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
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Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	29/04/2022	Date of issue:	29/04/2022

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** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.*

[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#]Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

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Sample ID: ADAS-3065	Condition on Receipt: Medium Sediment	Volume: Passed
Client Identifier: Pond 2 St Asaph, 424.05075.00154 Phase 0003	Description: pond water samples in preservative	
Date of Receipt: 25/04/2022	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	0 of 2	Real Time PCR	27/04/2022
Degradation Control[§]	Within Limits	Real Time PCR	27/04/2022
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	27/04/2022
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)[#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
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Date of preparation:	29/04/2022	Date of issue:	29/04/2022

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[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#]Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

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Sample ID: ADAS-3066	Condition on Receipt: Medium Sediment	Volume: Passed
Client Identifier: Pond 5 St Asaph, 424.05075.00154 Phase 0003	Description: pond water samples in preservative	
Date of Receipt: 25/04/2022	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	0 of 2	Real Time PCR	27/04/2022
Degradation Control[§]	Within Limits	Real Time PCR	27/04/2022
Great Crested Newt*	2 of 12 (GCN positive)	Real Time PCR	27/04/2022
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)[#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
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Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	29/04/2022	Date of issue:	29/04/2022

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[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#]Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

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Sample ID: ADAS-3067	Condition on Receipt: Medium Sediment	Volume: Passed
Client Identifier: Pond 29 St Asaph, 424.05075.00154 Phase 0003	Description: pond water samples in preservative	
Date of Receipt: 25/04/2022	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	27/04/2022
Degradation Control[§]	Evidence of degradation	Real Time PCR	27/04/2022
Great Crested Newt*	Indeterminate	Real Time PCR	27/04/2022
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)[#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
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Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	29/04/2022	Date of issue:	29/04/2022

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[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#]Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

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Sample ID: ADAS-3069	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: Pond 34 St Asaph, 424.05075.00154 Phase 0003	Description: pond water samples in preservative	
Date of Receipt: 25/04/2022	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	27/04/2022
Degradation Control[§]	Within Limits	Real Time PCR	27/04/2022
Great Crested Newt*	2 of 12 (GCN positive)	Real Time PCR	27/04/2022
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)[#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
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Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	29/04/2022	Date of issue:	29/04/2022

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[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#]Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

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Sample ID: ADAS-3070	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: Pond 7 St Asaph, 424.05075.00154 Phase 0003	Description: pond water samples in preservative	
Date of Receipt: 25/04/2022	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	27/04/2022
Degradation Control[§]	Within Limits	Real Time PCR	27/04/2022
Great Crested Newt*	1 of 12 (GCN positive)	Real Time PCR	27/04/2022
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)[#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
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Date of preparation:	29/04/2022	Date of issue:	29/04/2022

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[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#]Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

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Sample ID: ADAS-3071	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: Pond 1 St Asaph, 424.05075.00154 Phase 0003	Description: pond water samples in preservative	
Date of Receipt: 25/04/2022	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	27/04/2022
Degradation Control[§]	Within Limits	Real Time PCR	27/04/2022
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	27/04/2022
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)[#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
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Date of preparation:	29/04/2022	Date of issue:	29/04/2022

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[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#]Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

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Sample ID: ADAS-3072	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: Pond 25 St Asaph, 424.05075.00154 Phase 0003	Description: pond water samples in preservative	
Date of Receipt: 25/04/2022	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	28/04/2022
Degradation Control[§]	Within Limits	Real Time PCR	28/04/2022
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	28/04/2022
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)[#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
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Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	29/04/2022	Date of issue:	29/04/2022

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[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#]Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

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Sample ID: ADAS-3073	Condition on Receipt: Medium Sediment	Volume: Passed
Client Identifier: Pond 22 St Asaph, 424.05075.00154 Phase 0003	Description: pond water samples in preservative	
Date of Receipt: 25/04/2022	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	27/04/2022
Degradation Control[§]	Within Limits	Real Time PCR	27/04/2022
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	27/04/2022
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)[#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:		Signed:	
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	29/04/2022	Date of issue:	29/04/2022

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[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#]Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

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Sample ID: ADAS-3073	Condition on Receipt: Medium Sediment	Volume: Passed
Client Identifier: Pond 4 St Asaph, 424.05075.00154 Phase 0003	Description: pond water samples in preservative	
Date of Receipt: 25/04/2022	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	27/04/2022
Degradation Control[§]	Within Limits	Real Time PCR	27/04/2022
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	27/04/2022
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)[#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
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[#]Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

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Sample ID: ADAS-3075	Condition on Receipt: Medium Sediment	Volume: Passed
Client Identifier: Pond 16 St Asaph, 424.05075.00154 Phase 0003	Description: pond water samples in preservative	
Date of Receipt: 25/04/2022	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	27/04/2022
Degradation Control[§]	Within Limits	Real Time PCR	27/04/2022
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	27/04/2022
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)[#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
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Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	29/04/2022	Date of issue:	29/04/2022

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[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#]Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

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Sample ID: ADAS-3076	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: Pond 8 St Asaph, 424.05075.00154 Phase 0003	Description: pond water samples in preservative	
Date of Receipt: 25/04/2022	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	27/04/2022
Degradation Control[§]	Within Limits	Real Time PCR	27/04/2022
Great Crested Newt*	1 of 12 (GCN positive)	Real Time PCR	27/04/2022
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)[#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:		Signed:	
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	29/04/2022	Date of issue:	29/04/2022

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[#]Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

Appendix 1: Interpretation of results

Sample Condition

Upon sample receipt we score your samples according to quality: good, low sediment, medium sediment, high sediment, white precipitate, and presence of algae.

There are three reasons as to why sediment should be avoided:

1. It is possible for DNA to persist within the sediment for longer than it would if it was floating in the water which could lead to a false positive result i.e. in this case GCN not recently present but present a long time ago
2. In some cases sediment can cause inhibition of the PCR analysis used to detect GCN eDNA within samples which could lead to an indeterminate result.
3. In some cases sediment can interfere with the DNA extraction procedure resulting in poor recovery of the eDNA which in turn can lead to an indeterminate result.

Algae can make the DNA extraction more difficult to perform so if it can be avoided then this is helpful.

Sometimes samples contain a white precipitate which we have found makes the recovery of eDNA very difficult. This precipitate can be present in such high amounts that it interferes with the eDNA extraction process meaning that we cannot recover the degradation control (nor most likely the eDNA itself) at sufficient levels for the control to be within the acceptable limits for the assay, therefore we have to classify these type of samples as indeterminate.

What do my results mean?

A positive result means that great crested newts are present in the water or have been present in the water in the recent past (eDNA degrades over around 7-21 days).

A negative result means that DNA from the great crested newt has not been detected in your sample.

On occasion an inconclusive result will be issued. This occurs where the DNA from the great crested newt has not been detected but the controls have indicated that either: the sample has been degraded and/or the eDNA was not fully extracted (poor recovery); or the PCR inhibited in some way. This may be due to the water chemistry or may be due to the presence of high levels of sediment in samples which can interfere with the DNA extraction process. A re-test could be performed but a fresh sample would need to be obtained. We have successfully performed re-tests on samples which have had high sediment content on the first collection and low sediment content (through improved sample collection) on the re-test. If water chemistry was the cause of the indeterminate then a re-test would most likely also return an inconclusive result.

The results will be recorded as indeterminate if the GCN result is negative and the degradation result is recorded as:

1. evidence of decay - meaning that the degradation control was outside of accepted limits
2. evidence of degradation or residual inhibition - meaning that the degradation control was outside of accepted limits but that this could have been due to inhibitors not being removed sufficiently by the dilution of inhibited samples (according to the technical advice note)

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Sample ID: ADAS-5883	Condition on Receipt: Good	Volume: Passed
Client Identifier: P36, St Asaph	Description: pond water samples in preservative	
Date of Receipt: 04/07/2022	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	08/07/2022
Degradation Control[§]	Within Limits	Real Time PCR	08/07/2022
Great Crested Newt*	9 of 12 (GCN positive)	Real Time PCR	08/07/2022
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)[#]	4 of 4	Real Time PCR	As above for GCN

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Date of preparation:	11/07/2022	Date of issue:	11/07/2022

eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.*

[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#]Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

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Sample ID: ADAS-5884	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: P51, St Asaph	Description: pond water samples in preservative	
Date of Receipt: 04/07/2022	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	07/07/2022
Degradation Control[§]	Within Limits	Real Time PCR	07/07/2022
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	07/07/2022
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)[#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:		Signed:	
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	11/07/2022	Date of issue:	11/07/2022

eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.*

[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#]Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

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Sample ID: ADAS-5893	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: P50, St Asaph	Description: pond water samples in preservative	
Date of Receipt: 04/07/2022	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	0 of 2	Real Time PCR	08/07/2022
Degradation Control[§]	Within Limits	Real Time PCR	08/07/2022
Great Crested Newt*	12 of 12 (GCN positive)	Real Time PCR	08/07/2022
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)[#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
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Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	11/07/2022	Date of issue:	11/07/2022

eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.*

[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#]Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

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Sample ID: ADAS-5882	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: P23, St Asaph	Description: pond water samples in preservative	
Date of Receipt: 04/07/2022	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	08/07/2022
Degradation Control[§]	Within Limits	Real Time PCR	08/07/2022
Great Crested Newt*	3 of 12 (GCN positive)	Real Time PCR	08/07/2022
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)[#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:		Signed:	
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	11/07/2022	Date of issue:	11/07/2022

eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.*

[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#]Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

Appendix 1: Interpretation of results

Sample Condition

Upon sample receipt we score your samples according to quality: good, low sediment, medium sediment, high sediment, white precipitate, and presence of algae.

There are three reasons as to why sediment should be avoided:

1. It is possible for DNA to persist within the sediment for longer than it would if it was floating in the water which could lead to a false positive result i.e. in this case GCN not recently present but present a long time ago
2. In some cases sediment can cause inhibition of the PCR analysis used to detect GCN eDNA within samples which could lead to an indeterminate result.
3. In some cases sediment can interfere with the DNA extraction procedure resulting in poor recovery of the eDNA which in turn can lead to an indeterminate result.

Algae can make the DNA extraction more difficult to perform so if it can be avoided then this is helpful.

Sometimes samples contain a white precipitate which we have found makes the recovery of eDNA very difficult. This precipitate can be present in such high amounts that it interferes with the eDNA extraction process meaning that we cannot recover the degradation control (nor most likely the eDNA itself) at sufficient levels for the control to be within the acceptable limits for the assay, therefore we have to classify these type of samples as indeterminate.

What do my results mean?

A positive result means that great crested newts are present in the water or have been present in the water in the recent past (eDNA degrades over around 7-21 days).

A negative result means that DNA from the great crested newt has not been detected in your sample.

On occasion an inconclusive result will be issued. This occurs where the DNA from the great crested newt has not been detected but the controls have indicated that either: the sample has been degraded and/or the eDNA was not fully extracted (poor recovery); or the PCR inhibited in some way. This may be due to the water chemistry or may be due to the presence of high levels of sediment in samples which can interfere with the DNA extraction process. A re-test could be performed but a fresh sample would need to be obtained. We have successfully performed re-tests on samples which have had high sediment content on the first collection and low sediment content (through improved sample collection) on the re-test. If water chemistry was the cause of the indeterminate then a re-test would most likely also return an inconclusive result.

The results will be recorded as indeterminate if the GCN result is negative and the degradation result is recorded as:

1. evidence of decay - meaning that the degradation control was outside of accepted limits
2. evidence of degradation or residual inhibition - meaning that the degradation control was outside of accepted limits but that this could have been due to inhibitors not being removed sufficiently by the dilution of inhibited samples (according to the technical advice note)

Appendix G Great Crested Newt eDNA Survey Results 2024

Results of Great Crested Newt Surveys 2022 - 2024

St Asaph Solar Farm

Anesco

SLR Project No.: 406.065274.00001

29 April 2025

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Sample ID: ADAS-6345	Condition on Receipt: Good	Volume: Passed
Client Identifier: P60, St Asaph	Description: pond water samples in preservative	
Date of Receipt: 02/07/2024	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	12/07/2024
Degradation Control[§]	Within Limits	Real Time PCR	12/07/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	12/07/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)[#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
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Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	16/07/2024	Date of issue:	16/07/2024

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** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.*

[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#]Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

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Sample ID: ADAS-6347	Condition on Receipt: Algae Present	Volume: Passed
Client Identifier: P63, St Asaph	Description: pond water samples in preservative	
Date of Receipt: 02/07/2024	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	0 of 2	Real Time PCR	12/07/2024
Degradation Control[§]	Within Limits	Real Time PCR	12/07/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	12/07/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)[#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
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Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	16/07/2024	Date of issue:	16/07/2024

eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.*

[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#]Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

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Sample ID: ADAS-6348	Condition on Receipt: Medium Sediment	Volume: Passed
Client Identifier: P48, St Asaph	Description: pond water samples in preservative	
Date of Receipt: 02/07/2024	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	12/07/2024
Degradation Control[§]	Within Limits	Real Time PCR	12/07/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	12/07/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)[#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
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Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	16/07/2024	Date of issue:	16/07/2024

eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.*

[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#]Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

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Sample ID: ADAS-6349	Condition on Receipt: Good	Volume: Passed
Client Identifier: P59, St Asaph	Description: pond water samples in preservative	
Date of Receipt: 02/07/2024	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	12/07/2024
Degradation Control[§]	Within Limits	Real Time PCR	12/07/2024
Great Crested Newt*	1 of 12 (GCN positive)	Real Time PCR	12/07/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)[#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:		Signed:	
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	16/07/2024	Date of issue:	16/07/2024

eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

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[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#]Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

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Sample ID: ADAS-6350	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: P54, St Asaph	Description: pond water samples in preservative	
Date of Receipt: 02/07/2024	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	12/07/2024
Degradation Control[§]	Within Limits	Real Time PCR	12/07/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	12/07/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)[#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
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Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	16/07/2024	Date of issue:	16/07/2024

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[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#]Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

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Sample ID: ADAS-6351	Condition on Receipt: Algae Present	Volume: Passed
Client Identifier: P69, St Asaph	Description: pond water samples in preservative	
Date of Receipt: 02/07/2024	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	11/07/2024
Degradation Control[§]	Within Limits	Real Time PCR	11/07/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	11/07/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)[#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
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Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	16/07/2024	Date of issue:	16/07/2024

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[#]Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

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Sample ID: ADAS-6352	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: P68, St Asaph	Description: pond water samples in preservative	
Date of Receipt: 02/07/2024	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	12/07/2024
Degradation Control[§]	Within Limits	Real Time PCR	12/07/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	12/07/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)[#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:		Signed:	
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	16/07/2024	Date of issue:	16/07/2024

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[#]Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

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Sample ID: ADAS-6353	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: P67, St Asaph	Description: pond water samples in preservative	
Date of Receipt: 02/07/2024	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	12/07/2024
Degradation Control[§]	Within Limits	Real Time PCR	12/07/2024
Great Crested Newt*	12 of 12 (GCN positive)	Real Time PCR	12/07/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)[#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:		Signed:	
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	16/07/2024	Date of issue:	16/07/2024

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[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

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[#]Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

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Sample ID: ADAS-6354	Condition on Receipt: White Precipitate	Volume: Passed
Client Identifier: P65, St Asaph	Description: pond water samples in preservative	
Date of Receipt: 02/07/2024	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	0 of 2	Real Time PCR	12/07/2024
Degradation Control[§]	Within Limits	Real Time PCR	12/07/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	12/07/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)[#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
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Position:	Director: Biotechnology	Position:	MD: Biotechnology
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Sample ID: ADAS-6356	Condition on Receipt: Good	Volume: Passed
Client Identifier: P66, St Asaph	Description: pond water samples in preservative	
Date of Receipt: 02/07/2024	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	12/07/2024
Degradation Control[§]	Within Limits	Real Time PCR	12/07/2024
Great Crested Newt*	11 of 12 (GCN positive)	Real Time PCR	12/07/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)[#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
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Sample ID: ADAS-6357	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: P32, St Asaph	Description: pond water samples in preservative	
Date of Receipt: 02/07/2024	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	12/07/2024
Degradation Control[§]	Within Limits	Real Time PCR	12/07/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	12/07/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)[#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:		Signed:	
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	16/07/2024	Date of issue:	16/07/2024

eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.*

[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#]Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

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Sample ID: ADAS-6360	Condition on Receipt: Good	Volume: Passed
Client Identifier: P61, St Asaph	Description: pond water samples in preservative	
Date of Receipt: 02/07/2024	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	12/07/2024
Degradation Control[§]	Within Limits	Real Time PCR	12/07/2024
Great Crested Newt*	6 of 12 (GCN positive)	Real Time PCR	12/07/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)[#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:		Signed:	
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	16/07/2024	Date of issue:	16/07/2024

eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.*

[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#]Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

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Sample ID: ADAS-6361	Condition on Receipt: Algae Present	Volume: Passed
Client Identifier: P2, St Asaph	Description: pond water samples in preservative	
Date of Receipt: 02/07/2024	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	1 of 2	Real Time PCR	12/07/2024
Degradation Control[§]	Within Limits	Real Time PCR	12/07/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	12/07/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)[#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:		Signed:	
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	16/07/2024	Date of issue:	16/07/2024

eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.*

[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#]Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

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Sample ID: ADAS-6366	Condition on Receipt: Algae Present	Volume: Passed
Client Identifier: P20, St Asaph	Description: pond water samples in preservative	
Date of Receipt: 02/07/2024	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	0 of 2	Real Time PCR	12/07/2024
Degradation Control[§]	Within Limits	Real Time PCR	12/07/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	12/07/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)[#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:		Signed:	
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	16/07/2024	Date of issue:	16/07/2024

eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.*

[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#]Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

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Sample ID: ADAS-6368	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: P31, St Asaph	Description: pond water samples in preservative	
Date of Receipt: 02/07/2024	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	12/07/2024
Degradation Control[§]	Within Limits	Real Time PCR	12/07/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	12/07/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)[#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:		Signed:	
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	16/07/2024	Date of issue:	16/07/2024

eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.*

[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#]Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

Appendix 1: Interpretation of results

Sample Condition

Upon sample receipt we score your samples according to quality: good, low sediment, medium sediment, high sediment, white precipitate, and presence of algae.

There are three reasons as to why sediment should be avoided:

1. It is possible for DNA to persist within the sediment for longer than it would if it was floating in the water which could lead to a false positive result i.e. in this case GCN not recently present but present a long time ago
2. In some cases sediment can cause inhibition of the PCR analysis used to detect GCN eDNA within samples which could lead to an indeterminate result.
3. In some cases sediment can interfere with the DNA extraction procedure resulting in poor recovery of the eDNA which in turn can lead to an indeterminate result.

Algae can make the DNA extraction more difficult to perform so if it can be avoided then this is helpful.

Sometimes samples contain a white precipitate which we have found makes the recovery of eDNA very difficult. This precipitate can be present in such high amounts that it interferes with the eDNA extraction process meaning that we cannot recover the degradation control (nor most likely the eDNA itself) at sufficient levels for the control to be within the acceptable limits for the assay, therefore we have to classify these type of samples as indeterminate.

What do my results mean?

A positive result means that great crested newts are present in the water or have been present in the water in the recent past (eDNA degrades over around 7-21 days).

A negative result means that DNA from the great crested newt has not been detected in your sample.

On occasion an inconclusive result will be issued. This occurs where the DNA from the great crested newt has not been detected but the controls have indicated that either: the sample has been degraded and/or the eDNA was not fully extracted (poor recovery); or the PCR inhibited in some way. This may be due to the water chemistry or may be due to the presence of high levels of sediment in samples which can interfere with the DNA extraction process. A re-test could be performed but a fresh sample would need to be obtained. We have successfully performed re-tests on samples which have had high sediment content on the first collection and low sediment content (through improved sample collection) on the re-test. If water chemistry was the cause of the indeterminate then a re-test would most likely also return an inconclusive result.

The results will be recorded as indeterminate if the GCN result is negative and the degradation result is recorded as:

1. evidence of decay - meaning that the degradation control was outside of accepted limits
2. evidence of degradation or residual inhibition - meaning that the degradation control was outside of accepted limits but that this could have been due to inhibitors not being removed sufficiently by the dilution of inhibited samples (according to the technical advice note)

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Sample ID: ADAS-6355	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: P23, St Asaph	Description: pond water samples in preservative	
Date of Receipt: 02/07/2024	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	17/07/2024
Degradation Control[§]	Within Limits	Real Time PCR	17/07/2024
Great Crested Newt*	2 of 12 (GCN positive)	Real Time PCR	17/07/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)[#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:		Signed:	
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	17/07/2024	Date of issue:	17/07/2024

eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.*

[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#]Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

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Sample ID: ADAS-6358	Condition on Receipt: Algae Present	Volume: Passed
Client Identifier: P29, St Asaph	Description: pond water samples in preservative	
Date of Receipt: 02/07/2024	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	17/07/2024
Degradation Control[§]	Within Limits	Real Time PCR	17/07/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	17/07/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)[#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:		Signed:	
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	17/07/2024	Date of issue:	17/07/2024

eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.*

[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#]Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

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Sample ID: ADAS-6359	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: P25, St Asaph	Description: pond water samples in preservative	
Date of Receipt: 02/07/2024	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	17/07/2024
Degradation Control[§]	Within Limits	Real Time PCR	17/07/2024
Great Crested Newt*	1 of 12 (GCN positive)	Real Time PCR	17/07/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)[#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:		Signed:	
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	17/07/2024	Date of issue:	17/07/2024

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** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.*

[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#]Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

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Sample ID: ADAS-6362	Condition on Receipt: Algae Present	Volume: Passed
Client Identifier: P4, St Asaph	Description: pond water samples in preservative	
Date of Receipt: 02/07/2024	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	17/07/2024
Degradation Control[§]	Within Limits	Real Time PCR	17/07/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	17/07/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)[#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:		Signed:	
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	17/07/2024	Date of issue:	17/07/2024

eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

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[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#]Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

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Sample ID: ADAS-6363	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: P22, St Asaph	Description: pond water samples in preservative	
Date of Receipt: 02/07/2024	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	17/07/2024
Degradation Control[§]	Within Limits	Real Time PCR	17/07/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	17/07/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)[#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:		Signed:	
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	17/07/2024	Date of issue:	17/07/2024

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[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#]Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

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Sample ID: ADAS-6364	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: P43, St Asaph	Description: pond water samples in preservative	
Date of Receipt: 02/07/2024	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	17/07/2024
Degradation Control[§]	Within Limits	Real Time PCR	17/07/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	17/07/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)[#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:		Signed:	
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	17/07/2024	Date of issue:	17/07/2024

eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

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[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#]Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

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Sample ID: ADAS-6367	Condition on Receipt: Medium Sediment	Volume: Passed
Client Identifier: P28, St Asaph	Description: pond water samples in preservative	
Date of Receipt: 02/07/2024	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	17/07/2024
Degradation Control[§]	Within Limits	Real Time PCR	17/07/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	17/07/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10^{-4} ng/µL)[#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by:	Dr Helen Rees	Report Issued by:	Dr Ben Maddison
Signed:		Signed:	
Position:	Director: Biotechnology	Position:	MD: Biotechnology
Date of preparation:	17/07/2024	Date of issue:	17/07/2024

eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.*

[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#]Additional positive controls (10^{-1}, 10^{-2}, 10^{-3} ng/µL) are also routinely run, results not shown here.

Appendix 1: Interpretation of results

Sample Condition

Upon sample receipt we score your samples according to quality: good, low sediment, medium sediment, high sediment, white precipitate, and presence of algae.

There are three reasons as to why sediment should be avoided:

1. It is possible for DNA to persist within the sediment for longer than it would if it was floating in the water which could lead to a false positive result i.e. in this case GCN not recently present but present a long time ago
2. In some cases sediment can cause inhibition of the PCR analysis used to detect GCN eDNA within samples which could lead to an indeterminate result.
3. In some cases sediment can interfere with the DNA extraction procedure resulting in poor recovery of the eDNA which in turn can lead to an indeterminate result.

Algae can make the DNA extraction more difficult to perform so if it can be avoided then this is helpful.

Sometimes samples contain a white precipitate which we have found makes the recovery of eDNA very difficult. This precipitate can be present in such high amounts that it interferes with the eDNA extraction process meaning that we cannot recover the degradation control (nor most likely the eDNA itself) at sufficient levels for the control to be within the acceptable limits for the assay, therefore we have to classify these type of samples as indeterminate.

What do my results mean?

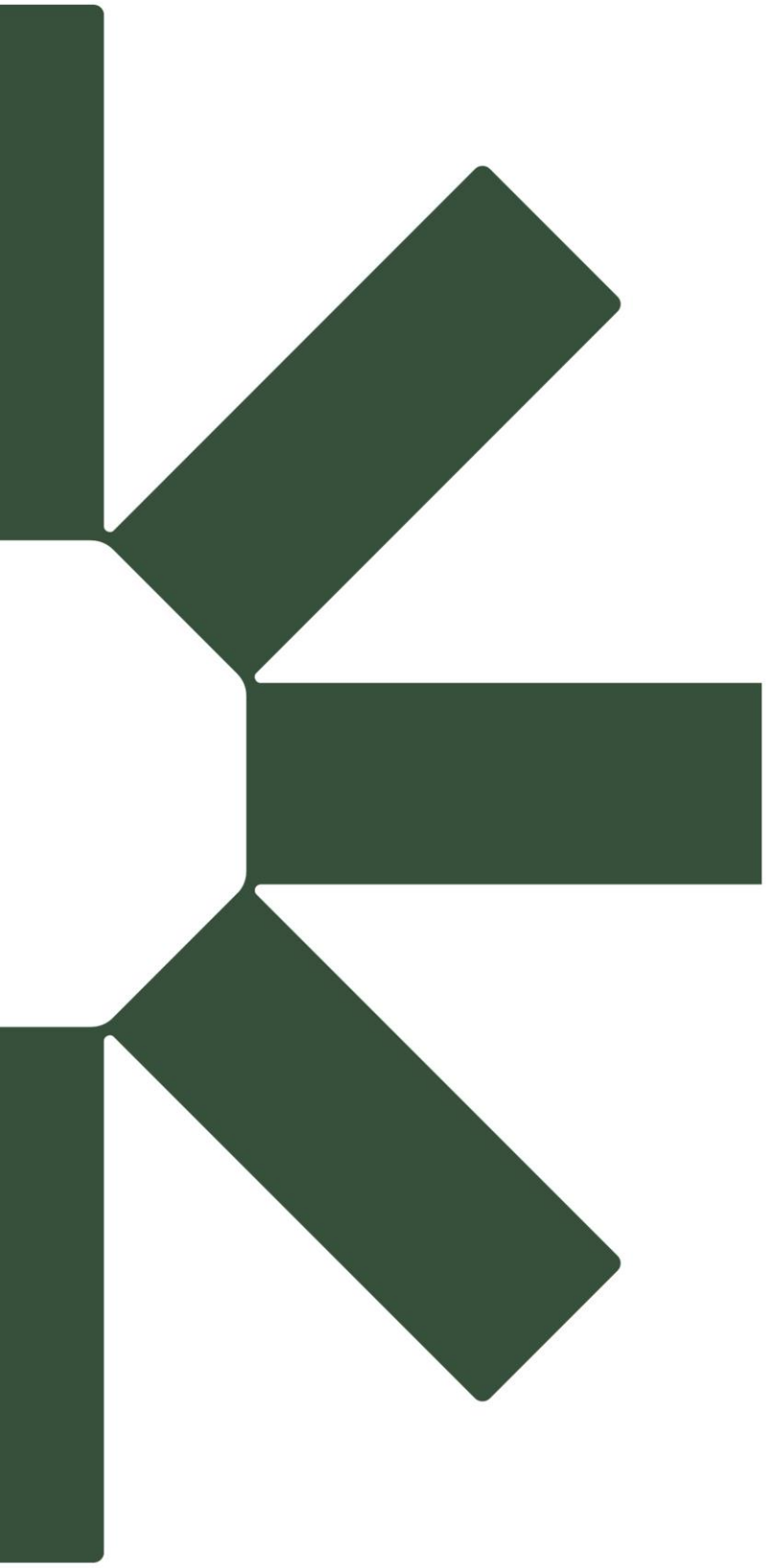
A positive result means that great crested newts are present in the water or have been present in the water in the recent past (eDNA degrades over around 7-21 days).

A negative result means that DNA from the great crested newt has not been detected in your sample.

On occasion an inconclusive result will be issued. This occurs where the DNA from the great crested newt has not been detected but the controls have indicated that either: the sample has been degraded and/or the eDNA was not fully extracted (poor recovery); or the PCR inhibited in some way. This may be due to the water chemistry or may be due to the presence of high levels of sediment in samples which can interfere with the DNA extraction process. A re-test could be performed but a fresh sample would need to be obtained. We have successfully performed re-tests on samples which have had high sediment content on the first collection and low sediment content (through improved sample collection) on the re-test. If water chemistry was the cause of the indeterminate then a re-test would most likely also return an inconclusive result.

The results will be recorded as indeterminate if the GCN result is negative and the degradation result is recorded as:

1. evidence of decay - meaning that the degradation control was outside of accepted limits
2. evidence of degradation or residual inhibition - meaning that the degradation control was outside of accepted limits but that this could have been due to inhibitors not being removed sufficiently by the dilution of inhibited samples (according to the technical advice note)



Making Sustainability Happen