Technical Appendix: Results of Great Crested Newt Surveys

Bold Forest Garden Village

St Helens Borough Council

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1.0 Introduction

SLR Consulting Limited (SLR) was commissioned by St. Helens Borough Council (SHBC) to undertake great crested newt (*Triturus cristatus*) (GCN) surveys of ponds located within Bold Forest Garden Village, located north of Gorsey Lane in St Helens, Merseyside (central Ordnance Survey (OS) grid reference: SJ 53825 92292), hereafter referred to as 'the Site'. These surveys are required to inform a masterplan framework for the Site which is included as an allocation for residential development in the St Helens Borough Council Local Plan Up To 2037 (the Local Plan)[1]. This report has been produced to present the results of the GCN surveys only. It will accompany an Ecological Impact Assessment (EcIA) which will consider the implications of these survey results on masterplan design and mitigation requirements.

1.1 Relevant Legislation

In England, GCNs are protected under the Conservation of Habitats and Species Regulations 2017 (as amended)[2] which consolidates the Conservation of Habitats and Species Regulations 2010 with subsequent amendments, which defines European protected species. GCNs are also protected under the Wildlife and Countryside Act (WCA) 1981[3], as amended by the Countryside and Rights of Way (CRoW) Act 2000[4] and the Natural Environment and Rural Communities (NERC) Act 2006[5].

These pieces of legislation combine to give substantial protection to GCNs and their resting places. In summary, it is an offence to:

- Deliberately/intentionally kill, injure or take a GCN;
- Take or destroy the eggs of a GCN;
- Possess or control any live or dead specimen or anything derived from a GCN;
- Recklessly damage, destroy or obstruct access to any structure or place used for shelter or protection by a GCN; and
- Deliberately/intentionally or recklessly disturb GCNs.

This legislation applies to all life-stages of the species, from egg through larvae to sub-adults and adult newts.

GCN is additionally listed on Section 41 of the NERC Act 2006[5] as a species of principal importance (SPI) for nature conservation.

1.2 Evidence of Technical Competence and Experience

The surveys were led by SLR Senior Ecologist Shona Redman BSc (Hons) MSc, assisted by Seasonal Ecologists Oran Coey BSc PGD and Phillippa Watt BSc (Hons) MSc, and The Mersey Forest staff members Airren Martin and Lauren Taylor. Shona is a Full member of the Chartered Institute of Ecology and Environmental Management (CIEEM) (MCIEEM) and has over seven years' experience as a professional ecologist. She holds a Natural England Level 1 class licence to survey for GCN and also holds a separate licence allowing the use of box traps. Oran and Philippa are qualifying members of CIEEM.

[1] https://sthelens.gov.uk/media/4315/St-Helens-Borough-Local-Plan-up-to-2037/pdf/Local_Plan_Written_Statement_-_FINAL_adoption_version.pdf?m=1658409100420

[2] https://www.legislation.gov.uk/uksi/2017/1012/contents

[3] https://www.legislation.gov.uk/ukpga/1981/69/contents

[4] https://www.legislation.gov.uk/ukpga/2000/37/contents

[5] https://www.legislation.gov.uk/ukpga/2006/16/contents

This report was authored by Shona Redman and has been subject to Quality Assurance review as per SLR's policies by Dr Kate Vincent. Kate is a Technical Director with over 20 years' experience in private, public and academic sectors. She is a Full member of CIEEM and a Chartered Environmentalist (CEnv).

1.3 Validity of data

According to CIEEM advice[6], survey data are valid for a period of 12 to 18 months from the date of the survey. The report highlights any circumstances where data may be valid for less than 18 months. Between 18 months and 3 years a professional ecologist will need to undertake a site visit and may also need to update desk study information (effectively updating the PEA) and then review the validity of the report.

[6] Chartered Institute of Ecology and Environmental Management (2019), Advice Note on the Lifespan of Ecological Reports & Surveys. CIEEM, Winchester, Hampshire.

2.0 Methodology

2.1 Desk Study

A desk study was conducted in July 2024 by The Mersey Forest. The methodology is provided within their Preliminary Ecological Appraisal (PEA) report[7]. A summary of the amphibian records returned within 2km of the Site is provided in this report.

2.2 Field Surveys

A total of 23 ponds were surveyed across the Site, comprising habitat suitability index (HSI) assessments, eDNA surveys, and presence/likely-absence and population size-class surveys. Further details on each methodology are provided in Sections 2.2.1 to 2.2.3.1 below.

Each pond (except those found to be dry) was subject to at least one presence/likely-absence survey whilst waiting for the eDNA results to ensure that surveys could be completed within the required timescales, should the ponds be positive for GCN eDNA. Where ponds were indeterminate or positive for eDNA further surveys were undertaken up to a total of four or six visits, respectively.

Survey dates, personnel and weather conditions for the surveys are presented in Tables 1 and 2.

Table 1: Survey dates

Pond no.	eDNA date	Presence/likely-absence and population size-class surveys					
		Survey 1 date	Survey 2 date	Survey 3 date	Survey 4 date	Survey 5 date	Survey 6 date
P3.1	15/04/2025	15/04/2025	N/A – eDNA negative				
P3.2	15/04/2025	15/04/2025	N/A – eDNA negative				
P3.3	01/05/2025	01/05/2025	N/A – eDNA negative				
P3.4	15/04/2025	15/04/2025	N/A – eDNA negative				
P3.5	15/04/2025	15/04/2025	01/05/2025	08/05/2025	N/A - Dry	N/A – GCN assumed absent	
P3.6	16/04/2025	22/04/2025	06/05/2025	13/05/2025	28/05/2025	N/A – GCN assumed absent	
P5.1	15/04/2025	15/04/2025	N/A – eDNA negative				
P5.2	15/04/2025	15/04/2025	N/A – eDNA negative				
P6.1	N/A – Pond dry						
P6.2	15/04/2025	14/04/2025	06/05/2025	12/05/2025	27/05/2025	N/A – GCN assumed absent	
P6/8	15/04/2025	14/04/2025	06/05/2025	12/05/2025	27/05/2025	02/06/2025	09/06/2025
P7.1	15/04/2025	14/04/2025	06/05/2025	12/05/2025	27/05/2025	N/A – GCN assumed absent	
P7.2	15/04/2025	14/04/2025	06/05/2025	12/05/2025	27/05/2025	N/A – GCN assumed absent	

[7] The Mersey Forest (2024), Bold Forest Garden Village Preliminary Ecological Appraisal and Biodiversity Net Gain Feasibility Report v3.

Pond no.	eDNA date	Presence/likely-absence and population size-class surveys					
		Survey 1 date	Survey 2 date	Survey 3 date	Survey 4 date	Survey 5 date	Survey 6 date
P9.1	15/04/2025	16/04/2025	07/05/2025	13/05/2025	28/05/2025	N/A – GCN assumed absent	
P9.2	15/04/2025	16/04/2025	07/05/2025	13/05/2025	28/05/2025	N/A – GCN assumed absent	
P11	16/04/2025	22/04/2025	N/A – eDNA negative				
P13	15/04/2025	16/04/2025	07/05/2025	12/05/2025	28/05/2025	N/A – GCN assumed absent	
P-LWS-1	01/05/2025	01/05/2025	N/A – eDNA negative				
P-LWS-2	16/04/2025	22/04/2025	01/05/2025	08/05/2025	15/05/2025	N/A – GCN assumed absent	
P-LWS-3	N/A – Pond dry						
P-LWS-4	01/05/2025	01/05/2025	N/A – eDNA negative				
P-LWS-5/6	01/05/2025	01/05/2025	08/05/2025	15/05/2025	29/05/2025	02/06/2025	09/06/2025
P-LWS-7	16/04/2025	22/04/2025	08/05/2025	15/05/2025	29/05/2025	02/06/2025	09/06/2025
P-LWS-8	01/05/2025	01/05/2025	08/05/2025	15/05/2025	29/05/2025	N/A – GCN assumed absent	
P-LWS-9	16/04/2025	22/04/2025	08/05/2025	15/05/2025	29/05/2025	N/A – GCN assumed absent	

Table 2: Personnel and weather conditions

Survey date	Surveyors	Minimum overnight temperature (°C)	Wind (Beaufort)	Precipitation
14/04/2025	Shona Redman assisted by Airren Martin	7	2 - 3	None
15/04/2025	Shona Redman assisted by Airren Martin	7	5	None
16/04/2025	Shona Redman assisted by Airren Martin	5	3	None
22/04/2025	Shona Redman assisted by Pip Watt	7	4	None
01/05/2025	Shona Redman assisted by Airren Martin	9	3	None
06/05/2025	Shona Redman and Tom Redman	7	2	None
07/05/2025	Shona Redman assisted by Oran Coey	7	3	None
08/05/2025	Shona Redman assisted by Lauren Taylor	5	3	None
12/05/2025	Shona Redman assisted by Lauren Taylor	11	4	None
13/05/2025	Shona Redman assisted by Oran Coey	9	4	None
15/05/2025	Shona Redman assisted by Lauren Taylor	7	3	None
27/05/2025	Shona Redman assisted by Pip Watt	10	2	None

Survey date	Surveyors	Minimum overnight temperature (°C)	Wind (Beaufort)	Precipitation
28/05/2025	Shona Redman assisted by Pip Watt	12	2	None
29/05/2025	Shona Redman assisted by Lauren Taylor	15	4	None
02/06/2025	Shona Redman assisted by Lauren Taylor	11	3	None
09/06/2025	Shona Redman assisted by Lauren Taylor	12	3	None

2.2.1 Habitat Suitability Index (HSI) Surveys

A total of 26 ponds were identified within the Site by The Mersey Forest[7] and their locations are presented in Figure 1. This report uses the same pond numbers as the PEA report for consistency. Eighteen of the ponds were subject to a Habitat Suitability Index (HSI) assessment by The Mersey Forest between April and September 2024, to gauge their potential to support GCN. A summary of these assessments is provided in this report.

Six ponds were dry during The Mersey Forest 2024 surveys and two could not be accessed so were not subject to a HSI assessment. However, five of these (P9.1, P9.2, P-LWS-4, P-LWS-5/6 and P-LWS-8) were found to contain water during SLR's 2025 surveys and HSI assessments were undertaken, the results of which are provided in this report. Ponds LWS-5 and LWS-6 were found to be connected and as such, were assessed and surveyed as a single pond, hereafter referred to as 'P-LWS-5/6'.

GCN HSI scores are calculated using ten parameters[8]: site 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 following categorical scale is then used to estimate the overall suitability of the water body concerned:

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

There are further ponds within 500m of the Site. However, these were not within the scope of the surveys to information masterplan design. They will, however, require consideration for individual planning applications submitted within the masterplan area.

[8] Oldham, R.S., Keeble, J., Swan, M.J.S., and Jeffcote, M. (2000) Evaluating the Suitability of Habitat for the Great Crested Newt (*Triturus cristatus*). Herpetological Journal 10: 143-155.

2.2.2 eDNA Surveys

In total, 23 ponds were surveyed for GCN using the environmental DNA (eDNA) technique following the standard protocol[9], provided they held water at the time of survey. This involved the collection of water samples from the ponds by licensed (or experienced and accredited) surveyors. The samples were then sent to a specialist laboratory for analysis using qPCR.

In accordance with the approved protocol, twenty samples were collected from the margins of the pond using sterile equipment. The water at each sampling area was gently stirred using a sterile ladle before samples were taken, to mix up DNA (if present) which tends to sink, whilst ensuring that sediment on the bottom of the pond was not disturbed, as this is 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 England approved method contained within Biggs *et al*. (2014). According to Biggs *et al*. (2014), 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.

To avoid possible contamination, the surveyor avoided entering the water. Latex gloves were worn when sampling and only sterile equipment came into contact with the water.

All samples were taken, or directly supervised, by licensed ecologist Shona Redman (see Section 1.2).

2.2.3 Presence / Likely-absence and Population Size-class Surveys

Ponds that were positive or indeterminate for GCN eDNA were subject to further survey. The methodology for these ponds were the same but the survey effort varied depending upon the aim of these surveys. For ‘indeterminate’ ponds, four survey visits were undertaken to establish if GCN were present or likely-absent in each pond. For ‘positive’ ponds, six survey visits were undertaken to establish the population size-class of GCN present. Where GCN was recorded in any of the four visits to ‘indeterminate’ ponds, then an additional two visits would have been undertaken to complete the six visits required to assess population size.

Surveys were carried out according to Natural England survey guidelines[10], which outline the survey standards as follows:

- four or six survey visits between mid-March and mid-June;
- survey in suitable weather;
- at least half of the survey visits between mid-April and mid-May; and
- survey using (where practical) a minimum of three methods, including egg searching, netting, torching and bottle trapping.

All surveys were led by GCN licensed ecologist Shona Redman (see Section 1.2).

2.2.3.1 Field Methods

Torching

This technique was carried out at night, when newts are most active. Negative results are only meaningful is the surveys are carried out in suitable weather. Ideal weather conditions

[9] Biggs, J et al. (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.

[10] English Nature (2001), Great Crested Newt Mitigation Guidelines. English Nature, Peterborough.

are given by Natural England[10] as a night-time air temperature of more than 5℃, little or no wind, and no rain. Torchlight surveys involved walking slowly around the edge of the pond and scanning the water with a high-powered spotlight (Clulite, 1,000,000 candle power) where access and safety permitted. Using this method, GCN can be easily identified from smooth or palmate newts (*Lissotriton helvetica*) in clear water and counted. Bright light may cause newts to seek cover where they will be undetected, but the technique is appropriate to establish presence and for estimating populations. The species, sex (if possible), number of newts, and survey times were determined and recorded.

Bottle trapping

This method involved trapping newts at night and if not carried out correctly, can be harmful to the trapped newts. Because of this, strict guidelines from Natural England[10] were followed.

The method is reliable for detecting presence of GCN, and is especially useful in weedy or turbid water where visibility is poor or the vegetation is too dense for torch surveys to give reliable results. 'Funnel traps' constructed from plastic bottles attached to bamboo canes were immersed in the pond in the evening and removed early the following morning. Newts enter through the funnel entrance but cannot find their way back out again. The recommended density of traps is one trap for every 2m of margin, placed 2m from the edge; though this depends on habitat suitability and substrate as well as the shore incline and depth of pond. Traps were checked early the next morning before the temperature rose, and the trapped newts were sexed, counted and released. This technique is appropriate to establish presence and for estimating populations

To minimise risks to any newts, all bottles were deployed with an air bubble and none were left for more than 12 hours despite the guidelines stating bottles with an air bubble can be left for up to 17 hours (except during periods of hot weather). Trapping was not used when ponds were too shallow, the margins were unsafe to walk on or the night air temperature was due to drop below 5℃.

Floating versions of these traps were deployed for the final three visits in P-LWS-7. These bottles work the same as normal funnel traps but can be thrown into the pond from a distance and then tied onto a bamboo cane around the pond perimeter. This pond had been unsafe to trap traditionally due to deep, sinking mud around the margin and these floating traps were used in an attempt to collect population size-class data for the pond as it was positive for eDNA and GCN had not been recorded via torching.

Egg search

This method involved searching the leaves of aquatic vegetation for the distinctive eggs of GCN, which is very effective for detecting presence and confirming breeding. However, egg searching can be difficult or unreliable in deep water or heavily vegetated ponds with small newt populations. This method was used in daylight during bottle-trap deployment / retrieval or netting when surveyors were by the pond margins and were likely to observe folded vegetation if present. This technique is appropriate to establish presence but not for estimating populations.

Netting

Netting was carried out using a long-handled dig-net with a fine mesh. The perimeter of the pond was walked, and 15 minutes of netting was carried out for every 50m of water-margin. The method is less effective than bottle trapping and torching when surveying for adult GCN, but is very useful when searching for larvae. This method was used as an alternative or extra method of survey when weather or other constraints did not allow efficient or safe bottle trapping. If a pond had significance quantities of dead leaf litter on the bottom, netting was not carried out due to the amount of disturbance that would be caused and subsequent

impact on the water quality of the pond. This technique is appropriate to establish presence but not for estimating populations.

2.2.3.2 Population Estimate

Population size-class estimates are calculated from the maximum number of GCN caught or seen using one survey method during one visit. The maximum count breaks down into three size-classes, as presented in Table 3.

Table 3: Population size-classes

Maximum count recorded from any single survey method	Population size-class
1-9	Small
10-99	Medium
100+	Large

2.3 Limitations

P6.1 and P-LWS-3 were found to be dry or not exist, and they were therefore not subject to survey.

Many of the ponds were surrounded by dense vegetation or steep banks, meaning that water samples for the eDNA surveys were not taken from the full pond perimeter and this is likely reflected in the number of 'indeterminate' results that were returned because of water samples only being collected on the more open sides, where farm run-off is more likely to contaminate the water. Presence/likely-absence surveys were undertaken of these ponds to determine whether GCN were present in the absence of conclusive eDNA results.

P6/8 has deep water and steep, vegetated banks. It was only surveyed from distance during the first population size-class visit due to surveyors being unfamiliar with the pond and being unable to find a safe way of accessing the pond margin in the dark. The pond was next accessed in daylight to identify a safe means of access and surveys were completed in full on all remaining visits. GCN were only recorded during two visits to this pond, with counts of seven and two, and the greater of these was recorded on visit 4. The first survey is considered unlikely to have recorded enough GCN that it would have instead been classified as a medium population (10+ GCN). This is not considered a significant limitation to the population size-class assessment of this pond.

The presence/likely-absence and population size-class surveys were undertaken at the correct time of year in suitable weather conditions. However, they were limited by the following factors which are outlined more specifically for each pond in Appendix C:

- a turbidity score of 4 or above limiting effectiveness of torching;
- a vegetation cover score of 4 or above limiting effectiveness of torching;
- shallow water preventing use of bottle trapping or netting;
- dense vegetation preventing access to the full pond perimeter; and
- receding water levels creating deep areas of mud/debris around the pond margin which was unsafe to walk on.

Where possible, additional survey methods were used to supplement a constrained survey method and increase the chances of detecting GCN, if present. However, in some ponds this was not possible due to multiple limiting factors being present. P-LWS-7 was positive for eDNA but was unsafe to access for bottle trapping or netting due to deep mud/debris around

the margin. Surveys of this pond were altered from visit 4 onwards to use floating traps which can be thrown into a pond from a safe distance. Despite this, no GCN were recorded and an assumption has been made regarding the population size-class present.

Although there were limitations to the presence/likely-absence surveys, the data collected are considered sufficient to inform masterplan development. Planning applications that come forward in future years under the masterplan will require their own suite of GCN surveys, including consideration of off-site ponds, which will collect additional data on the presence or likely-absence of GCN and ensure any GCN populations are adequately protected and mitigated for in each development.

3.0 Results

3.1 Desk Study

The desk study returned records of four amphibian species within 2km of the Site:

- GCN – 76 records between 2005 and 2021 including records on-site within ponds in and around Tunstalls Farm Local Wildlife Site (LWS);
- Common toad (*Bufo bufo*) – 69 records between 1919 and 2022 (SPI);
- Common frog (*Rana temporaria*) – 65 records between 1980 and 2020; and
- Smooth newt (*Lissotriton vulgaris*) – 95 records between 1997 and 2016.

3.2 Habitat Suitability Index (HSI) Surveys

A photograph of each pond and the result of the HSI assessments are provided in Table 4. Only P9.1, P9.2, P-LWS-4, P-LWS-5/6 and P-LWS-8 were assessed by SLR in 2025. The remainder of the data presented were collected by The Mersey Forest in 2024, and are still considered valid. In summary, three ponds were assessed as having 'average' suitability, thirteen as 'below average' and seven as 'poor'. HSI results are also displayed in Figure 2.

Factor scores used to calculate the overall HSI scores are included as Appendix A.

Table 4: Habitat Suitability Index survey results and pond photographs

Pond	Location	Photograph	HSI Score and Rating
P3.1	OSGR: SJ 53102 91705 Parcel 3		0.58 Below Average
P3.2	OSGR: SJ 53121 91823 Parcel 3		0.48 Poor

Pond	Location	Photograph	HSI Score and Rating
P3.3	OSGR: SJ 53157 91831 Parcel 3		0.49 Poor
P3.4	OSGR: SJ 53338 91805 Parcel 3		0.53 Below Average
P3.5	OSGR: SJ 53136 91992 Parcel 3		0.59 Below Average
P3.6	OSGR: SJ 53580 91933 On boundary between Parcel 2 and Parcel 3		0.56 Below Average

Pond	Location	Photograph	HSI Score and Rating
P5.1	OSGR: SJ 52987 91900 Parcel 5		0.58 Below Average
P5.2	OSGR: SJ 53036 92062 Parcel 5		0.56 Below Average
P6.1	OSGR: SJ 53836 92414 Parcel 6		N/A – Pond dry
P6.2	OSGR: SJ 53923 92354 Parcel 6		0.56 Below Average

Pond	Location	Photograph	HSI Score and Rating
P6/8	OSGR: SJ 54281 92195 On boundary between Parcel 6 and Parcel 8		0.59 Below Average
P7.1	OSGR: SJ 53763 92449 On boundary between Parcel 6 and Parcel 7		0.59 Below Average
P7.2	OSGR: SJ 53762 92417 On boundary between Parcel 6 and Parcel 7		0.69 Average
P9.1	OSGR: SJ 54452 92547 Parcel 9		0.41 Poor

Pond	Location	Photograph	HSI Score and Rating
P9.2	OSGR: SJ 54452 92547 Parcel 9		0.41 Poor
P11	OSGR: SJ 53263 91703 On boundary between Parcel 3 and Parcel 11		0.56 Below Average
P13	OSGR: SJ 54232 92909 Parcel 13		0.44 Poor
P-LWS-1	OSGR: SJ 53275 91905 On boundary between Parcel 3 and Tunstalls Farm LWS		0.29 Poor

Pond	Location	Photograph	HSI Score and Rating
P-LWS-2	OSGR: SJ 53265 92001 Tunstalls Farm LWS		0.68 Average
P-LWS-3	OSGR: SJ 53106 92139 Tunstalls Farm LWS		N/A – No pond present
P-LWS-4	OSGR: SJ 53305 92192 Tunstalls Farm LWS		0.44 Poor
P-LWS-5/6	OSGR: SJ 53364 92136 Tunstalls Farm LWS		0.61 Average

Pond	Location	Photograph	HSI Score and Rating
P-LWS-7	OSGR: SJ 53482 92042 On boundary between Parcel 3 and Tunstalls Farm LWS		0.51 Below Average
P-LWS-8	OSGR: SJ 53538 91980 On boundary between Parcel 3 and Tunstalls Farm LWS		0.55 Below Average
P-LWS-9	OSGR: SJ 53381 92267 Tunstalls Farm LWS		0.59 Below Average

3.3 eDNA Surveys

GCN eDNA results are summarised in Table 5. Full results are included in Appendix B.

In summary, three ponds (P6/8, P-LWS-5/6 and P-LWS-7) tested positive for the presence of GCN eDNA. Nine ponds tested negative for the presence of GCN eDNA and the remaining eleven ponds were indeterminate, likely due to chemical run-off from the fields interfering with the water chemistry, meaning that control DNA in the tests could not be detected accurately.

Table 5: Great Crested Newt eDNA results

Pond no.	eDNA result
P3.1	Negative
P3.2	Negative
P3.3	Negative
P3.4	Negative
P3.5	Indeterminate
P3.6	Indeterminate
P5.1	Negative
P5.2	Negative
P6.1	N/A – Dry
P6.2	Indeterminate
P6/8	Positive
P7.1	Indeterminate
P7.2	Indeterminate
P9.1	Indeterminate
P9.2	Indeterminate
P11	Negative
P13	Indeterminate
P-LWS-1	Negative
P-LWS-2	Indeterminate
P-LWS-3	N/A – Dry
P-LWS-4	Negative
P-LWS-5/6	Positive
P-LWS-7	Positive
P-LWS-8	Indeterminate
P-LWS-9	Indeterminate

3.4 Presence / Likely-absence and Population Size-class Surveys

All of the survey results are included in Appendix C. In summary, small populations of GCN were recorded in P6/8 (maximum count 7; Photograph 1) and P-LWS-5/6 (maximum count 2). No GCN were recorded in P-LWS-7 despite a positive eDNA result and consequently a small population is assumed to be present in this pond.

No GCN were recorded in any of the eDNA indeterminate ponds during four survey visits and GCN are therefore assumed absent from these ponds.

A single male smooth newt was recorded in P3.2 and a single female smooth/palmate newt was recorded in P7.2. Common frog and common toad larvae were also both recorded in P7.2.

Photograph 3-1: Female GCN caught during bottle trapping of P6/8

3.5 Incidental Sightings

3.5.1 Invasive Species

Japanese knotweed (*Reynoutria japonica*) was observed on the southern bank of P6.2 and a large stand was observed just beyond the north-western boundary of the Site (Photograph 2). Giant Hogweed (*Heracleum mantegazzianum*) was observed in several locations around Tunstalls Farm including adjacent to the farm track, farm buildings and on spoil mounds (Photograph 3). Both species are invasive non-native species (INNS) listed under Schedule 9 of the WCA 1981 (as amended).

Photograph 2: Japanese knotweed on the southern bank of P6.2

Photograph 3: Giant hogweed observed at Tunstalls Farm

3.5.2 Notable Mammals

West European hedgehog (*Erinaceous europaeus*) was observed on several separate survey nights (Photograph 4). Specifically, they were recorded in Tunstalls Farm LWS, near P5.1 and near P7.2. Brown hare (*Lepus europaeus*) was observed in arable fields in the south and north-east of the Site. Both species are SPIs as listed under Section 41 of the NERC Act 2006.

Photograph 4: Hedgehog observed near P-LWS-2 during GCN surveys

Figure 1 Pond Location Plan

Technical Appendix: Results of Great Crested Newt Surveys

Bold Forest Garden Village

St Helens Borough Council

SLR Project No.: 410.066617.00001

25 June 2025

353000 353500 354000 354500

393000 392500 392000

Bold Road
Travers' Entry
Reginald Road
Brindley Road
Abbotsfield Road
Nells Road
Gorsey Lane
Crawford Close
Crawford Street

P13
P9.1
P9.2
P7.1
P7.2
P6.1
P6.2
P6/8
P-LWS-9
P-LWS-4
P-LWS-5/6
P-LWS-3
P5.2
P-LWS-7
P-LWS-2
P3.5
P-LWS-8
P3.6
P5.1
P-LWS-1
P3.2
P3.3
P3.4
P3.1
P11

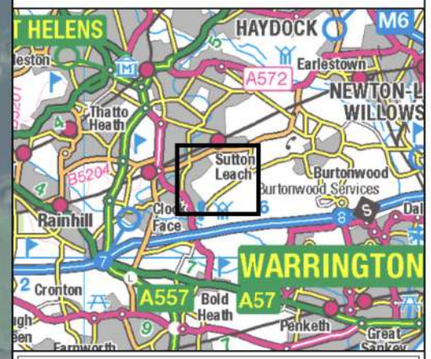
N

0 250 500
Metres

LEGEND

Site Boundary

Pond Location



ST HELENS BOROUGH COUNCIL

SLR

BOLD FOREST GARDEN VILLAGE

GREAT CRESTED NEWT SURVEY REPORT

POND LOCATIONS

FIGURE 1

Scale 1:7,000 @ A3

Date JUNE 2025

410.066257.00001.0016.0 Pond Locations

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Figure 2 HSI Results

Technical Appendix: Results of Great Crested Newt Surveys

Bold Forest Garden Village

St Helens Borough Council

SLR Project No.: 410.066617.00001

25 June 2025

353000
353500
354000
354500
393000
392500
392000

Bold Road
Travers' Entry
Reginald Road
Brindley Road
Abbotsfield Road
Nells Road
Gorsey Lane
Crawford Close
Crawford Street

P13 (0.44)
P9.1 (0.41)
P9.2 (0.41)
P7.1 (0.59)
P6.1 (n/a)
P7.2 (0.69)
P6.2 (0.56)
P6/8 (0.59)
P-LWS-9 (0.59)
P-LWS-4 (0.44)
P-LWS-5/6 (0.61)
P-LWS-3 (n/a)
P5.2 (0.56)
P-LWS-7 (0.51)
P-LWS-2 (0.68)
P3.5 (0.59)
P-LWS-8 (0.55)
P3.6 (0.56)
P-LWS-1 (0.29)
P5.1 (0.58)
P3.2 (0.48)
P3.3 (0.49)
P3.4 (0.53)
P3.1 (0.58)
P11 (0.56)

N

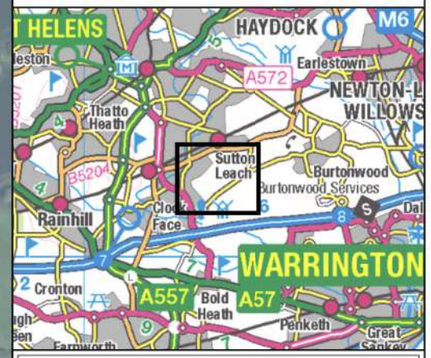
0 250 500
Metres

LEGEND

Site Boundary

HSI Category

- Average (0.6 - 0.69)
- Below Average (0.5 - 0.59)
- Poor (< 0.5)
- Not Surveyed (Dry)



ST HELENS BOROUGH COUNCIL

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BOLD FOREST GARDEN VILLAGE

GREAT CRESTED NEWT SURVEY REPORT

HSI RESULTS

FIGURE 2

Scale 1:7,000 @ A3

Date JUNE 2025

410.066257.00001.0017.0 HSI Results

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Figure 3 eDNA Results

Technical Appendix: Results of Great Crested Newt Surveys

Bold Forest Garden Village

St Helens Borough Council

SLR Project No.: 410.066617.00001

25 June 2025

353000
353500
354000
354500
393000
392500
392000
410.066257.00001.0018.0 eDNA Results

N

Bold Road
Travers' Entry
Reginald Road
Brindley Road
Abbotsfield Road
Nells Road
Gorsey Lane
Crawford Close
Crawford Street

P13
P9.1
P9.2
P7.1
P7.2
P6.1
P6.2
P6/8
P-LWS-9
P-LWS-4
P-LWS-5/6
P-LWS-3
P5.2
P-LWS-7
P-LWS-2
P3.5
P-LWS-8
P3.6
P5.1
P-LWS-1
P3.2
P3.3
P3.4
P3.1
P11

0
250
500
Metres

LEGEND

Site Boundary

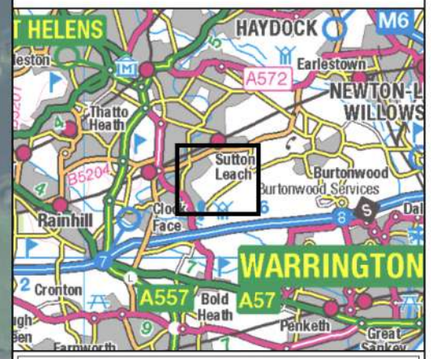
eDNA Result

Positive

Inconclusive

Negative

Sampling Not Possible (Dry)



ST HELENS BOROUGH COUNCIL

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BOLD FOREST GARDEN VILLAGE

GREAT CRESTED NEWT SURVEY REPORT

EDNA RESULTS

FIGURE 3

Scale 1:7,000 @ A3

Date JUNE 2025

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Figure 4 Population Size-class Results

Technical Appendix: Results of Great Crested Newt Surveys

Bold Forest Garden Village

St Helens Borough Council

SLR Project No.: 410.066617.00001

25 June 2025

353000 353500 354000 354500

393000 392500 392000

Bold Road
Travers' Entry
Reginald Road
Brindley Road
Abbotsfield Road
Nells Road
Gorsey Lane
Crawford Close
Crawford Street

P13
P9.1
P9.2
P7.1
P7.2
P6.1
P6.2
P6/8
P-LWS-9
P-LWS-4
P-LWS-5/6
P-LWS-3
P5.2
P-LWS-2
P-LWS-7
P3.5
P-LWS-8
P3.6
P5.1
P-LWS-1
P3.2
P3.3
P3.4
P3.1
P11

N

0 250 500
Metres

LEGEND

Site Boundary

Population Class

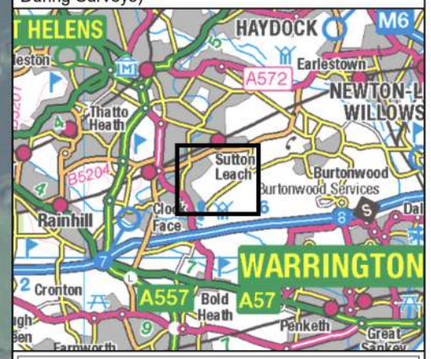
Small GCN Population

Assumed Small GCN Population

GCN Assumed Absent (No GCN Found)

Pond Dry

Results:
P6/8 and P-LWS-5 / P-LWS-6 = Small Population
P-LWS-7 = Assumed Small Population
P6.1 and P-LWS-3 = Pond Dry
All Other Ponds Assumed Absent (No GCN Found During Surveys)



ST HELENS BOROUGH COUNCIL

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BOLD FOREST GARDEN VILLAGE
GREAT CRESTED NEWT SURVEY REPORT
POPULATION SIZE/CLASS RESULTS

FIGURE 4

Scale 1:7,000 @ A3
Date JUNE 2025

410.066257.00001.0019.0 Population Size-Class Results

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Appendix A Habitat Suitability Index (HSI) Calculations

Technical Appendix: Results of Great Crested Newt Surveys

Bold Forest Garden Village

St Helens Borough Council

SLR Project No.: 410.066617.00001

25 June 2025

A.1 Habitat Suitability Index (HSI) Calculations

Table A-1: Habitat Suitability Index Assessments of Ponds within the BFGV Site.

Pond	Habitat Suitability Index Parameter Score										HSI Score
	SI1	SI2	SI3	SI4	SI5	SI6	SI7	SI8	SI9	SI10	
P3.1	1.00	0.8	1.00	0.33	0.4	0.67	0.67	1.00	0.33	0.3	0.58 (BA)
P3.2	1.00	0.2	1.00	0.33	0.2	0.67	0.67	1.00	0.33	0.3	0.48 (P)
P3.3	1.00	0.8	1.00	0.01	1.00	1.00	1.00	1.00	0.33	0.3	0.49 (P)
P3.4	1.00	0.6	1.00	0.33	0.2	0.67	0.67	1.00	0.33	0.3	0.53 (BA)
P3.5	1.00	0.4	1.00	0.33	1.00	0.67	0.67	1.00	0.33	0.3	0.59 (BA)
P3.6	1.00	0.2	1.00	0.33	1.00	0.67	0.67	1.00	0.33	0.3	0.56 (BA)
P5.1	1.00	0.4	1.00	0.33	0.8	0.67	0.67	1.00	0.33	0.3	0.58 (BA)
P5.2	1.00	0.2	1.00	0.33	1.00	0.67	0.67	1.00	0.33	0.3	0.56 (BA)
P6.1	-	-	-	-	-	-	-	-	-	-	N/A – Pond dry
P6.2	1.00	1.00	0.50	0.33	0.2	1.00	1.00	1.00	0.33	0.3	0.56 (BA)
P6/8	1.00	0.2	0.9	0.33	1	0.67	0.67	1.00	0.67	0.3	0.59 (BA)
P7.1	1.00	0.2	1.00	0.33	1.00	0.67	0.67	1.00	0.67	0.3	0.59 (BA)
P7.2	1.00	0.8	1.00	0.33	1.00	0.67	0.67	1.00	0.67	0.3	0.69 (A)
P9.1	1.00	0.2	0.1	0.33	0.2	1.00	1.00	1.00	0.33	0.3	0.41 (P)
P9.2	1.00	0.2	0.1	0.33	0.2	1.00	1.00	1.00	0.33	0.3	0.41 (P)
P11	1.00	0.2	1.00	0.33	1.00	0.67	0.67	1.00	0.33	0.3	0.56 (BA)
P13	1.00	1.00	0.9	0.01	1.00	0.67	0.67	0.72	0.33	0.3	0.44 (P)
P-LWS-1*	-	-	-	-	-	-	-	-	-	-	0.29 (P)
P-LWS-2*	-	-	-	-	-	-	-	-	-	-	0.68 (A)
P-LWS-3	-	-	-	-	-	-	-	-	-	-	N/A – No pond present
P-LWS-4	1.00	0.2	0.1	0.33	0.2	1.00	1.00	1.00	0.67	0.3	0.44 (P)
P-LWS-5/6	1.00	0.7	0.9	0.33	0.4	0.67	0.67	1.00	0.67	0.3	0.61 (A)
P-LWS-7*	-	-	-	-	-	-	-	-	-	-	0.51 (BA)
P-LWS-8	1.00	0.8	0.5	0.33	0.2	0.67	0.67	1.00	0.67	0.3	0.55 (BA)
P-LWS-9*	-	-	-	-	-	-	-	-	-	-	0.59 (BA)

(A) Average 0.6 - 0.69, (BA) Below Average 0.5 – 0.59, (P) Poor <0.5; (SI) Suitability Index.

* HSI breakdowns were not provided by The Mersey Forest for these ponds.

Appendix B Great Crested Newt eDNA Results

Technical Appendix: Results of Great Crested Newt Surveys

Bold Forest Garden Village

St Helens Borough Council

SLR Project No.: 410.066617.00001

25 June 2025

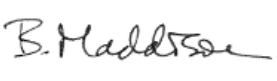
Client:
Tom Redman, SLR Consutling
1040079-015489-410, version 1

RSK ADAS Ltd
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

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

www.adas.uk

Sample ID: ADAS-8503	
Client Identifier: P3.5	Grid references/coordinates: Not Supplied
Description: pond water samples in preservative	Condition on Receipt: Low Sediment
Date of Receipt : 23/04/2025	Volume: Passed

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	30/04/2025
Degradation Control[§]	Evidence of degradation	Real Time PCR	30/04/2025
Great Crested Newt*	Indeterminate	Real Time PCR	30/04/2025
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:	01/05/2025	Date of issue:	01/05/2025

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 GCN if all of the replicates are negative; positive for GCN 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

[§] 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.

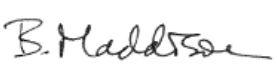
Client:
Tom Redman, SLR Consutling
1040079-015489-410, version 1

RSK ADAS Ltd
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

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

www.adas.uk

Sample ID: ADAS-8504	
Client Identifier: P3.1	Grid references/coordinates: Not Supplied
Description: pond water samples in preservative	Condition on Receipt: Low Sediment
Date of Receipt : 23/04/2025	Volume: Passed

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	29/04/2025
Degradation Control[§]	Within limits	Real Time PCR	29/04/2025
Great Crested Newt*	0 of 12 (negative)	Real Time PCR	29/04/2025
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:	01/05/2025	Date of issue:	01/05/2025

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 GCN if all of the replicates are negative; positive for GCN 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

[§] 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:
Tom Redman, SLR Consutling
1040079-015489-410, version 1

RSK ADAS Ltd
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

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

www.adas.uk

Sample ID: ADAS-8505	
Client Identifier: P3.2	Grid references/coordinates: Not Supplied
Description: pond water samples in preservative	Condition on Receipt: Good
Date of Receipt : 23/04/2025	Volume: Passed

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	28/04/2025
Degradation Control[§]	Within limits	Real Time PCR	28/04/2025
Great Crested Newt*	0 of 12 (negative)	Real Time PCR	28/04/2025
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:	01/05/2025	Date of issue:	01/05/2025

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 GCN if all of the replicates are negative; positive for GCN 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

[§] 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:
Tom Redman, SLR Consutling
1040079-015489-410, version 1

RSK ADAS Ltd
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

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

www.adas.uk

Sample ID: ADAS-8506	
Client Identifier: P-LWS-9	Grid references/coordinates: Not Supplied
Description: pond water samples in preservative	Condition on Receipt: Good
Date of Receipt : 23/04/2025	Volume: Passed

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	30/04/2025
Degradation Control[§]	Evidence of degradation	Real Time PCR	30/04/2025
Great Crested Newt*	Indeterminate	Real Time PCR	30/04/2025
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:	01/05/2025	Date of issue:	01/05/2025

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 GCN if all of the replicates are negative; positive for GCN 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

[§] 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:
Tom Redman, SLR Consutling
1040079-015489-410, version 1

RSK ADAS Ltd
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

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

www.adas.uk

Sample ID: ADAS-8507			
Client Identifier: P-LWS-7		Grid references/coordinates: Not Supplied	
Description: pond water samples in preservative		Condition on Receipt: Medium Sediment	
Date of Receipt : 23/04/2025		Volume: Passed	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	29/04/2025
Degradation Control[§]	Within limits	Real Time PCR	29/04/2025
Great Crested Newt*	1 of 12 (positive)	Real Time PCR	29/04/2025
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:	01/05/2025	Date of issue:	01/05/2025

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 GCN if all of the replicates are negative; positive for GCN 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

[§] 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:
Tom Redman, SLR Consutling
1040079-015489-410, version 1

RSK ADAS Ltd
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

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

www.adas.uk

Sample ID: ADAS-8508			
Client Identifier: P11		Grid references/coordinates: Not Supplied	
Description: pond water samples in preservative		Condition on Receipt: Low Sediment	
Date of Receipt : 23/04/2025		Volume: Passed	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	29/04/2025
Degradation Control[§]	Within limits	Real Time PCR	29/04/2025
Great Crested Newt*	0 of 12 (negative)	Real Time PCR	29/04/2025
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:	01/05/2025	Date of issue:	01/05/2025

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 GCN if all of the replicates are negative; positive for GCN 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

[§] 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-8509			
Client Identifier: P-LWS-2		Grid references/coordinates: Not Supplied	
Description: pond water samples in preservative		Condition on Receipt: Medium Sediment	
Date of Receipt : 23/04/2025		Volume: Passed	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	29/04/2025
Degradation Control[§]	Evidence of degradation	Real Time PCR	29/04/2025
Great Crested Newt*	Indeterminate	Real Time PCR	29/04/2025
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:	01/05/2025	Date of issue:	01/05/2025

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 GCN if all of the replicates are negative; positive for GCN 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

[§] 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-8510			
Client Identifier: P3.6		Grid references/coordinates: Not Supplied	
Description: pond water samples in preservative		Condition on Receipt: Medium Sediment	
Date of Receipt : 23/04/2025		Volume: Passed	
Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	28/04/2025
Degradation Control[§]	Evidence of degradation	Real Time PCR	28/04/2025
Great Crested Newt*	Indeterminate	Real Time PCR	28/04/2025
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:	01/05/2025	Date of issue:	01/05/2025

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 GCN if all of the replicates are negative; positive for GCN 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

[§] 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-8511	
Client Identifier: P13	Grid references/coordinates: Not Supplied
Description: pond water samples in preservative	Condition on Receipt: Low White Precipitate
Date of Receipt : 23/04/2025	Volume: Passed

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	29/04/2025
Degradation Control[§]	Evidence of degradation	Real Time PCR	29/04/2025
Great Crested Newt*	Indeterminate	Real Time PCR	29/04/2025
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:	01/05/2025	Date of issue:	01/05/2025

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 GCN if all of the replicates are negative; positive for GCN 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

[§] 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-8512	
Client Identifier: P6.2	Grid references/coordinates: Not Supplied
Description: pond water samples in preservative	Condition on Receipt: Low Sediment
Date of Receipt : 23/04/2025	Volume: Passed

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	01/05/2025
Degradation Control[§]	Evidence of degradation	Real Time PCR	01/05/2025
Great Crested Newt*	Indeterminate	Real Time PCR	01/05/2025
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:	01/05/2025	Date of issue:	01/05/2025

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 GCN if all of the replicates are negative; positive for GCN 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

[§] 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-8513	
Client Identifier: P5.2	Grid references/coordinates: Not Supplied
Description: pond water samples in preservative	Condition on Receipt: Low Sediment
Date of Receipt : 23/04/2025	Volume: Passed

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	29/04/2025
Degradation Control[§]	Within limits	Real Time PCR	29/04/2025
Great Crested Newt*	0 of 12 (negative)	Real Time PCR	29/04/2025
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:	01/05/2025	Date of issue:	01/05/2025

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 GCN if all of the replicates are negative; positive for GCN 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

[§] 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-8514			
Client Identifier: P7.1		Grid references/coordinates: Not Supplied	
Description: pond water samples in preservative		Condition on Receipt: Medium Sediment	
Date of Receipt : 23/04/2025		Volume: Passed	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	28/04/2025
Degradation Control[§]	Evidence of degradation	Real Time PCR	28/04/2025
Great Crested Newt*	Indeterminate	Real Time PCR	28/04/2025
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:	01/05/2025	Date of issue:	01/05/2025

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 GCN if all of the replicates are negative; positive for GCN 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

[§] 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-8515	
Client Identifier: P3.4	Grid references/coordinates: Not Supplied
Description: pond water samples in preservative	Condition on Receipt: Medium Sediment
Date of Receipt : 23/04/2025	Volume: Passed

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	01/05/2025
Degradation Control[§]	Within limits	Real Time PCR	01/05/2025
Great Crested Newt*	0 of 12 (negative)	Real Time PCR	01/05/2025
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:	01/05/2025	Date of issue:	01/05/2025

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 GCN if all of the replicates are negative; positive for GCN 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

[§] 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-8516			
Client Identifier: P7.2		Grid references/coordinates: Not Supplied	
Description: pond water samples in preservative		Condition on Receipt: Medium Sediment	
Date of Receipt : 23/04/2025		Volume: Passed	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	29/04/2025
Degradation Control[§]	Evidence of degradation	Real Time PCR	29/04/2025
Great Crested Newt*	Indeterminate	Real Time PCR	29/04/2025
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:	01/05/2025	Date of issue:	01/05/2025

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 GCN if all of the replicates are negative; positive for GCN 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

[§] 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-8517	
Client Identifier: P5.1	Grid references/coordinates: Not Supplied
Description: pond water samples in preservative	Condition on Receipt: Low Sediment
Date of Receipt : 23/04/2025	Volume: Passed

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	28/04/2025
Degradation Control[§]	Within limits	Real Time PCR	28/04/2025
Great Crested Newt*	0 of 12 (negative)	Real Time PCR	28/04/2025
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:	01/05/2025	Date of issue:	01/05/2025

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 GCN if all of the replicates are negative; positive for GCN 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

[§] 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-8518	
Client Identifier: P6/8	Grid references/coordinates: Not Supplied
Description: pond water samples in preservative	Condition on Receipt: Good
Date of Receipt : 23/04/2025	Volume: Passed

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	01/05/2025
Degradation Control[§]	Evidence of degradation	Real Time PCR	01/05/2025
Great Crested Newt*	12 of 12 (positive)	Real Time PCR	01/05/2025
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:	01/05/2025	Date of issue:	01/05/2025

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 GCN if all of the replicates are negative; positive for GCN 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

[§] 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-8519	
Client Identifier: P9.1	Grid references/coordinates: Not Supplied
Description: pond water samples in preservative	Condition on Receipt: Low Sediment
Date of Receipt : 23/04/2025	Volume: Passed

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	29/04/2025
Degradation Control[§]	Evidence of degradation	Real Time PCR	29/04/2025
Great Crested Newt*	Indeterminate	Real Time PCR	29/04/2025
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:	01/05/2025	Date of issue:	01/05/2025

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 GCN if all of the replicates are negative; positive for GCN 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

[§] 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-8520	
Client Identifier: P9.2	Grid references/coordinates: Not Supplied
Description: pond water samples in preservative	Condition on Receipt: Low Sediment
Date of Receipt : 23/04/2025	Volume: Passed

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	29/04/2025
Degradation Control[§]	Evidence of degradation	Real Time PCR	29/04/2025
Great Crested Newt*	Indeterminate	Real Time PCR	29/04/2025
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:	01/05/2025	Date of issue:	01/05/2025

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 GCN if all of the replicates are negative; positive for GCN 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

[§] 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-8194	
Client Identifier: P-LWS-1	Grid references/coordinates: Not Supplied
Description: pond water samples in preservative	Condition on Receipt: Good
Date of Receipt : 06/05/2025	Volume: Passed

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	05/07/2025
Degradation Control[§]	Within limits	Real Time PCR	05/07/2025
Great Crested Newt*	0 of 12 (negative)	Real Time PCR	05/07/2025
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:	07/05/2025	Date of issue:	07/05/2025

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 GCN if all of the replicates are negative; positive for GCN 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

[§] 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-8195	
Client Identifier: P-LWS-8	Grid references/coordinates: Not Supplied
Description: pond water samples in preservative	Condition on Receipt: Medium Sediment
Date of Receipt : 06/05/2025	Volume: Passed

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	0 of 2	Real Time PCR	05/07/2025
Degradation Control[§]	Evidence of degradation or residual inhibition	Real Time PCR	05/07/2025
Great Crested Newt*	Indeterminate	Real Time PCR	05/07/2025
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:	07/05/2025	Date of issue:	07/05/2025

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 GCN if all of the replicates are negative; positive for GCN 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

[§] 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:
BFGV, Shona Redman, SLR Consulting
1040079-GO SLR, BFGV, version 1

RSK ADAS Ltd
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

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

www.adas.uk

Sample ID: ADAS-8197			
Client Identifier: P-LWS-4		Grid references/coordinates: Not Supplied	
Description: pond water samples in preservative		Condition on Receipt: Low Sediment	
Date of Receipt : 06/05/2025		Volume: Passed	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	05/07/2025
Degradation Control[§]	Within limits	Real Time PCR	05/07/2025
Great Crested Newt*	0 of 12 (negative)	Real Time PCR	05/07/2025
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:	07/05/2025	Date of issue:	07/05/2025

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 GCN if all of the replicates are negative; positive for GCN 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

[§] 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-8203	
Client Identifier: P3.3	Grid references/coordinates: Not Supplied
Description: pond water samples in preservative	Condition on Receipt: High Sediment
Date of Receipt : 06/05/2025	Volume: Passed

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	05/07/2025
Degradation Control[§]	Within limits	Real Time PCR	05/07/2025
Great Crested Newt*	0 of 12 (negative)	Real Time PCR	05/07/2025
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:	07/05/2025	Date of issue:	07/05/2025

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 GCN if all of the replicates are negative; positive for GCN 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

[§] 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:
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Sample ID: ADAS-8205			
Client Identifier: P-LWS-5		Grid references/coordinates: Not Supplied	
Description: pond water samples in preservative		Condition on Receipt: Low Sediment	
Date of Receipt : 06/05/2025		Volume: Passed	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	0 of 2	Real Time PCR	05/07/2025
Degradation Control[§]	Within limits	Real Time PCR	05/07/2025
Great Crested Newt*	2 of 12 (positive)	Real Time PCR	05/07/2025
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:	07/05/2025	Date of issue:	07/05/2025

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 GCN if all of the replicates are negative; positive for GCN 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

[§] 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 C Presence/likely-absence and Population Size-class Survey Results

Technical Appendix: Results of Great Crested Newt Surveys

Bold Forest Garden Village

St Helens Borough Council

SLR Project No.: 410.066617.00001

25 June 2025

C.1 Presence/likely-absence and Population Size-class Survey Results

Table A-2: Detailed Results of Presence/likely-absence and Population Size-class Surveys of Ponds within the BFGV Site

Survey Visit 1						
Pond no.	**Torch Survey**	**Bottle Trap Survey**	**Netting**	**Egg Search**	**Environmental Data**	
P3.1	0	0	N/A	0	Air Temp.	11°C
					Turbidity	3
					Veg.	1
					Accessibility	60%
P3.2	1 male smooth newt	0	N/A	0	Air Temp.	10°C
					Turbidity	3
					Veg.	0
					Accessibility	75%
P3.3	0	N/A – too shallow	0	0	Air Temp.	12°C
					Turbidity	5
					Veg.	0
					Accessibility	100%
P3.4	0	0	N/A	0	Air Temp.	11°C
					Turbidity	3
					Veg.	0
					Accessibility	5%
P3.5	0	N/A – too shallow	N/A – too shallow	0	Air Temp.	10°C
					Turbidity	4
					Veg.	0
					Accessibility	70%
P3.6	Stickleback	N/A – too shallow	N/A – deep leaf litter present	0	Air Temp.	11°C
					Turbidity	3
					Veg.	0
					Accessibility	60%
P5.1	0	N/A – too shallow	0	0	Air Temp.	10°C
					Turbidity	1
					Veg.	4 (only margins clear)
					Accessibility	75%
P5.2	Stickleback	N/A – too shallow	0	0	Air Temp.	10°C
					Turbidity	3
					Veg.	0
					Accessibility	75%

Survey Visit 1						
P6.1	N/A – Pond dry. No surveys required					
P6.2	0	0	N/A - deep leaf litter present	0	Air Temp.	9°C
					Turbidity	4 (only margins clear)
					Veg.	0
					Accessibility	60%
P6/8	0	N/A – Steep vegetated banks unsafe to access in dark	N/A – Steep vegetated banks unsafe to access in dark	N/A – Steep vegetated banks unsafe to access in dark	Air Temp.	11°C
					Turbidity	3
					Veg.	1
					Accessibility	20% from a distance
P7.1	0	0	N/A - deep leaf litter present	0	Air Temp.	11°C
					Turbidity	4
					Veg.	0
					Accessibility	50%
P7.2	0	Common toad larvae	N/A - deep leaf litter present	0	Air Temp.	11°C
					Turbidity	4 (only margins clear)
					Veg.	0
					Accessibility	50%
P9.1	0	N/A – forecast below 5°C overnight	N/A – too shallow	0	Air Temp.	7°C
					Turbidity	3
					Veg.	0
					Accessibility	60%
P9.2	0	N/A – forecast below 5°C overnight	N/A – too shallow	0	Air Temp.	7°C
					Turbidity	2
					Veg.	0
					Accessibility	80%
P11	Stickleback	N/A – too shallow	N/A – deep leaf litter present	0	Air Temp.	11°C
					Turbidity	4
					Veg.	0
					Accessibility	75%
P13	0	N/A – forecast below 5°C overnight	0	0	Air Temp.	7°C
					Turbidity	5
					Veg.	1
					Accessibility	15%
P-LWS-1	0	N/A – too shallow	N/A – deep leaf litter present	0	Air Temp.	12°C
					Turbidity	3
					Veg.	4 (fallen catkins)
					Accessibility	90%

Survey Visit 1						
P-LWS-2	0	N/A – margins unsafe to walk on	N/A – margins unsafe to walk on	0	Air Temp.	11°C
					Turbidity	4
					Veg.	0
					Accessibility	100%
P-LWS-3	N/A – Pond dry. No surveys required					
P-LWS-4	0	N/A – too shallow	N/A – deep leaf litter present	0	Air Temp.	13°C
					Turbidity	4 (only margins clear)
					Veg.	4 (fallen leaves / catkins)
					Accessibility	40%
P-LWS-5/6	0	0	N/A - deep leaf litter present	0	Air Temp.	13°C
					Turbidity	4 (only margins clear)
					Veg.	3 (fallen leaves / catkins)
					Accessibility	30%
P-LWS-7	0	N/A – margins unsafe to walk on	N/A – margins unsafe to walk on	0	Air Temp.	11°C
					Turbidity	4
					Veg.	0
					Accessibility	50%
P-LWS-8	0	N/A – too shallow	N/A – deep leaf litter present	0	Air Temp.	13°C
					Turbidity	1
					Veg.	4 (fallen leaves / catkins)
					Accessibility	20%
P-LWS-9	0	0	N/A	0	Air Temp.	11°C
					Turbidity	3
					Veg.	1
					Accessibility	30%

Survey Visit 2						
Pond no.	**Torch Survey**	**Bottle Trap Survey**	**Netting**	**Egg Search**	**Environmental Data**	
P3.1	N/A – eDNA negative. No further surveys required					
P3.2	N/A – eDNA negative. No further surveys required					
P3.3	N/A – eDNA negative. No further surveys required					
P3.4	N/A – eDNA negative. No further surveys required					
P3.5	0	N/A – too shallow	N/A – too shallow	0	Air Temp.	12°C
					Turbidity	4
					Veg.	0

Survey Visit 2						
					Accessibility	75%
P3.6	0	N/A – too shallow and margins unsafe to walk on	N/A – deep leaf litter present	0	Air Temp.	10°C
					Turbidity	3
					Veg.	3 (fallen leaves / catkins)
					Accessibility	60%
P5.1	N/A – eDNA negative. No further surveys required					
P5.2	N/A – eDNA negative. No further surveys required					
P6.2	0	0	N/A - deep leaf litter present	0	Air Temp.	11°C
					Turbidity	5
					Veg.	1
					Accessibility	60%
P6/8	0	1 female GCN 1 male GCN	N/A – presence already confirmed	N/A – presence already confirmed	Air Temp.	11°C
					Turbidity	5
					Veg.	3
					Accessibility	50%
P7.1	0	0	N/A - deep leaf litter present	0	Air Temp.	12°C
					Turbidity	5
					Veg.	0
					Accessibility	50%
P7.2	0	0	N/A - deep leaf litter present	0	Air Temp.	12°C
					Turbidity	5
					Veg.	0
					Accessibility	50%
P9.1	0	N/A – too shallow	N/A – too shallow	0	Air Temp.	12°C
					Turbidity	3
					Veg.	0
					Accessibility	100%
P9.2	0	N/A – too shallow	N/A – too shallow	0	Air Temp.	12°C
					Turbidity	1
					Veg.	3
					Accessibility	100%
P11	N/A – eDNA negative. No further surveys required					
P13	Fish	0	0	0	Air Temp.	11°C
					Turbidity	5
					Veg.	1
					Accessibility	15% for trapping
P-LWS-1	N/A – eDNA negative. No further surveys required					
P-LWS-2	0			0	Air Temp.	12°C

Survey Visit 2						
		N/A – margins unsafe to walk on	N/A – margins unsafe to walk on		Turbidity	5
					Veg.	0
					Accessibility	100%
P-LWS-4	N/A – eDNA negative. No further surveys required					
P-LWS-5/6	0	0	N/A – presence already confirmed	N/A – presence already confirmed	Air Temp.	11°C
					Turbidity	4
					Veg.	2
					Accessibility	30%
P-LWS-7	0	N/A – margins unsafe to walk on	N/A – margins unsafe to walk on and presence already confirmed	N/A – presence already confirmed	Air Temp.	11°C
					Turbidity	3
					Veg.	2
					Accessibility	50%
P-LWS-8	0	N/A – too shallow	N/A – deep leaf litter present	0	Air Temp.	11°C
					Turbidity	3
					Veg.	4
					Accessibility	20%
P-LWS-9	0	0	N/A - deep leaf litter present	0	Air Temp.	12°C
					Turbidity	4
					Veg.	2
					Accessibility	30%

Survey Visit 3						
Pond no.	**Torch Survey**	**Bottle Trap Survey**	**Netting**	**Egg Search**	**Environmental Data**	
P3.5	0	N/A – too shallow	N/A – too shallow	0	Air Temp.	10°C
					Turbidity	4
					Veg.	0
					Accessibility	100%
P3.6	0	N/A – too shallow	N/A – deep leaf litter present	0	Air Temp.	15°C
					Turbidity	3
					Veg.	3 (fallen catkins)
					Accessibility	100%
P6.2	0	0	N/A - deep leaf litter present	0	Air Temp.	16°C
					Turbidity	4
					Veg.	3 (fallen leaves / catkins)
					Accessibility	70%
P6/8	0	0	N/A – presence	N/A – presence	Air Temp.	16°C
					Turbidity	4

Survey Visit 3						
			already confirmed	already confirmed	Veg.	3
					Accessibility	60%
P7.1	0	0	N/A - deep leaf litter present	0	Air Temp.	17°C
					Turbidity	4
					Veg.	3 (fallen leaves / catkins)
					Accessibility	50%
P7.2	1 female smooth / palmate newt	Common toad larvae	N/A - deep leaf litter present	0	Air Temp.	16°C
					Turbidity	4
					Veg.	1 (fallen leaves / catkins)
					Accessibility	70%
P9.1	0	N/A – too shallow	N/A – too shallow	0	Air Temp.	15°C
					Turbidity	1
					Veg.	1 (fallen catkins)
					Accessibility	100%
P9.2	0	N/A – too shallow	N/A – too shallow	0	Air Temp.	15°C
					Turbidity	1
					Veg.	4 (fallen catkins)
					Accessibility	100%
P13	Fish	0	0	0	Air Temp.	15°C
					Turbidity	5
					Veg.	1
					Accessibility	15% for trapping
P-LWS-2	0	0	0	0	Air Temp.	10°C
					Turbidity	5
					Veg.	0
					Accessibility	100%
P-LWS-5/6	0	0	N/A – presence already confirmed	N/A – presence already confirmed	Air Temp.	11°C
					Turbidity	4
					Veg.	2
					Accessibility	30%
P-LWS-7	0	N/A – margins unsafe to walk on	N/A – margins unsafe to walk on and presence already confirmed	N/A – presence already confirmed	Air Temp.	11°C
					Turbidity	4
					Veg.	3 (fallen leaves / catkins)
					Accessibility	50%
P-LWS-8	0	N/A – too shallow	N/A – deep leaf litter present	0	Air Temp.	11°C
					Turbidity	3
					Veg.	4 (fallen catkins)

Survey Visit 3						
					Accessibility	50%
P-LWS-9	0	0	N/A - deep leaf litter present	0	Air Temp.	12°C
					Turbidity	4
					Veg.	2 (fallen leaves / catkins)
					Accessibility	40%

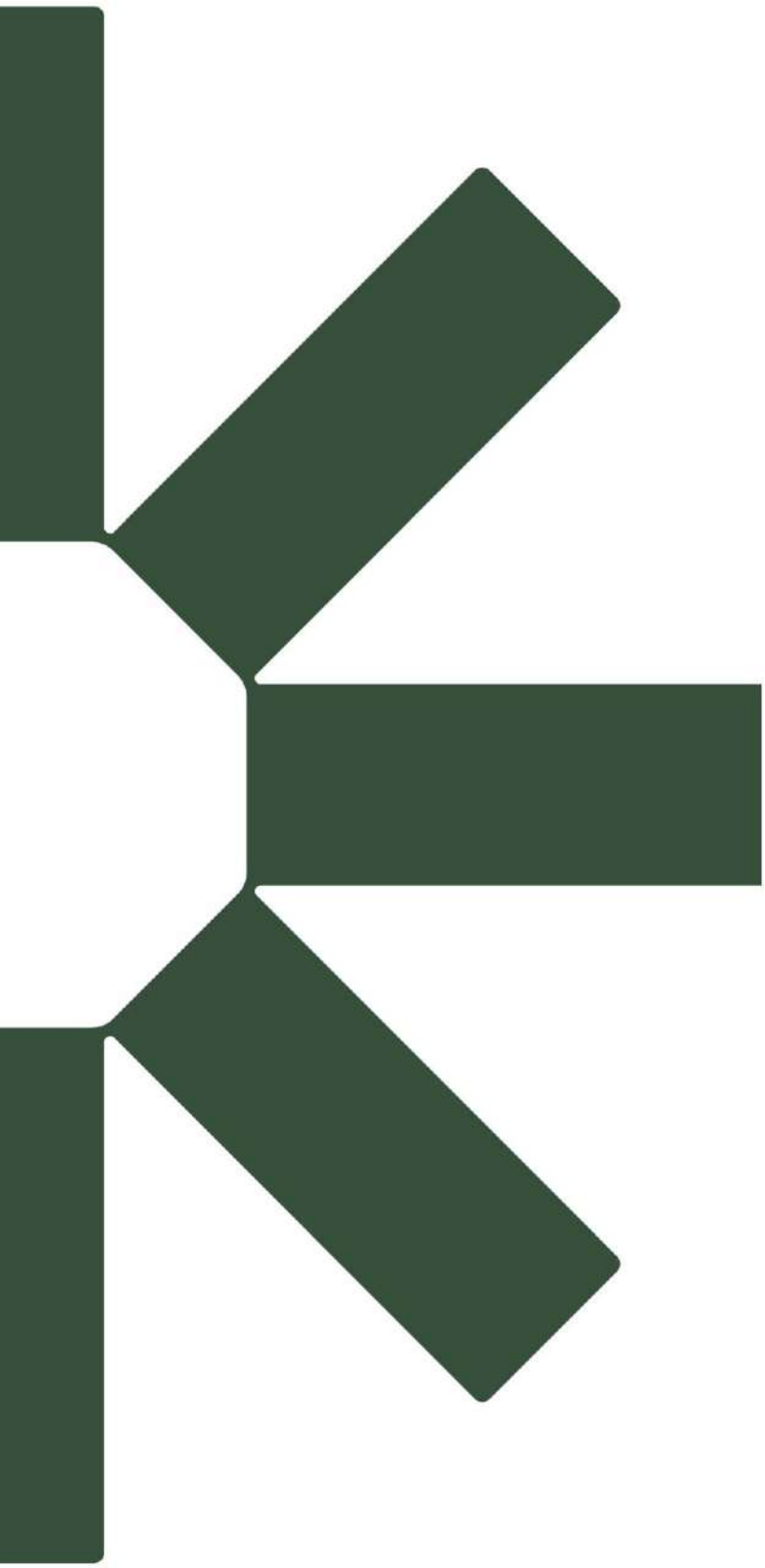
Survey Visit 4						
Pond no.	**Torch Survey**	**Bottle Trap Survey**	**Netting**	**Egg Search**	**Environmental Data**	
P3.5	N/A – Pond dry. No further surveys required. GCN assumed absent					
P3.6	0	N/A – too shallow	N/A – deep leaf litter present	0	Air Temp.	14°C
					Turbidity	4
					Veg.	1 (fallen leaves)
					Accessibility	100%
P6.2	0	0	N/A - deep leaf litter present	0	Air Temp.	11°C
					Turbidity	4-5
					Veg.	0
					Accessibility	75%
P6/8	0	2 female GCN 5 male GCN	N/A – presence already confirmed	N/A – presence already confirmed	Air Temp.	11°C
					Turbidity	4
					Veg.	4
					Accessibility	60%
P7.1	0	0	N/A - deep leaf litter present	0	Air Temp.	11°C
					Turbidity	4
					Veg.	0
					Accessibility	50%
P7.2	0	Common frog larvae	N/A - deep leaf litter present	0	Air Temp.	11°C
					Turbidity	4
					Veg.	0
					Accessibility	80%
P9.1	0	N/A – too shallow	N/A – too shallow	0	Air Temp.	15°C
					Turbidity	0
					Veg.	0
					Accessibility	80%
P9.2	0	N/A – too shallow	N/A – too shallow	0	Air Temp.	15°C
					Turbidity	0
					Veg.	1
					Accessibility	90%
P13	0	0	0	0	Air Temp.	14°C

Survey Visit 4						
					Turbidity	5
					Veg.	1
					Accessibility	15% for trapping
P-LWS-2	0	0	0	0	Air Temp.	10°C
					Turbidity	5
					Veg.	0
					Accessibility	100%
P-LWS-5/6	2 female GCN	0	N/A – presence already confirmed	N/A – presence already confirmed	Air Temp.	16°C
					Turbidity	4
					Veg.	2 (but some localised areas of margin 5)
					Accessibility	30%
P-LWS-7	0	0 (floating traps used)	N/A – margins unsafe to walk on and presence already confirmed	N/A – presence already confirmed	Air Temp.	15°C
					Turbidity	3
					Veg.	3 (fallen leaves)
					Accessibility	50%
P-LWS-8	0	N/A – too shallow	N/A – deep leaf litter present	0	Air Temp.	15°C
					Turbidity	3
					Veg.	2 (fallen leaves)
					Accessibility	80%
P-LWS-9	0	0	N/A - deep leaf litter present	0	Air Temp.	16°C
					Turbidity	4
					Veg.	1 (fallen leaves)
					Accessibility	50%

Survey Visit 5						
Pond no.	**Torch Survey**	**Bottle Trap Survey**	**Netting**	**Egg Search**	**Environmental Data**	
P3.6	N/A – GCN assumed absent after four visits. No further surveys required					
P6.2	N/A – GCN assumed absent after four visits. No further surveys required					
P6/8	0	0	N/A – presence already confirmed	N/A – presence already confirmed	Air Temp.	12°C
					Turbidity	4
					Veg.	4
					Accessibility	60%
P7.1	N/A – GCN assumed absent after four visits. No further surveys required					
P7.2	N/A – GCN assumed absent after four visits. No further surveys required					
P9.1	N/A – GCN assumed absent after four visits. No further surveys required					
P9.2	N/A – GCN assumed absent after four visits. No further surveys required					
P13	N/A – GCN assumed absent after four visits. No further surveys required					

Survey Visit 5						
P-LWS-2	N/A – GCN assumed absent after four visits. No further surveys required					
P-LWS-5/6	0	0	N/A – presence already confirmed	N/A – presence already confirmed	Air Temp.	14°C
					Turbidity	4
					Veg.	1 (fallen leaves)
					Accessibility	30%
P-LWS-7	0	0 (floating traps used)	N/A – presence already confirmed	N/A – presence already confirmed	Air Temp.	13°C
					Turbidity	3
					Veg.	1 (fallen leaves)
					Accessibility	50%
P-LWS-8	N/A – GCN assumed absent after four visits. No further surveys required					
P-LWS-9	N/A – GCN assumed absent after four visits. No further surveys required					

Survey Visit 6						
Pond no.	**Torch Survey**	**Bottle Trap Survey**	**Netting**	**Egg Search**	**Environmental Data**	
P6/8	0	0	N/A – presence already confirmed	N/A – presence already confirmed	Air Temp.	13°C
					Turbidity	4
					Veg.	4
					Accessibility	60%
P-LWS-5/6	0	0	N/A – presence already confirmed	N/A – presence already confirmed	Air Temp.	13°C
					Turbidity	2
					Veg.	1
					Accessibility	30%
P-LWS-7	0	0 (floating traps used)	N/A – presence already confirmed	N/A – presence already confirmed	Air Temp.	13°C
					Turbidity	2
					Veg.	1
					Accessibility	50%



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