

Billingshurst

784-B031362

Appendix 9.1G: Great Crested Newts

Highwood Ventures 5 Limited and Vivid Housing Limited

January 2026

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INTRODUCTION

1.1 PURPOSE OF THE REPORT

The purpose of this report is to:

- Present the relevant legislation and policy in relation to GCN;
- Describe the methods used to carry out the desk and field-based GCN assessments;
- Provide any GCN records on or within 2 km of the site;
- Assess the suitability of waterbodies onsite and within a 500 m radius for GCN;
- Determine the presence or likely absence of GCN;
- Identify if any additional surveys are required to inform this assessment.

This report should be reviewed (and reassessed if necessary) should there be any changes to the red line boundary or development proposals which this report was based on.

Note that scientific names are provided at the first mention of each species and common names (where appropriate) are then used throughout the rest of the report for ease of reading.

2.0 METHODOLOGY

2.1 DESK STUDY

The desktop study comprised two elements:

- An updated data search obtained from Sussex Biological Records Centre (SxBRC) in January 2026 including records of GCN within 2 km of the site boundary of both land parcels combined. Only records from within the past 10 years are considered within this report; and
- Online element including a 2 km search using the MAGIC (<https://magic.defra.gov.uk>) website and Ordnance Survey (OS) and Aerial Imagery (<https://www.bing.com/maps>). This included a search for any designated sites within 2 km supporting GCN or Natural England (NE) licence data including mitigation licencing. The search was conducted in January 2026.

2.2 HSI ASSESSMENT

Habitat Suitability Index (HSI) assessments were carried out on accessible waterbodies on the site and within a 500 m radius (Figure 1) on 29th June 2022 by Tetra Tech Associate Ecologist Vivienne Greenough CEcol MCIEEM (NE Level 1 GCN licence: 2015-16380-CLS-CLS). A HSI was also undertaken on the 19th May 2025 by Tetra Tech Project Ecologist Katie Hepburn (NE level 1 GCN licence: 2022-10469-CL08-GCN) for WB16 only once the area had been added to the RLB.

All surveys were conducted in accordance with Tetra Tech's Biosecurity Policy (Tetra Tech, 2019).

The HSI approach provides an objective method for assessing the suitability of a waterbody as habitat to support GCN (Oldham, 2001). The system provides an index between 0 and 1, with 0 indicating likely

unsuitable habitat and 1 optimal habitat. Ten suitability indices are used to calculate the index score, each representing a factor considered to affect GCN. These factors are listed and briefly explained below:

1. *Location*: i.e. the geographical location of the waterbody within the British Isles. Lowlands are generally thought to be most suitable; suitability declines with increases in altitude;
2. *Pond area*: i.e., the water surface area of a pond. Suitability peaks at approximately 800 m²;
3. *Pond drying*: how often a particular pond dries out. Ponds which dry out more frequently are less suitable;
4. *Water quality*: an indication of water quality based on the invertebrate diversity and aquatic plants present. High invertebrate diversity indicates high water quality and suitability;
5. *Shade*: an estimate of the total shaded perimeter of a pond. Shoreline shade below 60% is optimal;
6. *Fowl*: indication of impact by waterfowl. High waterfowl numbers are generally considered detrimental;
7. *Fish*: indication of fish abundance. High fish numbers are generally considered detrimental;
8. *Pond count*: based on the density of ponds occurring within 1 km of a particular pond. Suitability is positively correlated with pond density;
9. *Terrestrial habitat*: based on the availability of suitable habitat in the pond vicinity, e.g. rough grassland, scrub and woodland; and
10. *Macrophytes*: based on an estimate of the percentage cover by emergent and aquatic vegetation. Suitability peaks at between 70% and 80% cover.

The results are then compared against a categorical scale (ARG UK, 2010) to give an overall rating as follows:

- <0.5 = Poor
- 0.5 – 0.59 = Below Average
- 0.6 – 0.69 = Average
- 0.7 – 0.79 = Good
- >0.8 = Excellent

2.3 EDNA SURVEY

From the 16 waterbodies, 10 were not suitable for eDNA surveying due to either being dry (WB2) or being inaccessible to survey teams (WB1, WB3, WB4, WB5, WB6, WB7, WB12 and WB13), as well as WB16 not being within the RLB at the time. Environmental DNA (eDNA) surveys were therefore undertaken on the six remaining accessible waterbodies on 29th June 2022 (WB8, WB9, WB10, WB11, WB14 and WB15). The samples were collected from these waterbodies and securely sent to SureScreen Scientifics Ltd for laboratory analysis

Sample Collection Protocol

The eDNA sampling was carried out in accordance with the stringent survey methodologies defined within Natural England's accepted protocol (Biggs J, 2014). A total of 20 water samples were taken from around the perimeter of each waterbody, where access allowed. Where access around the waterbody was restricted (e.g. by fencing, vegetation, steep banks), samples were taken from as much of the accessible perimeter as possible and access noted as a limitation.

All water samples were mixed together and six representative 15 ml samples pipetted into tubes containing 35 ml ethanol. Each tube was shaken for 10 seconds to mix the sample and ethanol preservative.

eDNA Analysis

Samples were securely sent to the SureScreen Scientifics laboratory on the and the eDNA testing was carried out by SureScreen Scientifics, following their approved analysis methodology (detailed in **Appendix G.1**).

2.4 PRESENCE / LIKELY ABSENCE SURVEYS

Population size class (PSC) surveys were attempted in the following year 2023 on two waterbodies which returned positive eDNA results for GCN (WB8 and WB15). PSC includes six surveys and is used to classify the metapopulation (low, moderate and high), however, this was not possible due to both waterbodies drying after the fourth visit. As such, this is considered to be presence / likely absence surveys instead which were undertaken in accordance with standard best practice guidance as set out in the GCN Mitigation Guidelines (English Nature, 2001). All four survey visits were between mid-March to June and comprised of bottle trapping, torching and egg search methods .

Bottle Trapping

The technique comprised two site visits: an evening visit to set-up bottle traps, followed by a morning visit to collect the traps. Bottle traps are adapted 2-litre plastic bottles which are used for overnight capture of newts to assess presence of target species. They had suitably sized air bubbles and were equally spaced around the waterbody margins and left overnight. They were not unchecked for more than 17 hours and were collected before 11am the following day (English Nature, 2001). All surveys were undertaken when the predicted overnight air temperature exceeded 5°C.

Torching Survey

This technique involved a visual search for individual newts inhabiting each waterbody. Shortly after dusk, the surveyors walked around the waterbody and systematically shined a powerful torch on the water surface, counting the number and species of individual newts that were recorded. Close attention was given to marginal vegetation and open areas (which could be used for newt mating displays). To maximise the reliability of this technique, all torch surveys were conducted on evenings where the overnight air temperature exceeded 5°C, when newts are generally considered to be most active.

Egg Search

GCN eggs were searched for amongst submerged, floating and other aquatic vegetation. When laying their eggs, GCN fold leaves of aquatic plants around the egg creating a neat fold. This behaviour demonstrates that GCN are breeding in a particular waterbody. GCN eggs can be easily distinguished from other newt species by their size, shape and colour. Egg numbers cannot be used to estimate population size due to predation and high mortality rates. To limit disturbance, this method was ceased as soon as a GCN egg was positively identified in a waterbody.

GCN presence / likely absence surveys were carried out by Tetra Tech Consultant Ecologist Katie Hepburn (NE level 1 GCN licence: 2022-10469-CL08-GCN) and Tetra Tech Senior Ecologist Jon D'Arcy NE level 1 GCN

licence: 2017-30085-CLS-CLS). **Table 1** outlines the survey dates and weather conditions during each survey visit.

Table 1. Survey Dates and Weather Conditions

Survey No.	Waterbody	Date	Time (torch)		Air Temp (°C)		Wind Speed (Beaufort)	Cloud Cover (%)	Precipitation (%)
			Start	End	Initial	Final			
1	WB8	26/04/2023	21:40	22:20	12	11	0	100	0
	WB15		21:00	21:30					
2	WB8	03/05/2023	21:00	21:30	11	9	1	50	0
	WB15		21:30	22:00					
3	WB8	10/05/2023	21:30	22:20	16	12	2	80	0
	WB15		21:00	21:30	15		0	60	
4	WB8	17/05/2023	21:30	22:00	14	12	3	90	0
	WB15		22:00	22:30					

2.5 LIMITATIONS

At the time of the HSI (June 2022) eight off-site waterbodies could not be accessed so were not assessed for their GCN suitability (WB1, WB3, WB4, WB5, WB6, WB7, WB12 and WB13). Although accessible, WB2 was dry and therefore was not attributed a HSI score as it is considered unsuitable for GCN. Due to iterations of the site boundary between 2022 and 2025, WB16 was not included within the 2022 HSI assessment but was surveyed on 19th May 2025. The inaccessible waterbodies (listed above) were all located off-site and it is considered that these will not be impacted by the proposed development, with the exception of WB7.

WB7 is located approximately 40 m from the RLB, closest to a hedgerow and arable field within the site. It is fully surrounded by very dense unmanaged scrub, obscuring all visibility and access to the water. It is likely that the lack of management has made WB7 unsuitable for GCN due to the severity of shading, making mating and a viable population highly unlikely. Furthermore, a road system (A29, A272 and connecting roundabout) travels to the east and west, and there is a large Recycling Plant with associated roads and car parking to the north. These are barriers to GCN dispersal. The next nearest waterbody to WB7 is WB16 approximately 200 m northwards (onsite), on the other side of the Recycling Plant. Based on the above, WB7 is considered to be isolated from all known waterbodies and is unlikely to hold a viable GCN population. As such, the lack of access is not considered to be a significant survey limitation.

The eDNA survey of the six suitable and accessible waterbodies was conducted on 29th June 2022, which is during the accepted survey season to detect GCN eDNA. Samples were then stored appropriately and sent by secure courier to SureScreen Scientifics, where they arrived on 8th July 2022. All six waterbodies surveyed returned a conclusive result.

The 2023 HSI surveys found WB8, WB11, WB14 and WB15 to be suitable to support GCN and therefore eDNA sampling was carried out from various points along the length of the waterbody, where accessible. Dense vegetation around WB14 meant eDNA samples could only be collected from one bank side.

The presence / likely absence surveys were carried out in April and June 2023, which is within the GCN survey season. This data is however beyond the usual accepted lifespan of data to remain valid (CIEEM,

2019). However, updated UK Habitat Classification surveys conducted in May 2025 confirmed that the waterbodies identified on the site (WB8, WB9, WB10 and WB11) were either dry or choked with overgrown vegetation, so largely unsuitable habitat for GCN, the results of the previous assessment can therefore be used to indicate the baseline and assess impacts.

Notwithstanding the limitations highlighted above, the survey effort applied is considered sufficient to meet the aims of the survey and this report, in accordance with the aforementioned guidelines.

3.0 RESULTS

3.1 DESK STUDY

Records for amphibians within a 2 km radius of the site boundary returned by SxBRC are summarised in **Table 2**, below.

Table 2. Amphibian records within 2 km radius of the site boundary

Species	Number of records	Recorded individuals	Latest recorded year	Closest record
Common Frog, <i>Rana temporaria</i>	6	6	2023	Onsite
Common Toad, <i>Bufo bufo</i>	12	>131	2018	1.3 km southwest
Great crested newt	17	202	2024	1.3km southeast
Palmate newt <i>Lissotriton helveticus</i>	2	31	2015	1.3 km southwest
Smooth newt, <i>Lissotriton vulgaris</i>	7	3	2016	1.3km southwest

A search of MAGIC did not identify any known statutory designated sites or non-statutory sites which are designated or are known to support GCN, within 2 km. The two SSSIs within 2km are designated for dragonfly richness and river quality, and geological interest respectively. MAGIC returned three EPSMLs granted for GCN for the same site within a 2 km radius of the site boundary, as shown in **Table 3**.

Table 3: Summary survey licence and EPSLs within 2 km radius of the site boundary.

Reference	Indicative Distance and Direction	Licensable Actions / Data Information
2016-27022-EPS-MIT-2	1.7 km south-east	Damage and destroy resting site

3.2 HSI ASSESSMENT

A summary of the HSI assessment for accessible waterbodies is given in **Table 4** below and shown in **Figure 1**. Detailed HSI scores are given in **Appendix G.1**.

Table 4: Summary of HSI results

Waterbody ID	OS grid reference	Habitat description	HSI Score
WB8	TQ 07309 26116	A waterbody located within cattle-grazed pasture. Banks have grown up with long grasses, nettle <i>Urtica dioica</i> and water hemlock <i>Oenanthe crocata</i> .	0.64 – Average
WB9	TQ 06945 26037	A pooled area of still water located between WB8 and WB10. Edges are shallow leading on to cattle-grazed pasture. One alder <i>Alnus glutinosa</i> tree is present on the north bank, and marginal vegetation comprises of reed canary grass <i>Phalaris arundinacea</i> , bulrush <i>Typha latifolia</i> and nettle.	0.78 – Good
WB10	TQ 07104 25891	A small pond located adjacent to a residential driveway, on the corner of an arable field. Water surface is covered in duckweed <i>Lemna sp.</i> . Yellow iris <i>Iris pseudacorus</i> is present within the waterbody on one edge.	0.78 - Good
WB11	TQ 06921 25982	A waterbody located within cattle-grazed pasture. No woody bankside vegetation, but banks are grown with broadleaved dock <i>Rumex obtusifolius</i> , field bindweed <i>Convolvulus arvensis</i> , nettle, soft rush <i>Juncus effusus</i> and reed canary grass.	0.69 – Average
WB14	TQ 07466 26136	A slowly flowing channelised waterbody along a mature oak treeline, bisecting two arable fields, choked with overgrown nettle, bramble <i>Rubus fruticosus</i> and willow <i>Salix sp.</i> .	0.69 – Average
WB15	TQ 09019 26749	A narrow wet ditch between two arable fields. The banks on either side are approximately 8 m wide and comprise of Yorkshire fog <i>Holcus lanatus</i> with square-stalked willowherb <i>Epilobium tetragonum</i> .	0.70 – Good
WB16	TQ 08125 26260	A large lake with designated pegs for fishing. Mature trees around the bank and waterlily <i>Nymphaea alba</i> present in the centre.	0.30 - Poor

3.3 EDNA SURVEY

Two waterbodies (WB8 and WB15) returned a positive eDNA result, as both waterbody samples had one out of a series of twelve qPCR replicates returning a positive result. The remaining four waterbodies surveyed were negative (WB9, WB10, WB11 and WB14), as none of the twelve qPCR replicates returned a positive result. The full eDNA results report from SureScreen Scientifics is provided in **Appendix G.2**. Note that WB8 is mislabelled by SureScreen Scientifics as WB18.

3.4 PRESENCE / LIKELY ABSENCE SURVEY

No GCN or other amphibians were recorded during the four surveys which would determine presence / likely absence. Over the course of the surveys, the water levels within the waterbodies decreased and they eventually became dry (see photographs in **Table 5**) and unsuitable to support breeding GCN.

Table 5: Photographs of surveyed waterbodies in early and mid-season.

Waterbody ID	Photograph early-2023	Photograph late-2023
WB8	 <i>Photograph taken during torch survey 10th May 2023.</i>	 <i>Photograph taken 14th Sept 2023.</i>
WB15	 <i>Photograph taken 10th May 2023.</i>	 <i>Photograph taken 4th Oct 2023.</i>

As GCN were not recorded within the four presence / likely absence surveys, and the water levels within the waterbodies becoming too shallow to bottle trap, the additional two surveys required to undertake a PSC estimate were not conducted.

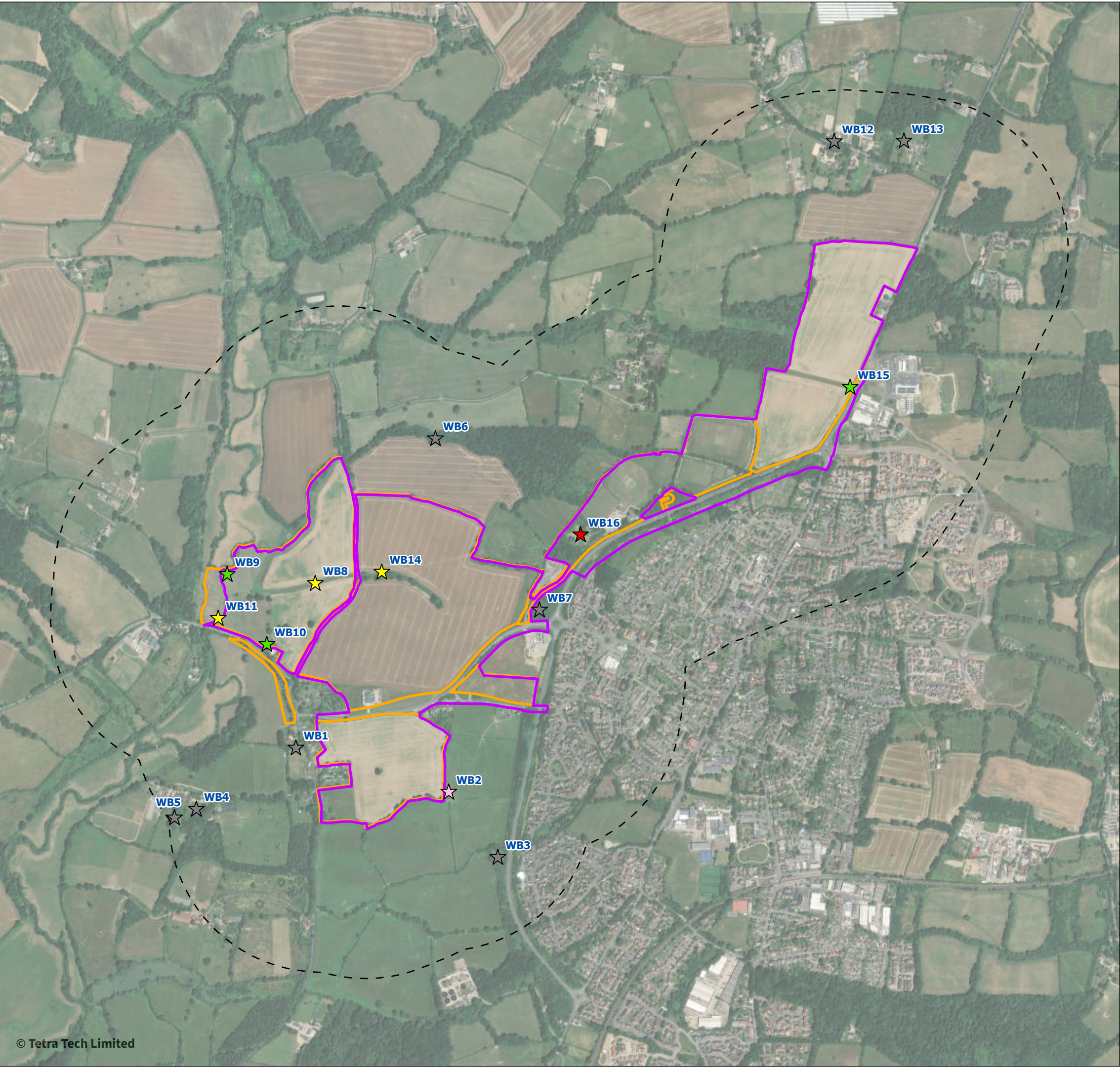
4.0 REFERENCES

- ARG UK. (2010). *Great Crested Newt Habitat Suitability Index. Advice Note 5*. ARG UK.
- Biggs J, E. N. (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*. Oxford: Freshwater Habitats Trust.
- CIEEM. (2019). *Advice Note on the Lifespan of Ecological Reports and Surveys*. Winchester.
- English Nature. (2001). *Great Crested Newt Mitigation Guidelines*. Peterborough.
- Oldham. (2001). *Evaluating the suitability of a habitat for the great crested newt (Triturus cristatus)*, *The Herpetological Journal*.
- Tetra Tech. (2019). *Biosecurity Policy*.

5.0 FIGURES

Figure 1 – Waterbody Location Plan and Habitat Suitability Index Results

Figure 2 – eDNA Survey Results Plan



Waterbody Location & HSI Survey Results Plan

Billingshurst



Highwood Homes Ltd Legend

- EclA boundary
- Survey area
- Survey area buffer (500 m)

HSI Category

- ★ Good
- ★ Average
- ★ Poor
- ★ Dry
- ★ Not accessed

Drawn by: CHRIS.DAWE

Checked by: Georgie Davey

Figure No. 1

Revision No. A

23 January 2026

0 140 280 420 560 Meters
Scale 1:12,385 @A3

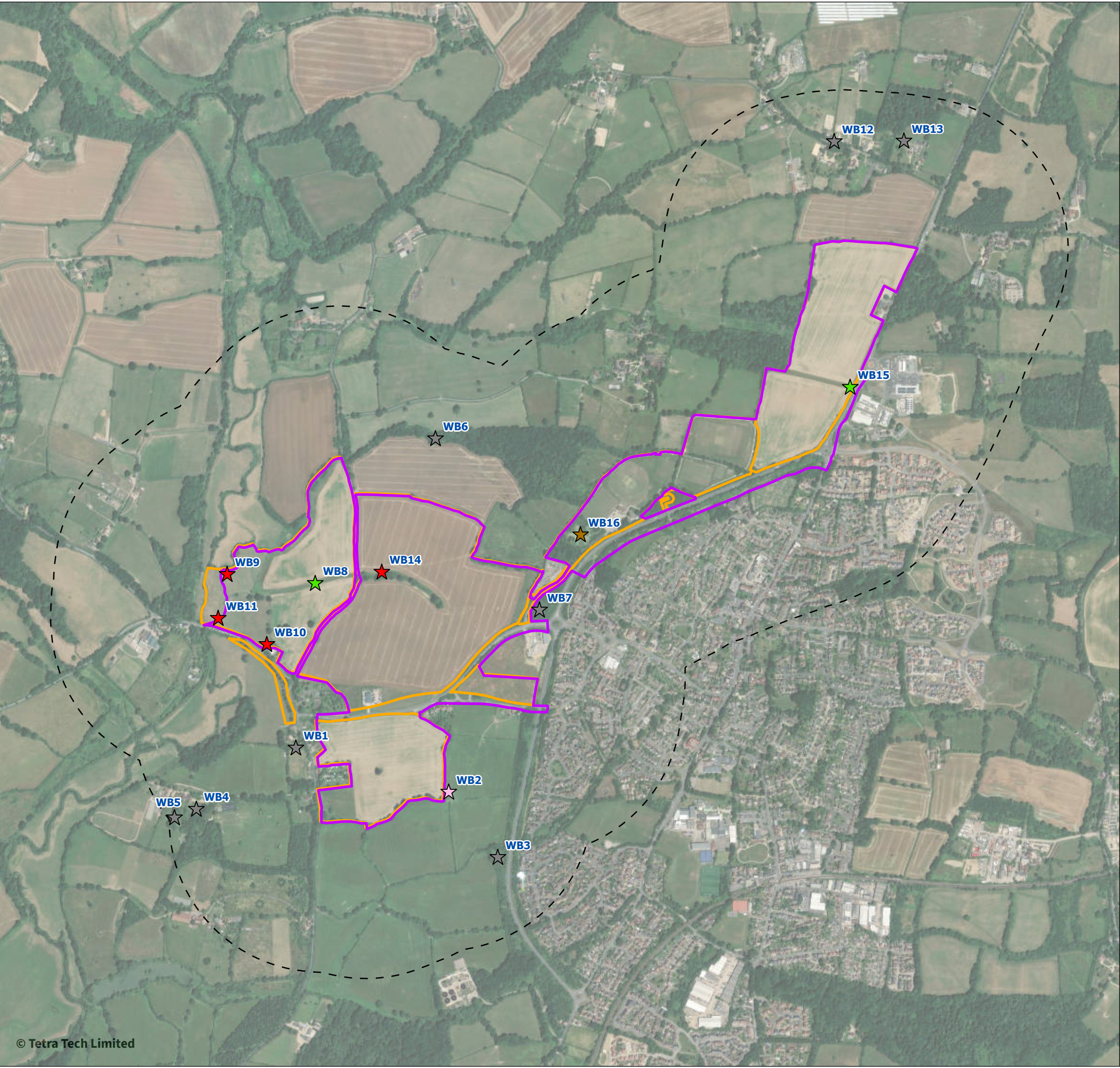
British National Grid
NGR: 508055E 126260N

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eDNA Survey Results Plan

Billingshurst



Highwood Homes Ltd

Legend

- EclA boundary
- Survey area
- Survey area buffer (500 m)

eDNA Results

- Positive
- Negative
- Dry
- Not surveyed
- Not accessed

Drawn by: CHRIS.DAWE
Checked by: Georgie Davey

Figure No. 2
Revision No. A
23 January 2026

0 140 280 420 560 Meters
Scale 1:12,385 @A3

British National Grid
NGR: 508055E 126260N

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6.0 APPENDICES

Appendix G.1 – HSI Results

Appendix G.2– eDNA Results

APPENDIX G.1: HSI RESULTS

Waterbodies WB1, WB2, WB3, WB4, WB5, WB6, WB7, WB12 and WB13 were not accessible and therefore not surveyed.

WB8			
Habitat Suitability Index			
			SI value
SI1. Map location	A/B/C	A	1.00
SI2. Surface area	rectangle/ellipse/irregular		
	length (m)	410	
	width (m)	1	
	OR estimate (m ²) if irregular		
	area (m ²) =	410	0.82
SI3. Dessication rate	never/rarely/sometimes/frequently	sometimes	0.50
SI4. Water quality	good/moderate/poor/bad	moderate	0.67
SI5. Shade	% of margin shaded 1m from bank	95	0.30
SI6. Waterfowl	absent/major/minor	absent	1.00
SI7. Fish population	absent/possible/minor/major	possible	0.67
SI8. Pond density	number of ponds within 1km	15	1.00
SI9. Terrestrial habitat	good/moderate/poor/isolated	moderate	0.67
SI10. Macrophyte cover	%	0	0.31
Photograph			HSI = 0.64
			
Date assessment undertaken			29.06.22

WB9				
Habitat Suitability Index				
SI1.	Map location	A/B/C	A	SI value 1.00
SI2.	Surface area	rectangle/ellipse/irregular		
		length (m)		
		width (m)		
		OR estimate (m ²) if irregular		
		area (m ²) =	201	0.40
SI3.	Dessication rate	never/rarely/sometimes/frequently	never	0.90
SI4.	Water quality	good/moderate/poor/bad	moderate	0.67
SI5.	Shade	% of margin shaded 1m from bank	25	1.00
SI6.	Waterfowl	absent/major/minor	minor	0.67
SI7.	Fish population	absent/possible/minor/major	possible	0.67
SI8.	Pond density	number of ponds within 1km	15	1.00
SI9.	Terrestrial habitat	good/moderate/poor/isolated	good	1.00
SI10.	Macrophyte cover	%	50	0.81
Photograph			HSI =	0.78
			Date assessment undertaken	29.06.22

Habitat Suitability Index

Photograph



HSI = 0.78

Date assessment undertaken	29.06.22
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Habitat Suitability Index

Photograph



HSI = 0.69

Date assessment undertaken	29.06.22
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Habitat Suitability Index

Photograph



HSI = 0.69

Date assessment undertaken	29.06.22
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WB15				
Habitat Suitability Index				
SI1.	Map location	A/B/C	A	SI value 1.00
SI2.	Surface area	rectangle/ellipse/irregular		
		length (m)		
		width (m)		
		OR estimate (m ²) if irregular		
		area (m ²) =	221	0.44
SI3.	Dessication rate	never/rarely/sometimes/frequently	sometimes	0.50
SI4.	Water quality	good/moderate/poor/bad	good	1.00
SI5.	Shade	% of margin shaded 1m from bank	80	0.60
SI6.	Waterfowl	absent/major/minor	absent	1.00
SI7.	Fish population	absent/possible/minor/major	absent	1.00
SI8.	Pond density	number of ponds within 1km	15	1.00
SI9.	Terrestrial habitat	good/moderate/poor/isolated	moderate	0.67
SI10.	Macrophyte cover	%	0	0.31
Photograph			HSI =	0.70
			Date assessment undertaken	29.06.22

WB16				
Habitat Suitability Index				
SI1.	Map location	A/B/C	A	SI value 1.00
SI2.	Surface area	rectangle/ellipse/irregular		
		length (m)		
		width (m)		
		OR estimate (m ²) if irregular	4648	
		area (m ²) =	4648	0.37
SI3.	Dessication rate	never/rarely/sometimes/frequently	never	0.90
SI4.	Water quality	good/moderate/poor/bad	poor	0.33
SI5.	Shade	% of margin shaded 1m from bank	30	1.00
SI6.	Waterfowl	absent/major/minor	major	0.01
SI7.	Fish population	absent/possible/minor/major	major	0.01
SI8.	Pond density	number of ponds within 1km	15	1.00
SI9.	Terrestrial habitat	good/moderate/poor/isolated	good	1.00
SI10.	Macrophyte cover	%	30	0.61
Photograph			HSI =	0.30
			Date assessment undertaken	19.05.25

APPENDIX G.2: EDNA RESULTS

Folio No: E15029
Report No: 1
Purchase Order: 7006860
Client: Tetra Tech
Contact: Vivienne Greenough

TECHNICAL REPORT

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

SUMMARY

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

RESULTS

Date sample received at Laboratory: 08/07/2022
Date Reported: 22/07/2022
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
5379	WB10 Billingshurst		Pass	Pass	Pass	Negative	0
5631	WB15 Billingshurst		Pass	Pass	Pass	Positive	1
5636	WB18 Billingshurst		Pass	Pass	Pass	Positive	1
5637	WB14 Billingshurst		Pass	Pass	Pass	Negative	0
5642	WB9 Billingshurst		Pass	Pass	Pass	Negative	0
5644	WB11 Billingshurst		Pass	Pass	Pass	Negative	0

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



Reported by: Jennifer Higginbottom

Approved by: Chelsea Warner

METHODOLOGY

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

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

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

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

INTERPRETATION OF RESULTS

SIC: **Sample Integrity Check** [Pass/Fail]

When samples are received in the laboratory, they are inspected for any tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to inconclusive results.

DC: **Degradation Check** [Pass/Fail]

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

IC: **Inhibition Check** [Pass/Fail]

The presence of inhibitors within a sample are assessed using a DNA marker. If inhibition is detected, samples are purified and re-analysed. Inhibitors cannot always be removed, if the inhibition check fails, the sample should be re-collected.

Result: **Presence of GCN eDNA** [Positive/Negative/Inconclusive]

Positive: GCN DNA was identified within the sample, indicative of GCN presence within the sampling location at the time the sample was taken or within the recent past at the sampling location.

Positive Replicates: Number of positive qPCR replicates out of a series of 12. If one or more of these are found to be positive the pond is declared positive for GCN presence. It may be assumed that small fractions of positive analyses suggest low level presence, but this cannot currently be used for population studies. In accordance with Natural England protocol, even a score of 1/12 is declared positive. 0/12 indicates negative GCN presence.

Negative: GCN eDNA was not detected or is below the threshold detection level and the test result



should be considered as evidence of GCN absence, however, does not exclude the potential for GCN presence below the limit of detection.

