



Redrow

Land South of Burbage

UPDATE BAT AND GREAT CRESTED NEWT SURVEY REPORT

November 2023

FPCR Environment and Design Ltd

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1.0 INTRODUCTION

- 1.1 Redrow commissioned FPCR Environment and Design Ltd. to complete update bat and great crested newt (GCN) *Triturus cristatus* surveys at the site located to the south of Coventry Road, north of the M69 and to the east of Bullfurlong Lane, Burbage, Leicestershire (central OS grid reference SP 439 915) to supplement the Ecological Appraisal.
- 1.2 The site comprised nine grassland field compartments (eight supporting improved grassland and one semi-improved), Burbage Fields farm buildings and the properties and associated gardens of 65 and 65a Coventry Road. Most field compartments were enclosed by hedgerows, some associated trees, whilst a ditch and associated brook ran through the site. Additional habitats present within the site included small areas of dense and scattered scrub, small area of marshy grassland, bare ground, standard trees, hardstanding track/roads and a small area of amenity grassland in the east.
- 1.3 During survey work of the site undertaken in June 2021 (including building internal and external assessments) buildings B1a, B2c, B3c, B5a-d, B6-B7 and B65a, which are to be lost to the proposed development, were assessed as having negligible potential to support roosting bats. Buildings B1b, B2a, B2b, B3b, B4c and B65 were considered to have low potential to support roosting bats, whilst building B3a, B4a and B4b were assessed as providing moderate potential. Surveys undertaken on the buildings with low and moderate potential for roosting bats in 2021 recorded no bat roosts.
- 1.4 In addition, a suit of bat activity surveys, comprising transects and automated static detector surveys, were also undertaken in 2021.
- 1.5 Aquatic GCN surveys were undertaken in 2021 on a number of ponds within the site/within 250m of the site during which no GCN were recorded. An eDNA survey was undertaken on 21st April 2021 on P22, within the north of the site which returned a negative result for the presence of GCN eDNA.
- 1.6 The results of the bat and GCN surveys undertaken in 2021 are provided within the submitted ecological appraisal (FPCR. 2023. Land South of Burbage Ecological Appraisal).
- 1.7 This report details the results of update building nocturnal surveys undertaken on buildings with low and moderate potential to support roosting bats, update bat activity surveys (transect and automated static detector) and update GCN eDNA surveys for the site.

Development Proposals

- 1.8 The proposals for the site are a Hybrid planning application for:
 - (a) full planning application for 422 dwellings, public open space and associated infrastructure and
 - (b) an outline planning application for a residential care home and up to 11no. self-built plots.

2.0 METHODOLOGY

Roosting Bats – Nocturnal Building Survey

- 2.1 Update nocturnal dusk emergence and re-entry bat surveys of the buildings to be lost within the site were completed in July and August 2023. During the survey, bat surveyors were positioned such that all aspects of the buildings with potential to support bats could be observed (Figures 1 and 2). The dusk surveys commenced 15 minutes before sunset and ended 1.5 to 2 hours following sunset whilst the dawn surveys commenced 1.5 to 2 hours before sunrise and ended 15 minutes after sunrise.
- 2.2 During the nocturnal survey the species and location of any bat activity was recorded using Echo Meter Touch® bat detectors in conjunction with Echo Meter Touch® app. Post-survey, bat calls were analysed using Kaleidoscope© (Wildlife Acoustics) software package, by taking measurements of the peak frequency, inter-pulse interval, call duration and end frequency. The survey was conducted in appropriate conditions, i.e. ambient temperatures above 10°C and with little wind and no rain (Table 1).

Table 1 – Nocturnal survey dates 2023

Survey date	Building	Dusk / Dawn	Time (sunset/sunrise)	Temperature	Cloud cover %	Rain (0-10)	Wind (0-10)
19.07.23	B1b, B2a, B2b, B3a, B3b, B4a, B4b, B4c	Dusk	21:17	14-16°C	50-80	0	1-2
20.07.23	B65	Dawn	05:07	10°C	20-30	0	1
03.08.23	B3a, B3b	Dawn	05:28	14°C	50	0	0-1
18.08.23	B3a, B3b, B4a, B4b, B4c	Dawn	05:52	15°C	100	0	1-2
04.09.23	B4a, B4b, B4c	Dusk	19:47	21-26°C	0-10	0	1-4

Foraging/Commuting Bats – Update Transect

Update Activity Transect Surveys

- 2.3 An update transect survey was undertaken across the site on 4th May 2023, which comprised a single walked transect. The primary objective of this was to identify foraging areas, commuting routes and species utilisation of the development and adjacent area.
- 2.4 The transect survey methodology takes into account the statutory guidance from English Nature (now Natural England)¹ and further guidelines introduced by the BCT² and JNCC³ (appropriate at the time of survey). The survey effort was determined from recommendations provided in BCT guidance.
- 2.5 The transect route was determined prior to survey in order to cover all habitat types on site with point count stops included to identify activity levels around the features of potential value to bats that are to be most affected by the development proposals (i.e. hedgerows, trees, scrub etc.). Each

¹ Mitchell-Jones A.J., 2004. *Bat Mitigation Guidelines*. English Nature, Peterborough.

² Collins, J. 2016. *Bat Surveys for Professional Ecologists: Good Practice Guidelines* (3rd Edn). The Bat Conservation Trust. I

³ Mitchell-Jones A.J. and McLeish A.P. 2004. *The Bat Workers' Manual 3rd Edition*. JNCC, Peterborough.

point count was a minimum of five minutes long, during which time all bat activity was recorded. The transects commenced at sunset and was two hours in duration.

- 2.6 The transect was walked at a steady pace and when a bat passed by the species, time and behaviour was recorded on a site plan. This information helps to form a general view of the bat activity present on site and highlights what habitat types are associated with bat activity. Wildlife Acoustics Inc. Echo Meter Touch® bat detectors were used during transects in conjunction with Echo Meter Touch® app and Apple Inc. iPad®.
- 2.7 The transects were undertaken when conditions were suitable (i.e. when the ambient air temperature exceeded 10°C and there was little wind and no rain) (see Table 2).

Table 2: Bat Activity Transect Survey Conditions

Survey Date	Sunset	Temperature °C	Rain	Wind (0-5)	Cloud %
04.05.23	20:34	14	0	1	90

Update Automated Surveys

- 2.8 A single static passive recording broadband detector was deployed along hedgerow H24, of which small section will be lost to the proposed development, in May 2023 to supplement the manual transect survey in accordance with industry guidance⁴.
- 2.9 Passive monitoring was undertaken using an automated logging system (Wildlife Acoustics Inc. Song Meter® SM4BAT+ bat detectors) with the output saved to an internal storage device. The SM4BAT+ device was placed within a hedgerow which provides foraging/commuting opportunities for the local bat population which is to be partially affected by the proposed development (Figure 3).
- 2.10 The static detector was programmed to activate 30 minutes before dusk and record continuously until 30 minutes following sunrise. It was set to record over an extended period (minimum of five consecutive nights) of suitable and/or typical weather conditions.
- 2.11 The recorded data was analysed using the Kaleidoscope© software package. The automated static detector survey timings and weather conditions are provided Table 3.

GCN – eDNA SURVEY

- 2.12 An update eDNA survey was carried out on ponds, P1, P2, P3, P4, P7, P9, P10 and P21). eDNA sampling was carried out to determine the presence/absence of GCN in accordance with industry guidance. This methodology has been approved by Natural England for the determination of GCN presence/absence.
- 2.13 Sampling was completed on 26th April 2023, with P9 repeated on 6th June, by appropriately licenced ecologists who collected water samples using kits obtained from ADAS. The methodology comprised taking samples of agitated water from 20 locations around the pond perimeter and mixing thoroughly. 15ml of this water was then placed into each of 6 sterile sample tubes containing preservative, precipitates and a DNA sequence that was used for degradation control. The sample was stored in accordance with the protocols provided by the laboratory. The sample was then transported under suitable conditions to the ADAS laboratory for analysis.

⁴ Collins, J. (ed.), 2016. *Bat Surveys for Professional Ecologists: Good Practice Guidelines* (3rd edn). The Bat Conservation Trust.

LIMITATIONS

- 2.14 The overnight temperatures during the May static detector survey dipped below 10°C briefly on the evenings of 5th/6th and 7th/8th (only for couple of hours each time). This was considered to be within the normal temperature range for this time of year and was only briefly below the appropriate conditions and as such it is considered unlikely to have significantly affected the overall results of the survey undertaken.

3.0 RESULTS

Roosting Bats – Nocturnal Building Survey

- 3.1 During the update building surveys undertaken, four common pipistrelle roost locations *Pipistrellus pipistrellus* were recorded across buildings B3a, B3b and B4a. The details of these roosts are provided in Table 4, whilst the locations are shown in photos 1 – 3 and in Figure 1. All roost occasions were recorded to be of individual or low number of bats.

Table 4: Bat roost survey results

Survey date	Building	Elevation	Emergence /re-entry	Time	Species and number of individuals	Location
19/07/23	B3a	South-east	Emergence	21:58	1 x Common pipistrelle	Gap in roof tiles near apex of roof
19/07/23	B3b	South-west	Emergence	21:59	1 x Common pipistrelle	Missing roof tile
18/08/23	B4a	Eastern	Re-entry	05:44	1 x Common pipistrelle	Under gutter directly below chimney
04/09/23	B4a	Southern	Emergence	20:19 and 20:31	2 x Common pipistrelle	From roof tiles near chimney (exact location unclear)

- 3.2 No bat emergencies or re-entries were recorded within buildings B1, B2 or B65 during the survey work undertaken.
- 3.3 Incidental bat activity recorded during the update building surveys comprised foraging and commuting common pipistrelle and Nathusius' pipistrelle *Pipistrellus nathusii* and occasional commuting noctule *Nyctalus noctule*, soprano pipistrelle *Pipistrellus pygmeus*, unidentified *Myotis* sp. and brown long-eared *Plecotus auratus*.



Photograph 1: B3a and B3b- bat roost locations.



Photograph 2: B4a eastern elevation- bat roost location.



Photograph 2: B4a southern elevation- bat roost location

Foraging/Commuting Bats – Update Transect

- 3.4 The following section provides a summary of the results recorded during the update transect and static survey undertaken in May 2023. Details of the survey data are provided in the associated plans and Appendices as indicated.

Activity Transect Survey (Figure 3)4th May 2023

- 3.5 Bat activity was recorded on 14 occasions within the application site boundary. Similarly, to previous surveys undertaken across the site (2021), bat activity was dominated by common pipistrelle with occasional noctule (two occasions). In addition, a single commuting brown long-eared bat was recorded in the west of the site. No other bat species were recorded during the update transect.

Static Detector Surveys (Appendix A and Figure 3)5th May – 9th May 2023

- 3.6 The static detector was located along hedgerow H24 in the centre of the site. A total of 169 registrations were recorded across the 5 survey nights, which is lower than activity levels recorded in 2021.
- 3.7 Activity recorded comprised a similar composition to activity recorded across the 2021 static surveys, with new species record. Activity in 2023 was dominated by common pipistrelle, with other species recorded including noctule, unidentified *Nyctalus* sp., *Myotis* sp., brown long-eared bat and unidentified *Pipistrelle* sp. Table 5 provides details on numbers recorded.

Table 5: Bat Static Summary Table

Species	Registrations	Percentage of Total (approximate)
Common Pipistrelle	104	61%
Noctule	37	22%
<i>Nyctalus</i> Species	18	11%
<i>Myotis</i> Species	3	2%
Brown Long-eared	2	1%
<i>Pipistrelle</i> Species	5	3%
Total	169	-

GCN – eDNA Survey26th April 2023

- 3.8 All the ponds surveyed on 26th April returned negative eDNA results, with the exception of pond P9. Given the lack of GCN recorded during the 2021 surveys and the majority of ponds returning a negative result in 2023, pond P9 was resurveyed in June. The second survey on pond P9 returned a negative result for the presence of GCN eDNA (Figure 4).

4.0 DISCUSSION AND RECOMMENDATIONS

- 4.1 All bats and their roosts are afforded full legal protection under the Conservation of Habitats and Species Regulations 2017 (as amended) and the Wildlife & Countryside Act 1981 (as amended). The purpose of the legislation is to maintain and restore protected species to a situation where their populations are favourable.

- 4.2 Under Regulation 41 of the Conservation of Habitats and Species Regulations 2017 (as amended) it is illegal to deliberately capture, injure or kill; deliberately disturb (including intentionally or recklessly) any UK bat species. This includes disturbance which impairs their ability to: breed and rear young, to migrate or hibernate, or adversely impacts their local distribution or abundance.

Roosting Bats

- 4.3 Single common pipistrelles were recorded roosting in buildings B3a and B3b, whilst three common pipistrelles in building B4a, across two locations. Given the low numbers recorded roosting, it is considered that the buildings support occasional roosts for individual/small numbers of common pipistrelle which are of low conservation concern. Following this, the loss of the buildings and roosts is considered to have a low scale of impact.
- 4.4 A Natural England Licence will be required to facilitate the removal/demolition of buildings B3a, B3b and B4a, which will result in the loss of the recorded roosts. As a requirement of the licence, replacement roost sites will likely be provided within the site boundary to ensure the Favourable Conservation Status of the recorded species is maintained. This could take the form of bat boxes on new buildings or retained trees within the site.
- 4.5 With the implementation of appropriate precautionary working methods, sensitive mitigation and enhancement measures, to be agreed via the licencing process, it is considered that significant adverse impacts to the local bat population will be avoided, and overall enhancements feasible at the site level.
- 4.6 The opportunity exists to further enhance roosting opportunities within the site for bats through the provision of bat boxes above and beyond that required under the licence, which can be erected on retained trees or on buildings within the site. Suitable designs include low profile WoodStone Bat Box, Vivara Pro WoodStone Bat and Vivara Pro Build-in WoodStone bat tube (or equivalent). These should be installed in pairs or groups of three at a height of at least 3m above ground in uncluttered locations and facing E/SE/S/SW/W to provide a range of alternate roosting opportunities.

Foraging and Commuting Bats

- 4.7 As per the previous survey work undertaken across the site, the bat assemblage recorded during the update 2023 surveys comprised common and widespread species.
- 4.8 Very little bat activity was recorded along the two hedgerows scheduled to be lost to the proposed development (H6 and H13), with only a single occasion of commuting noctule at the northern end of H13 during the May transect. Similarly, very low levels of bat activity were recorded along the other hedgerows where sections will be lost, with the activity recorded comprising species commonly recorded within urban environments. No new species were recorded within the site during the update survey work undertaken.
- 4.9 Given the species recorded and activity levels, the conclusion reached within the submitted Ecological Appraisal that loss of hedgerows H6 and H13 and the retention/proposed provision of suitable foraging and commuting habitats within and around the edges of the site is not anticipated to significantly impact the local bat population remains valid.
- 4.10 As per the Ecological Appraisal, to minimise additional potential effects over the construction/operational periods in the long-term, a sympathetic lighting scheme is recommended,

particularly adjacent to retained habitats and in the vicinity of newly created green space. This should consider the following design measures:

- No use of lighting at night during the construction period in areas where this will illuminate retained or created suitable commuting or foraging habitats including hedgerows/trees, the brook corridor and ponds.
- The lighting scheme including road lighting will direct lighting to where it is needed, using deflectors where appropriate to minimise light spill beyond the intended target area.
- Any upward lighting will be avoided or otherwise minimised via the use of baffles/cowls.
- Dark corridors (<1.0 lux) will be designed to incorporate retained habitats of value to bats for foraging, potential roosting and commuting purposes including the brook corridor and hedgerows. Newly created tree/shrub/hedgerow planting and ponds will also be sufficiently buffered to ensure a similarly a low light level is maintained in the vicinity of these features.
- Lighting will be appropriately designed to avoid light spill onto buffer zones.
- Lighting will either comprise LED luminaires as these have a sharp cut-off, lower intensity, good colour rendition and dimming capability, or low-pressure sodium lights or alternate design that emits light at one wavelength and as such has a low attraction to insects.
- Luminaires should feature peak wavelengths higher than 550nm to avoid the component of light most disturbing to bats⁵.
- All luminaires should lack UV elements when manufactured. The use of metal halide, fluorescent sources will be avoided⁴.

Great Crested Newts

- 4.11 The positive eDNA result returned for pond P9 is considered to be a false positive given the previous survey work undertaken, results of other ponds in 2023 and the negative result returned in June for the same pond.
- 4.12 No evidence for the presence of GCN were recorded within any of the other surveyed on-site or off-site ponds located within 250m of the site in 2023.
- 4.13 Given the lack of evidence for GCN recorded in 2021 and 2023, as per the submitted Ecological Appraisal, GCN are not considered to pose a constraint to the proposed development.

⁵ Institute of Lighting Professionals (ILP) and Bat Conservation Trust (BCT), 2023. Guidance Note GN08/23 Bats and Artificial Lighting at Night.

5.0 SUMMARY AND CONCLUSION

- 5.1 Common pipistrelle were recorded roosting within buildings B3a, B3b and B4a. Therefore, a licence will be required for the loss of these buildings/roost, under which working methods and mitigation are to be agreed with Natural England.
- 5.2 Bat activity recorded across the site was largely in line with previous survey data. Given the majority of suitable habitats are to be retained and significant green space to be provided within/at the boundaries of the site, along with the implementation of recommended appropriate lighting schemes, no significant impacts to the local bat population are anticipated as a result of the proposed development.
- 5.3 GCN are not considered likely to be present/utilising the habitats within the site and neighbouring ponds due to negative eDNA results of surveyed ponds.

APPENDIX A: UPDATE AUTOMATED DETECTOR RESULTS

Survey Dates	Survey Hours	Total Avg. per hour	Total Registrations	Common Pipistrelle			Noctule			Nyctalus Species			Myotis Species			Brown Long-eared			Pipistrellus Species		
				Period Total	Peak Count	Avg. per hour	Period Total	Peak Count	Avg. per hour	Period Total	Peak Count	Avg. per hour	Period Total	Peak Count	Avg. per hour	Period Total	Peak Count	Avg. per hour	Period Total	Peak Count	Avg. per hour
04/05/2023-09/05/2023	48:19:51	3.496	169	104	59	2.152	37	26	0.766	18	6	0.372	3	1	0.062	2	1	0.041	5	3	0.103

APPENDIX B: eDNA RESULTS

Client: Nick Grant,
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ADAS
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172 Chester Road
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Sample ID: ADAS-624 Condition on Receipt: High Sediment Volume: Passed
Client Identifier: P1, 9685 Description: pond water samples in preservative
Date of Receipt: 03/05/2023 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	04/05/2023
Degradation Control [§]	Within Limits	Real Time PCR	04/05/2023
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	04/05/2023
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: 05/05/2023 Date of issue: 05/05/2023

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

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

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

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

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

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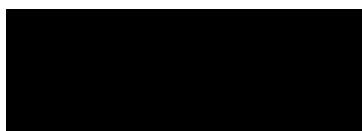
www.adas.uk

Sample ID: ADAS-629 Condition on Receipt: Good Volume: Passed
Client Identifier: P7, 9685 Description: pond water samples in preservative
Date of Receipt: 03/05/2023 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	04/05/2023
Degradation Control [§]	Within Limits	Real Time PCR	04/05/2023
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	04/05/2023
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

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

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Sample ID: ADAS-630 Condition on Receipt: High Sediment Volume: Passed
Client Identifier: P21, 9685 Description: pond water samples in preservative
Date of Receipt: 03/05/2023 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	05/05/2023
Degradation Control [§]	Within Limits	Real Time PCR	05/05/2023
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	05/05/2023
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[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

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Sample ID: ADAS-636 Condition on Receipt: Medium Sediment Volume: Passed
Client Identifier: P9, 9685 Description: pond water samples in preservative
Date of Receipt: 03/05/2023 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	05/05/2023
Degradation Control [§]	Within Limits	Real Time PCR	05/05/2023
Great Crested Newt*	1 of 12 (GCN positive)	Real Time PCR	05/05/2023
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

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

www.adas.uk

Sample ID: ADAS-640 Condition on Receipt: Good Volume: Passed

Client Identifier: P10, 9685 Description: pond water samples in preservative

Date of Receipt: 03/05/2023 Material Tested: eDNA from pond water samples

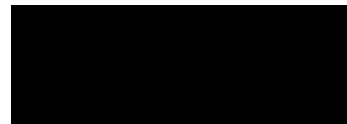
Determinant	Result	Method	Date of Analysis
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Degradation Control [§]	Within Limits	Real Time PCR	04/05/2023
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	04/05/2023
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Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 05/05/2023 Date of issue: 05/05/2023

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

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

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

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

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

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ADAS
Spring Lodge
172 Chester Road
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Sample ID: ADAS-641 Condition on Receipt: High Sediment Volume: Passed
Client Identifier: P4, 9685 Description: pond water samples in preservative
Date of Receipt: 03/05/2023 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	1 of 2	Real Time PCR	04/05/2023
Degradation Control [§]	Within Limits	Real Time PCR	04/05/2023
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	04/05/2023
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: 05/05/2023 Date of issue: 05/05/2023

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Sample ID: ADAS-645 Condition on Receipt: Good Volume: Passed
Client Identifier: P2, 9685 Description: pond water samples in preservative
Date of Receipt: 03/05/2023 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	04/05/2023
Degradation Control [§]	Within Limits	Real Time PCR	04/05/2023
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	04/05/2023
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

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Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 05/05/2023 Date of issue: 05/05/2023

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Sample ID: ADAS-747 Condition on Receipt: High Sediment Volume: Passed
Client Identifier: P3, 9685 Description: pond water samples in preservative
Date of Receipt: 03/05/2023 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	04/05/2023
Degradation Control [§]	Within Limits	Real Time PCR	04/05/2023
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	04/05/2023
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: 05/05/2023 Date of issue: 05/05/2023

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

Appendix 1: Interpretation of results

Sample Condition

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

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

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

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

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

What do my results mean?

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

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

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

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

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

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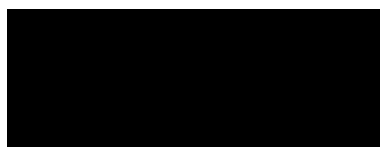
www.adas.uk

Sample ID: ADAS-764 Condition on Receipt: Low Sediment Volume: Passed
Client Identifier: P9, 9685 Description: pond water samples in preservative
Date of Receipt: 09/06/2023 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	13/06/2023
Degradation Control [§]	Within Limits	Real Time PCR	13/06/2023
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	13/06/2023
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: 14/06/2023 Date of issue: 14/06/2023

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

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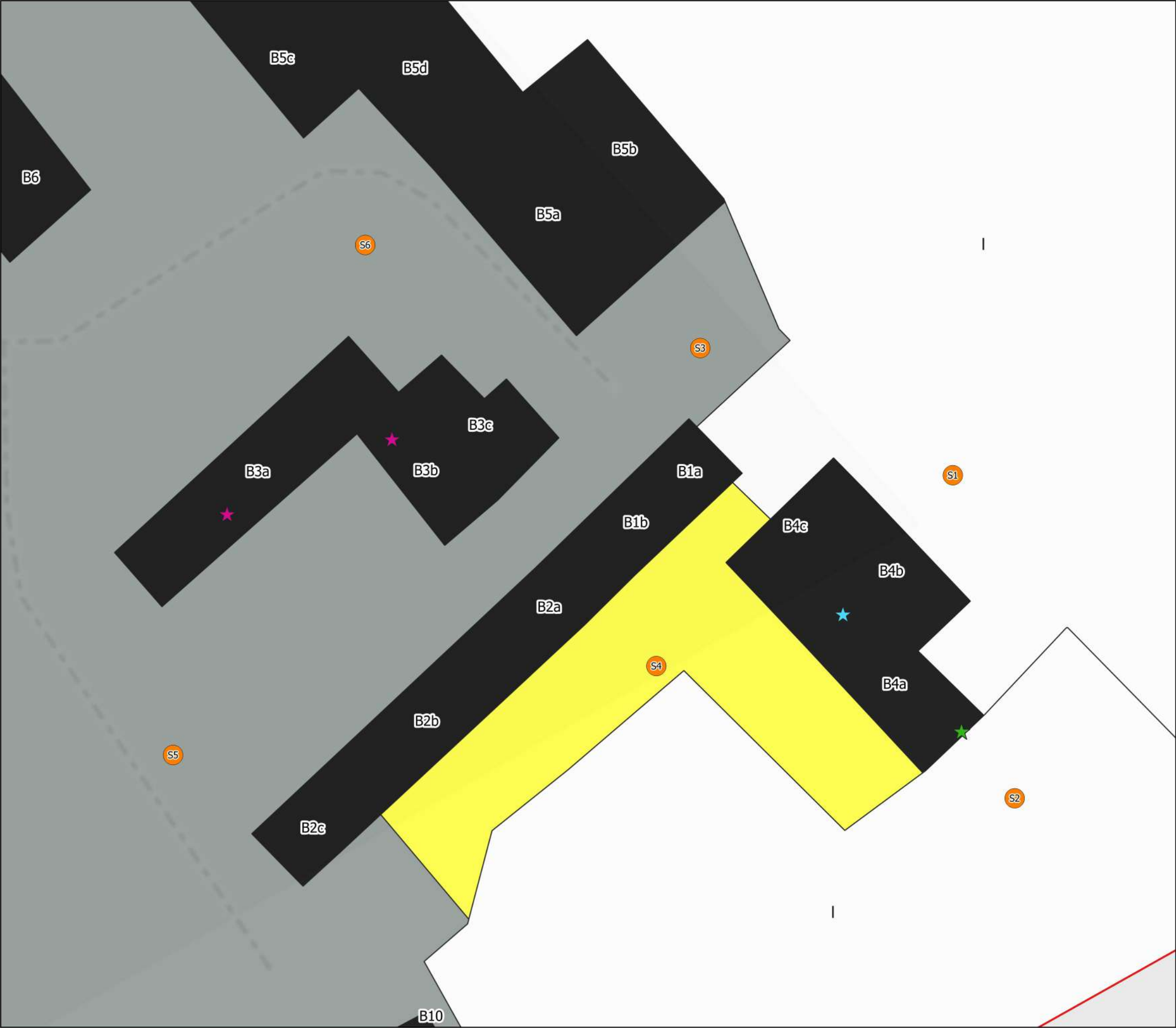
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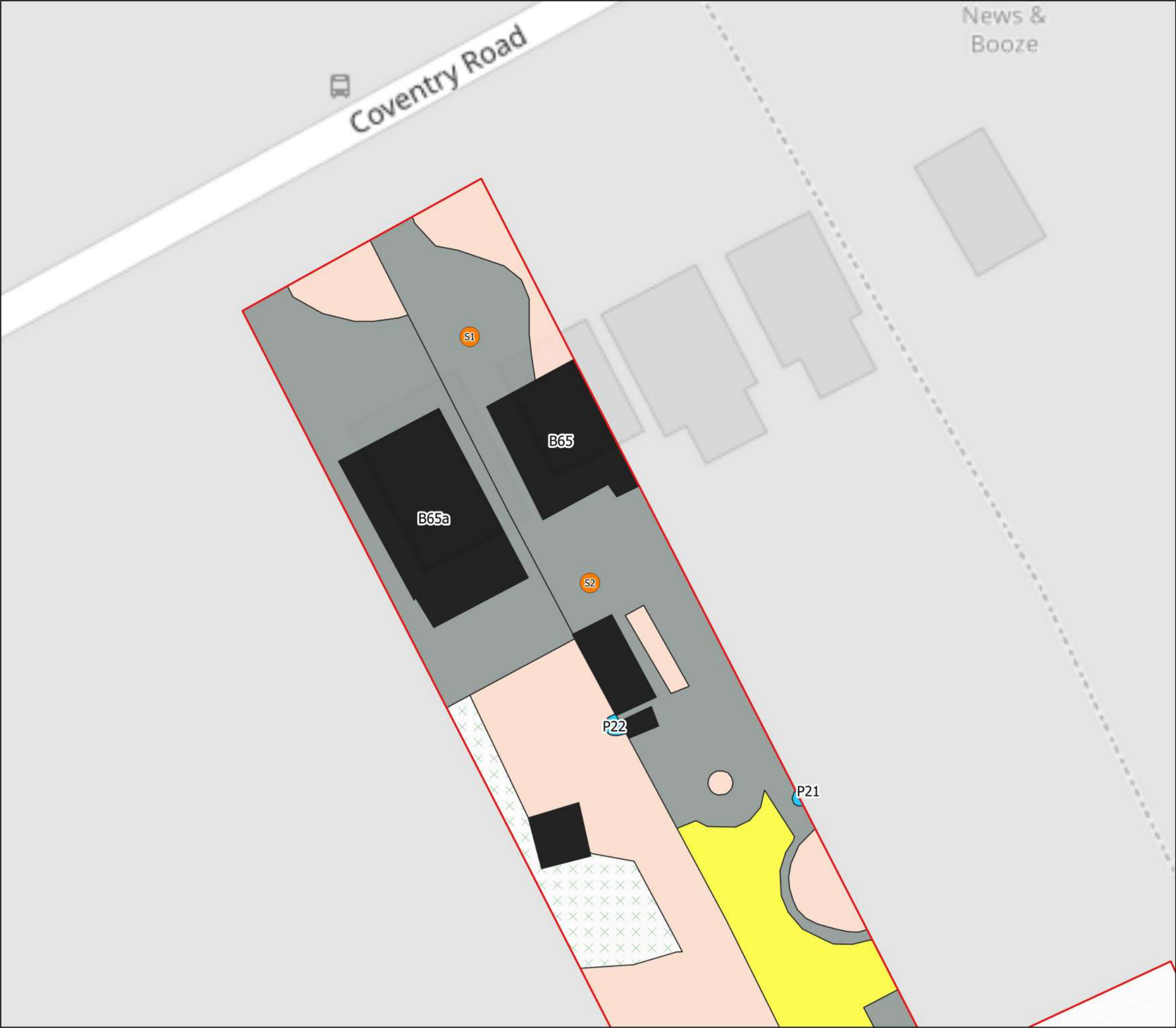
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Key:

- Site Boundary
- Building Surveyor Locations

Common Pipistrelle Roost

- 19.07.23
- 18.08.23
- 04.09.23

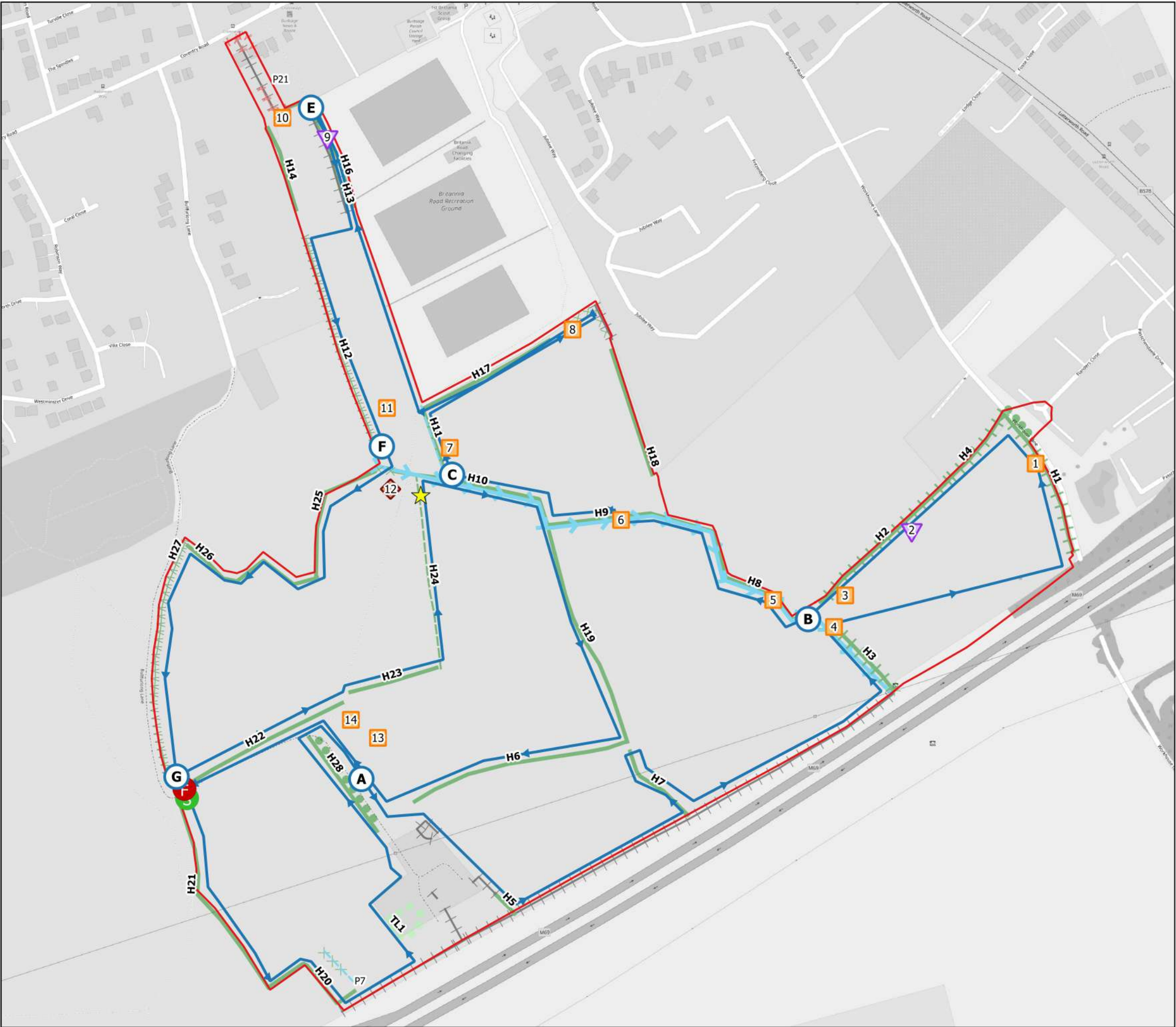


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Key:

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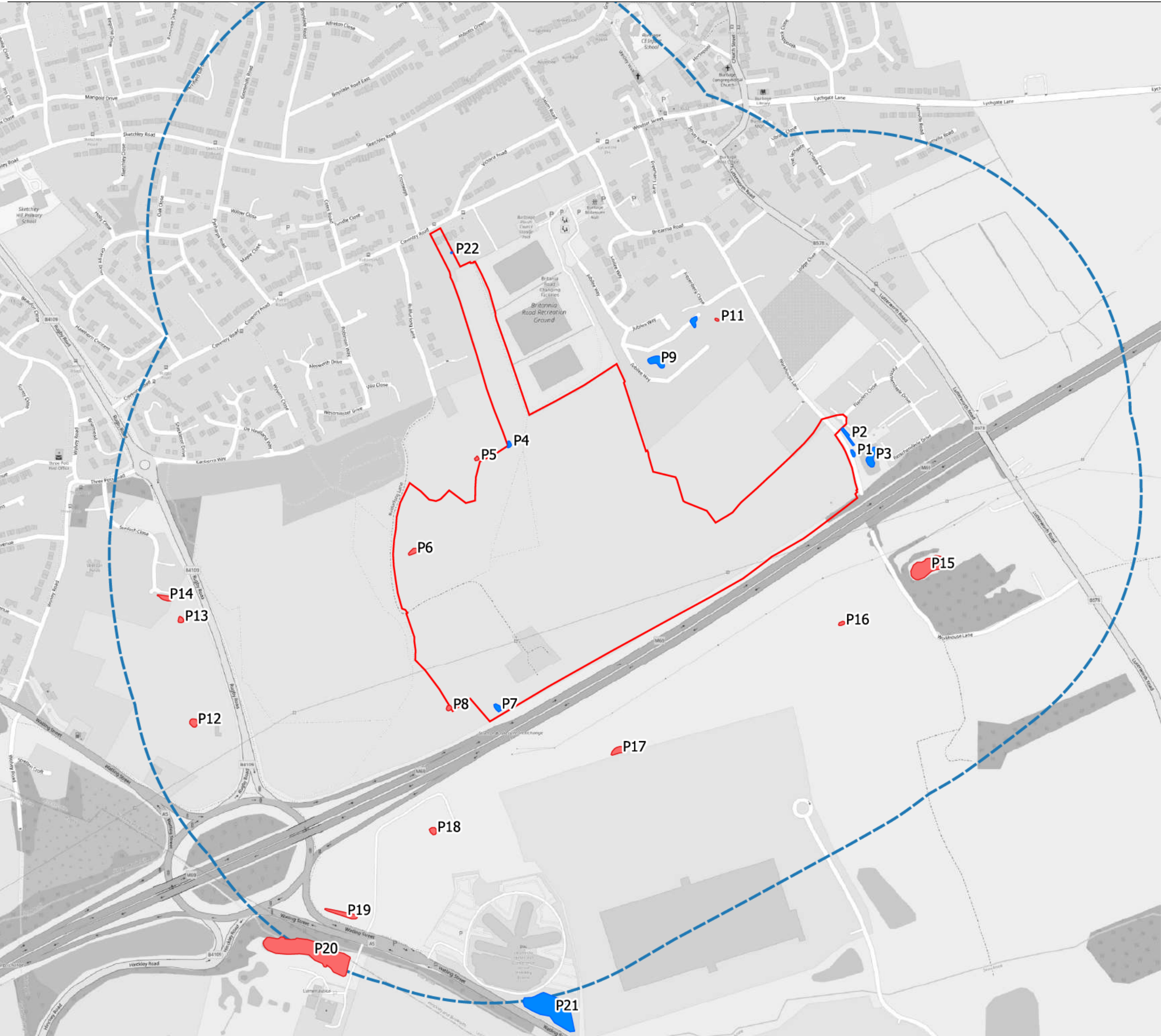
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Key:

- Site Boundary
- Transect Route
- Point Count Locations
- Start point
- Finish point
- Static Location
- Bat Contacts**
 - Common Pipistrelle
 - Brown Long-eared
 - Noctule

Ref.	Time	Species Name	Passes	Behaviour
PC A	20:44 - 20:49	No Bats	NULL	NULL
1	21:05	Common Pipistrelle	2	Commuting
2	21:07	Noctule	1	Commuting
3	21:08	Common Pipistrelle	1	Commuting
PC B	21:09 - 21:14	Ref. 4	NULL	NULL
4	21:12	Common Pipistrelle	3	Commuting
5	21:15	Common Pipistrelle	4	Foraging
6	21:20	Common Pipistrelle	Continuous	Foraging
PC C	21:25 - 21:30	Ref. 6	NULL	NULL
7	21:29	Common Pipistrelle	1	Commuting
8	21:34	Common Pipistrelle	1	Commuting
PC E	21:43 - 21:48	Ref. 9, 10	NULL	NULL
9	21:45	Noctule	2	Commuting
10	21:48	Common Pipistrelle	3	Commuting
PC F	21:54 - 21:59	Ref. 11, 12	NULL	NULL
11	21:55	Common Pipistrelle	Multiple	Foraging
12	21:57	Brown Long-eared	1	Commuting
13	22:28	Common Pipistrelle	Multiple	Foraging
14	22:29	Common Pipistrelle	1	Commuting



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Key

- Site Boundary
- 500m Buffer
- eDNA Results - Negative
- Not Surveyed